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Vitamin A and D deficiencies associated with incident tuberculosis in HIV-infected patients initiating antiretroviral therapy in multinational case-cohort study

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Abstract

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Conflicts of Interest

All other authors declare no conflicts of interest.

Introduction—Numerous micronutrients have immunomodulatory roles that may influence risk of tuberculosis (TB), but the association between baseline micronutrient deficiencies and incident TB after antiretroviral (ART) initiation in HIV-infected individuals is not well characterized.

Methods—We conducted a case-cohort study (n=332) within a randomized trial comparing three ART regimens in 1571 HIV treatment-naïve adults from nine countries. A subcohort of 30 patients was randomly selected from each country (n=270). Cases (n=77; main cohort=62, random subcohort=15) included patients diagnosed with TB by 96 weeks post-ART initiation. We determined pre-treatment concentrations of vitamin A, carotenoids, vitamin B6, vitamin B12, vitamin D, vitamin E, and selenium. We measured associations between pre-treatment micronutrient deficiencies and incident TB using Breslow-weighted Cox regression models.

Results—Median pre-treatment CD4+ T-cell count was 170 cells/mm³; 47.3% were female; and 53.6% Black. In multivariable models after adjusting for age, sex, country, treatment arm, prior TB, baseline CD4 count, HIV viral load, body mass index, and C-reactive protein, pre-treatment deficiency in vitamin A (adjusted hazard ratio, aHR 5.33, 95% confidence interval, CI 1.54–18.43) and vitamin D (aHR 3.66, 95%CI 1.16–11.51) were associated with TB post-ART.

Conclusion—In a diverse cohort of HIV-infected adults from predominantly low- and middle-income countries, deficiencies in vitamin A and vitamin D at ART initiation were independently associated with increased risk of incident TB in the ensuing 96 weeks. Vitamin A and D may be important modifiable risk factors for TB in high-risk HIV infected patients starting ART in resource-limited, highly-TB-endemic settings.

Keywords

Micronutrients; tuberculosis; HIV; case-cohort study; antiretroviral treatment; resource-limited settings

Introduction

Tuberculosis (TB) is the leading single-agent infectious cause of death worldwide, killing an estimated 1.5 million people in 2014 ¹. It is also one of the primary causes of early mortality in patients starting antiretroviral therapy (ART) in low- and middle-income countries (LMIC) ^{2,3}.

Micronutrient deficiencies are common in people living with HIV, particularly in LMIC. A lack of availability of micronutrient-rich foods, reduced dietary intake due to advanced HIV, excessive gastrointestinal losses from diarrhea and malabsorption, and chronic inflammation affect the overall nutrition of HIV-infected individuals. Non-specific markers of nutritional status, including low body mass index (BMI), low albumin, and anemia (both iron-deficiency and anemia of chronic disease) are associated with increased TB risk following ART initiation ^{4–11}. However, these general markers are incompletely predictive of TB; in particular, the role of deficiencies in individual micronutrients with immunomodulatory properties on susceptibility to new or reactivation TB is poorly understood. There is a need to accurately establish TB risk associated with individual micronutrient deficiencies. As patients in LMIC who survive the early months of ART experience high levels of subsequent survival ^{12,13}, finding modifiable nutritional risk factors could also lead to targeted

interventions aimed at reducing TB incidence and mortality during the critical early treatment period.

Several micronutrients play plausible roles in TB immunopathogenesis^{14–16}. Animal models highlight a plausible role of deficiencies and TB risk and clinical outcomes^{17,18}. Further, decreased levels of multiple micronutrients, including vitamins A^{19–21}; carotenoids²²; vitamin D^{23–29}, vitamin E^{30–32} and selenium^{32–36} are frequently observed in patients with TB and often correlate with severity of disease. However, previous studies have mostly been cross-sectional, focused on single micronutrients, or supplementation trials including patients with prevalent TB, and most did not adjust other important variables including BMI or inflammatory markers.

In the present study, we sought to assess multiple micronutrients at baseline prior to initiation of ART and look longitudinally at the outcome of incident TB in a diverse population from nine predominantly high-HIV, high-TB, LMIC settings.

Methods

Study participants

Our study population was sampled from the Prospective Evaluation of Antiretrovirals in Resource Limited Settings (PEARLS) trial (Adult AIDS Clinical Trials Group [ACTG] A5175, clinicaltrials.gov NCT00084136), an open-label, randomized trial evaluating the non-inferiority of once-daily versus twice-daily dosing of three ART regimens. The parent study is described in detail elsewhere³⁷. In brief, between May 2005 and July 2007, 1571 treatment-naïve HIV-1 infected adults with CD4+ T-cell counts <300 cells/mm³ from 9 countries, including Brazil (n=231), Haiti (n=100), India (n=255), Malawi (n=221), Peru (n=134), South Africa (n=210), Thailand (n=100), the United States (n=210), and Zimbabwe (n=110), were randomized to one of three ART treatment arms. Inclusion requirements included age ≥ 18 years, CD4+ T-cell count <300 cells/mm³, 7 days or less of cumulative ART use, negative pregnancy test and absence of significant derangement in baseline laboratory values (including haemoglobin <7.5 g/dL, estimated creatinine clearance <60 mL/min, aspartate aminotransferase or alanine aminotransferase >5 times upper limit of normal), all documented within 45 days of study entry. Participants with prevalent TB at study entry were excluded for our analysis, and patients with serious acute, chronic, or recurrent infections must have been clinically stable and completed at least 14 days of appropriate therapy.

We performed a case-cohort design to evaluate the association between micronutrient deficiencies and incident TB to allow efficient assessment of outcomes and as this design provides similar effect estimates and standard errors compared to full cohorts with cohorts of >1250 participants and sampling fraction >10%^{38–40}. Cases were defined as participants diagnosed with incident TB within 96 weeks after ART initiation. Two-hundred seventy randomly selected participants (30 per country, 10 per ART treatment arm) from the parent study and additional TB cases not in the random sub-cohort made up a full case-cohort sample of 332 participants used for this analysis. TB cases included 15 in the random sub-

cohort and 62 not in the random sub-cohort (77 total). The study population is described in Figure 1 and Table 1.

Study approval was obtained from the institutional review board and ethics committee at the Johns Hopkins University (JHU) and at each participating institution. Written informed consent was obtained from all participants.

Data Collection & Laboratory Assessment

Along with a detailed history and physical examination of the participants, a complete blood count (CBC), and HIV RNA viral load were obtained before initiating ART. CD4 count was obtained 7 to 45 days prior to initiation of ART. HIV RNA levels were measured by real-time PCR by the Amplicor HIV-1 Monitor Test, v1.5 (Roche, Branchburg, NJ, USA). Both the HIV RNA testing and CD4 count were done at externally quality-assured laboratories. History and physical examination were performed routinely at scheduled visits on weeks 2, 4, 8, then every 4 weeks through week 24, and then every 8 weeks through week 96. Incident TB was diagnosed according to standard ACTG definitions described elsewhere (Appendix 60, reference ³⁷).

Micronutrient Selection and Measurement

Micronutrients with a suspected or proven role in the pathogenesis of TB were selected for measurement and analysis. We did not include iron status or hypoalbuminemia as exposures of interest in this analysis as we have previously published on anemia, iron status, inflammation and risk of TB ^{11,41}. Micronutrient concentrations for study participants were measured using archived plasma or serum that had been collected within 14 days of starting ART (pre-ART) and stored at -80°C at labs certified by NIH (National Institute of Health) Division of AIDS using standardized protocols.

To minimize variation in measurements, all micronutrients were measured at a single laboratory at Johns Hopkins University that had extensive experience with micronutrient assays. Total (vitamin D2 and D3) serum 25-hydroxyvitamin D was measured using DiaSorin (Stillwater, MN). Serum vitamin B12 concentration was measured using Abbott AxSYM (Abbott Laboratories), an immunochemical automated analyser, and serum vitamin B6 using high-performance liquid chromatography (HPLC) with fluorescence detection. HPLC-ultraviolet was used to measure serum concentrations of retinol (vitamin A); the following carotenoids (α -carotene, β -carotene, β -cryptoxanthin, lutein, zeaxanthin, lycopene), and vitamin E (α and γ -tocopherol). A Perkins-Elmer AAnalyst 600 graphite furnace atomic absorption spectrometer was used to measure serum selenium concentrations. To adjust relevant micronutrient levels for inflammation status, we also measured C-reactive protein (CRP) using enzyme linked immunosorbent assay kits by R & D Systems at JHU ¹¹. External quality assurance for the laboratory measurements was done as per the NIH Division of AIDS and AIDS Clinical Trials Group (ACTG) Network laboratory quality Assurance guidelines ³⁷.

Statistical Analyses

We used cut-off values for micronutrient deficiency based on previous studies: $<1.05 \mu\text{mol/L}$ or $<0.7 \mu\text{mol/L}$ for vitamin A as different definitions have been proposed; $<19 \text{ nmol/L}$ for vitamin B6; $<148 \text{ pmol/L}$ for vitamin B12; $<20 \text{ ng/mL}$ for vitamin D (25-hydroxyvitamin D); $<9.3 \mu\text{mol/L}$ for α -tocopherol (vitamin E isoform); and $<85 \mu\text{g/L}$ for selenium^{42–45}. We also included vitamin D *insufficiency* (20 to $<32 \text{ ng/mL}$). No widely accepted cut-off values exist for deficiencies in γ -tocopherol (vitamin E isoform) or carotenoids (α -carotene, β -carotene, β -cryptoxanthin, lutein, zeaxanthin, and lycopene). We therefore measured total carotenoids by summing individual measurements and compared patients above or below the median total concentration, as well as above or below median γ -tocopherol levels to evaluate the association between *relative* deficiency and risk of TB. Only 1 patient met the criteria for α -tocopherol (vitamin E isoform) deficiency ($<9.3 \mu\text{mol/L}$) so we also compared groups above or below median concentration for risk of incident TB.

The primary objective in our analyses was to assess baseline micronutrient levels in a case-cohort sample and determine the relationship between baseline deficiencies and incident TB within 96 weeks of starting ART. Baseline characteristics of cases and non-cases were compared using the non-parametric Mann-Whitney U test for continuous variables or Fisher's exact test for discrete variables. Medians and interquartile ranges (IQR) were shown for continuous data and proportions for discrete data. Prior history of TB was obtained by self-report. Univariate and multivariable Cox regression analyses were used to ascertain associations between baseline (pre-ART) micronutrient deficiencies and incident TB post-ART initiation using stratified Breslow weights (stratified by country of origin and treatment arm). In our primary analysis, we included all variables thought to have a potential *a priori* association with incident tuberculosis, plus each micronutrient separately. To confirm that our results did not reflect model overfitting, we added variables to the model one at a time and checked hazards ratios for micronutrient deficiencies at each step. The primary multivariable model adjusted for age, gender, treatment arm, country of origin, prior history of TB, baseline CD4 count (cells/mm^3), baseline HIV $\log_{10}$ viral load, BMI and baseline CRP (which has been shown to influence measured serum concentrations of several micronutrient)^{46–48}. Age, CD4 count, viral load, BMI and CRP were analysed as continuous variables. In secondary, *post hoc* analysis, we also adjusted for other micronutrient deficiencies found to have a statistically significant association with incident TB. Further, as serum vitamin A can decrease during an acute phase response, we performed a secondary analysis restricted to cases diagnosed after 1 month. Kaplan-Meier estimates were used to calculate time to incident TB among the patients for selected micronutrients that either had an independent association or have been shown in our previous studies to be associated with TB. The log-rank test was used to evaluate univariate differences in groups for time to incident TB. Data were analysed using S-Plus version 8.

Results

Study Population characteristics

A total of 77 incident TB cases were identified in the PEARLS trials by 96 weeks after ART initiation (Figure 1). These included 55 pulmonary and 22 extrapulmonary TB cases. Pooled

median pre-treatment CD4+ T-cell count was 170 cells/mm³, 47.3% of patients were female, and 53.6% black. Among the 77 cases and 255 non-cases in the case-cohort sample, there were no significant differences in median age, gender, or treatment arm assignment (Table 1). Compared to non-cases, cases had lower median CD4 count (136 vs. 180 cells/mm³, $p=0.002$), a trend toward higher median log₁₀ HIV viral load (5.2 vs. 5.0, $p=0.07$), higher probability of prior TB (32% vs. 17%, $p=0.006$), and higher baseline prevalence of both anemia and hypoalbuminemia, as previously described ⁴¹. Cases and non-cases differed significantly with respect to study site/country of origin ($p<0.001$), with about three-quarters of TB cases from India (28.5% of total cases), Malawi (18.2%), or South Africa (27.3%). Among TB cases, the median time to diagnosis was 24 weeks; those who initiated ART at a lower CD4 cell count (<200 cells/mm³) had a shorter time to diagnosis ($p=0.01$) as compared to those with high baseline CD4 cell count (≥ 200 cells/mm³).

Micronutrient status pre-ART and incident TB

With regard to micronutrient status at ART initiation, cases had significantly lower baseline median levels of γ -tocopherol (2.18 vs. 2.67 $\mu\text{mol/L}$, $p=0.01$), total carotenoids (0.83 vs. 1.10 $\mu\text{mol/L}$, $p<0.001$) and selenium (62.74 vs. 84.25 $\mu\text{mol/L}$, $p<0.001$) than non-cases (Table 2). Vitamin A (1.35 vs. 1.53 $\mu\text{mol/L}$, $p=0.07$) and vitamin D (30 vs. 32 ng/mL, $p=0.07$) levels were marginally lower among cases. The median values of other micronutrients did not differ significantly between groups. Using established deficiency cutoffs, 7% of cases and 3% of non-cases were deficient in vitamin A (<0.7 $\mu\text{mol/L}$), 31% and 37% in vitamin B6 (<19 nmol/L), 6% and 9% in vitamin B12 (<148 pmol/L), 22% and 14% in vitamin D (<20 ng/mL), 2% and 0% in α -tocopherol (vitamin E isoform) [<9.3 $\mu\text{mol/L}$], and 76% and 51% in selenium (<85 mg/L). Baseline micronutrient deficiency prevalence in the sub-cohort was previously described ⁴⁹.

Deficiencies in serum vitamin A, vitamin D, and selenium were associated with increased hazards of incident TB in univariate analysis. After adjusting for potential confounders, pre-ART deficiencies of vitamin A (<0.7 $\mu\text{mol/L}$) [aHR 5.33, 95%CI 1.54–18.43] and vitamin D (<20 ng/mL) [aHR 3.66, 95%CI 1.16–11.51] remained significantly associated with subsequent risk of TB (Table 3). Vitamin D insufficiency (20 to <32 ng/mL), however, was not associated with increased risk of TB (aHR 0.90, 95% CI 0.29–2.81). Restricting the analysis to only patients with pulmonary TB, we found a similar association between incident TB for vitamin A deficiency (aHR 6.33, 95%CI 1.35–29.65, $p=0.02$) as well as for vitamin D deficiency (aHR 3.31, 95%CI 0.99–11.08, $p=0.053$). After adjusting for vitamin D status, pre-ART deficiency in vitamin A remained associated with elevated TB risk (aHR 4.81, 95%CI 1.33–17.36). Similarly, after adjusting for vitamin A status, deficiency in vitamin D was associated with increased TB risk (aHR 2.95, 95%CI 0.74–11.67), although this association was no longer statistically significant ($p=0.12$). Excluding 15 participants diagnosed with TB within 4 weeks, vitamin A deficiency remained associated with increased TB risk (aHR 3.25, 95%CI 0.77–13.79) but this finding did not reach statistical significance in this restricted group ($p=0.11$). No other measured micronutrients were associated with increased risk of TB in adjusted regression models. Lastly, participants with baseline deficiencies in vitamin A, vitamin D, and selenium who developed TB did so at

earlier times than the cases without baseline deficiencies (based on median time to development of TB) (Figure 2).

Discussion

In this case-cohort study of adults with advanced HIV Infection in nine mostly high-burden TB countries, individuals who developed TB after ART initiation were more likely to have pre-treatment deficiency in vitamin A and vitamin D than randomly selected patients who did not develop TB (i.e. non-cases) during follow-up. Deficiencies in vitamins A and D were each associated with more than tripling of the hazard of incident TB after ART initiation. Both associations remained significant after adjusting for body-mass index, inflammatory status, and other potential confounders. These findings provide new insight into micronutrient status and incident TB in a diverse multinational population initiating ART.

Micronutrient deficiencies are common in individuals with prevalent TB, with or without concomitant HIV infection. A number of supplementation trials have examined the effect of adjunctive micronutrients with standard chemotherapy on TB outcomes (reviewed in ⁵⁰) but few have assessed risk of development of TB in the era of ART. Notably, the potential immunologic role of micronutrients in prevention of incident tuberculosis is distinct from their role as adjunctive treatment in individuals with prevalent disease, although the latter has received greater focus ⁵¹. These previous trials had significant heterogeneity in patient populations, including proportion of HIV-infected patients and age categories, composition of multivitamin supplements, outcomes measured, and length of follow-up, limiting conclusions and generalizability of findings. Although many of these studies failed to find an additive clinical benefit of adjunctive micronutrient supplementation ⁵⁰, a large double-blinded, randomized-controlled trial (RCT) of 887 patients with pulmonary TB in Tanzania found a 45% reduction in TB recurrence in patients who received multivitamin supplementation (which included vitamins A, B complex, C, E, and selenium) compared to the placebo arm over a median follow-up period of 43 months, with an even greater reduction (63%) observed in HIV-infected patients ⁵². This finding is both clinically relevant given high rates of TB recurrence observed in resource-limited settings (32% of patients with incident TB in our cohort had a prior history of TB) ^{53–55} and because it suggests a potential protective role in TB of one or several micronutrients.

Vitamin D deficiency is frequently observed in individuals with TB, with additional data from (mostly HIV-uninfected) cohort studies suggesting an association between deficiency and risk of downstream disease ^{23,25,27,56–59}. Immunologically, vitamin D modulates both the innate and adaptive immune response – it promotes downregulation of Th1 immune responses but also toll-like receptor-mediated macrophage activation, cathelicidin-dependent phagosome maturation and fusion with lysosomes, and intracellular killing of *M. tuberculosis* ^{16,60–62}. Vitamin D insufficiency (defined as <30 ng/mL) was associated with a 66% (95%CI 4–164%) higher rate of relapse, defined in this study as *MTB* culture-positivity in culture-negative patients at 1 month of antituberculous therapy, in the above Tanzanian supplementation trial cohort ⁶³. In a separate Tanzanian cohort of HIV-infected adults initiation ART, Sudfeld *et al* observed that baseline 25-hydroxyvitamin D deficiency (<20 ng/mL), but not insufficiency (defined as 20–30 ng/mL), was associated with increased risk

of incident pulmonary TB after ART initiation, adjusting for age, gender, ART regimen, BMI, and CD4+ T-cell count, season, and WHO HIV disease stage (aHR 2.89, $p=0.027$) with a median follow-up of 20.6 months and 43 TB cases⁵⁷. Using similar *a priori* adjustment variables, we found a comparable relative increase in hazard of early TB following ART initiation for patients with baseline vitamin D deficiency (aHR 3.66, 95%CI 1.16–11.51). Our study provides further support that vitamin D is a significant predictor of TB post-ART and has additional strengths, including a larger TB case number, use of a diverse, multi-national patient population, evaluation of multiple micronutrient deficiencies, adjustment for inflammation (which affects serum micronutrient assessment), and rigorous patient follow-up and clinical assessment as a sub-analysis within an ACTG study.

Vitamin A deficiency is commonly observed in individuals with prevalent TB and is associated with disease severity, but data from cohort studies evaluating vitamin A deficiency state and risk of downstream TB in high-TB, high-HIV settings are limited^{10,19–21}. *In vitro* studies have demonstrated an ability of vitamin A to inhibit *M. tuberculosis* replication in culture and within macrophages^{64–66}. Vitamin A active metabolite (all-*trans* retinoic acid [ATRA])-mediated reduction in the cellular cholesterol concentration and increased lysosomal acidification has been proposed as a possible mechanism for vitamin A enhancement of *M. tuberculosis* killing in infected macrophages¹⁴. Our exploratory findings suggest a potential preventive role of vitamin A for development of TB in high-risk patient populations.

Regarding other measured micronutrients, a previous study of HIV-infected injection drug users in the United States found a 3-fold risk of mycobacterial disease (75% *M. tuberculosis*) over 2 years in patients with baseline deficiency in selenium (defined as <135 µg/L) after adjusting for ART use and CD4+ T-cell count³⁶, but this study did not adjust for BMI or inflammatory status (which decreases measured selenium concentrations)⁴⁷. Although we found an association between baseline selenium deficiency (<85 µg/L) and risk of TB in univariate analysis, no association was found in our adjusted analysis. Other measured antioxidant micronutrients, including B vitamins, carotenoids, and vitamin E, have established roles in innate and adaptive immunity¹⁵, but we also failed to find an association between deficiency or relative deficiency and increased risk of TB. The reason for the inverse association between lower-than-median concentrations of γ-tocopherol and TB is unclear.

Our study has some important limitations. Firstly, the number of TB cases may have been too small to sufficiently detect differences in micronutrients that have a weaker association with TB risk. Although it helped in eliminating reverse causation, for improved generalizability, studies with larger sample size may be needed. Our patient population was also relatively healthy before starting ART, without pre-morbid AIDS-defining conditions, severe anemia or other significant laboratory derangement, potentially diminishing strength of association between micronutrient deficiencies and TB and generalizability of study findings. Secondly, some of the micronutrients analysed do not have established cut-offs for deficiency (namely carotenoids and γ-tocopherol), and we therefore analysed those patients for relative deficiency as a crude measure of association. Thirdly, the study was not powered to test for interaction among the micronutrients. Fourthly, certain micronutrients with limited

circulating half-lives and are more prone to intra-individual variability of serum concentration^{67,68}, potentially attenuating the observed association between micronutrient status and TB risk. However, serial pre-ART blood collection to account for such variability was not feasible in this study. We also did not capture patient smoking status, which could influence micronutrient concentrations and TB risk. Lastly, this was an observational study intended to understand the role of micronutrients in increasing TB risk and not to identify or predict high-risk patients. However, with the identification of specific micronutrients and their role in subsequent TB status, this hypothesis-generating study provides additional insights into the potential role of vitamin D and vitamin A micronutrient supplementation and risk of developing TB among HIV-infected patients in resource limited, high-TB-burden settings. Several trials are now underway to assess the role of vitamin D supplementation and the risk of TB infection and disease in HIV-infected and uninfected individuals (NCT02880982, NCT01798680) and should shed light on vitamin D's role as a preventive therapy for acquisition of TB infection and risk of TB and TB/HIV progression^{69,70}

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Figure 1.
Patient population used in case-cohort analysis

¹PEARLS study described in ³⁵

²Case-cohort study description in ⁶⁴

Figure 2.
Kaplan-Meier analysis of time to TB for study participants with selected micronutrient deficiencies

Table 1

Baseline characteristics of TB case and non-case patients

Characteristic	Cases N=77	Non-cases N=255	P-value ^I
Baseline age, median (IQR) years	34 (29–39)	35 (29–41)	0.62
Males, n (%)	46 (60)	129 (51)	0.19
Country, n (%)			
Brazil	4 (5)	29 (11)	
Haiti	4 (5)	29 (11)	
India	22 (28)	27 (11)	
Malawi	14 (18)	27 (11)	
Peru	1 (1)	30 (12)	< 0.001
South Africa	21 (27)	26 (10)	
Thailand	3 (4)	29 (11)	
US	2 (3)	30 (11)	
Zimbabwe	6 (8)	28 (11)	
Race, n (%)			
Asian	26 (34)	57 (22)	
Black	44 (57)	134 (52)	
White	4 (5)	29 (11)	0.01
Other ²	3 (4)	35 (14)	
HIV treatment regimen ³ , n (%)			
3TC/ZDV/EFV	22 (29)	94 (37)	
ATV/FTC/DDI	31 (40)	83 (32)	0.35
FTC/TDF/EFV	24 (31)	78 (31)	
Baseline CD4 cell count, median (IQR) cells/mm ³	136 (60–201)	180 (90–231)	0.002
Baseline log ₁₀ viral load, median (IQR) copies/ml	5.2 (4.6–5.7)	5.0 (4.6–5.5)	0.07
Prior history of TB, n (%)	25 (32)	43 (17)	0.006
Baseline BMI, median (IQR) kg/m ²	21.6 (19.5–22.9)	22.3 (20.1–25.1)	0.02

Abbreviations: IQR-interquartile range, TB-tuberculosis

^IValues based on Fisher's exact test or Mann Whitney U test²Other races include American Indians / Alaskan Natives / unknown / more than one race³EFV+3TC/ZDV = efavirenz 600mg daily, lamivudine/zidovudine 150mg / 300mg twice daily

ATV+DDI+FTC = atazanavir 400mg daily, didanosine-EC 400mg daily, emtricitabine 200mg daily

EFV+FTC/TDF = efavirenz 600mg daily, emtricitabine/tenofovir 200mg / 300mg daily

Table 2

Serum micronutrient concentration of TB case and non-case patients before ART initiation

Micronutrient	Cases Median (IQR) N=77	Non-cases Median (IQR) N=255	P-value¹
Vitamin A (µmol/L)	1.35 (1.00–2.0)	1.53 (1.2–1.97)	0.07
Vitamin B6 (nmol/L) ²	24.55 (15.8–37.2)	23.5 (15.5–37.5)	0.50
Vitamin B12 (pmol/L) ³	316.5 (271 – 408)	338 (252 – 478)	0.39
Carotenoids (µmol/L) ⁴	0.83 (0.56–1.22)	1.10 (0.82–1.49)	<0.001
Vitamin D (ng/mL)	30 (21–35)	32 (24–39)	0.07
Vitamin E, α-tocopherol (µmol/L)	23.51 (19.35–27.44)	22.27 (18.84–26.98)	0.20
Vitamin E, γ-tocopherol (µmol/L)	2.18 (1.47–3.03)	2.67 (1.82–3.75)	0.01
Selenium (µg/L)	62.74 (50.23–84.42)	84.25 (58.52–99.9)	<0.001
CRP (mg/dL)	10.24 (4.3 – 34.39)	3.03 (1.24 – 9.32)	< 0.001

Abbreviations: ART-antiretroviral therapy, CRP-C-reactive protein, IQR-interquartile range, TB-tuberculosis

¹ P-values based on Mann Whitney U test² N=280 samples available for testing³ N=298 samples available for testing⁴ Carotenoids include α-carotene, β-carotene, β-cryptoxanthin, lutein, zeaxanthin & lycopene

Table 3

Univariate and multivariable associations between micronutrients and incident TB after ART initiation

Micronutrient	Crude HRR (95% CI)	P-value	Adjusted HRR ¹ (95% CI)	P-value
Vitamin A (retinol)				
Deficiency: < 0.7 µmol/L	7.14 (2.29–22.30)	0.001	5.33 (1.54–18.43)	0.01
Mild deficiency: 0.7–1.05 µmol/L	2.88 (0.52–16.0)	0.23	1.98 (0.31–12.72)	0.47
Normal: 1.05 µmol/L	Ref	-	Ref	-
Vitamin B6				
Deficiency: < 19 nmol/L	1.03 (0.44–2.40)	0.94	0.75 (0.30–1.89)	0.54
Sufficiency: 19 nmol/L	Ref		Ref	
Vitamin B12				
< 148 pmol/L	0.65 (0.11–3.87)	0.63	0.82 (0.11–6.11)	0.85
148 pmol/L	Ref		Ref	
Carotenoids ²				
< 1.09	2.01 (0.87–4.65)	0.10	1.4 (0.42–4.91)	0.56
> 1.09	Ref		Ref	
Vitamin D				
Deficiency: < 20 ng/mL	2.89 (0.98–8.53)	0.05	3.66 (1.16–11.51)	0.03
Insufficiency: 20–32 ng/mL	1.17 (0.47–2.92)	0.73	0.90 (0.29–2.81)	0.86
Sufficient: 32 ng/mL	Ref		Ref	
Vit E (γ-tocopherol)				
< 2.69	0.40 (0.16–1.01)	0.053	0.38 (0.15–0.97)	0.04
> 2.69	Ref		Ref	
Vit E (α-tocopherol)				
< 22.26	0.65 (0.22–1.90)	0.43	0.49 (0.17–1.40)	0.18
> 22.26	Ref		Ref	
Selenium				
< 85 µg/L	3.63 (1.59–8.28)	0.002	1.91 (0.69–5.30)	0.22
85 µg/L	Ref		Ref	

Abbreviations: CI-confidence interval, HR-hazard ratio, TB-tuberculosis

¹ Adjusted hazard ratio represents the independent risk of the added marker characteristic when adjusting for age, sex, country of origin, treatment arm, prior history of tuberculosis, baseline CD4+ T cell count, baseline HIV viral load, body mass index, baseline CRP² Carotenoids include α-carotene, β-carotene, β-cryptoxanthin, lutein, zeaxanthin & lycopene