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Tuberculosis After One Year of Combination Antiretroviral Therapy in Nigeria: A Retrospective Cohort Study

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Abstract

Our objective was to determine tuberculosis (TB) incidence and evaluate TB risk in adults after one or more years of use of combination antiretroviral therapy (cART) through a retrospective cohort study in Jos, Nigeria. We studied a cohort of HIV-infected adults treated with ART for at least 1 year. Based on immunologic and virologic responses to ART, patients were categorized into four groups: CD4 T cell count ≥ 350 cells/mm^3 and HIV-1 RNA level ≤ 400 copies/ml (group 1), CD4 T cell count ≥ 350 cells/mm^3 and HIV-1 RNA level > 400 copies/ml (group 2), CD4 T cell count < 350 cells/mm^3 and HIV-1 RNA level ≤ 400 copies/ml (group 3), and CD4 T cell count < 350 cells/mm^3 and HIV-1 RNA level > 400 copies/ml (group 4). Time to incident TB for the four groups was analyzed using the Kaplan–Meier method. Cox regression models were used to evaluate predictors of incident TB. In this cohort of 5,093 HIV-infected adults, of which 68.4% were female, with a mean age 35.1 years (standard deviation 9.1 years), we observed 98 cases of incident TB during 4 years and 3 months of follow-up. The overall TB incidence rate was 8.7 cases/1,000 patient-years of follow-up. Adjusted hazards for incident TB were 2.11 (95% CI 0.97–4.61), 2.05 (95% CI 1.10–3.79), and 3.65 (95% CI 1.15–5.06) in group 2, 3, and 4 patients, respectively, compared to group 1. Tuberculosis incidence in patients on ART is driven by poor immunologic and/or virologic response. Optimization of HIV treatment should be prioritized to reduce the burden of TB in this high-risk population.

Introduction

In countries with an HIV prevalence greater than 1%, the World Health Organization (WHO) estimates that persons with HIV infection are at 20 times greater risk for developing tuberculosis (TB) compared to those without HIV.[1] Despite substantial gains made in the management of HIV in the last decade, TB remains a major cause of death among HIV-infected persons.[2,3]

The risk of TB spans the spectrum of HIV disease and is greatest in the setting of immunosuppression resulting from depleted CD4 T cells.[4,5] Combination antiretroviral therapy (cART) reduces the incidence of TB by 70–90% in HIV-infected individuals; however, TB risk in this group remains higher than in the general population.[6–8] The exact CD4 T cell count threshold below which there is increased risk for TB remains unknown. An inverse relation between risk for TB and CD4 T cell count has, however, been demonstrated.[9,10] The influence of virologic response on TB risk, independent of CD4 T cell count, is also uncertain.

Between 8% and 26% of HIV-infected patients in sub-Saharan Africa die in the first year of ART use, with most deaths occurring in the first few months.[11] Most of these deaths are likely due to TB when adjustments are made for TB underdiagnosis and underreporting.[11] Consequently, early ART initiation and aggressive promotion of routine TB symptom screening during the early months of cART are among the interventions prescribed for the control of TB in HIV-infected patients.[12] In contrast, there is a relative dearth of data to formulate evidence-based interventions for TB

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control during long-term ART. Specifically, little is known about the incidence of TB after the first year of ART, and how the virologic and immunologic status attained after the first year of ART modulates subsequent TB risk.

To address this knowledge gap, we designed the current study taking advantage of data from the largest HIV treatment center in Nigeria. Our primary aim was to determine TB incidence after the receipt of at least 1 year of ART. In addition, we sought to determine the impact of immunologic and virologic status after 1 year of ART on subsequent risk for TB.

Materials and Methods

Study design and participants

We performed a retrospective cohort study of adult patients ($\geq$18 years) treated with ART for at least 1 year between January 2006 and March 2011 at the Jos University Teaching Hospital (JUTH) HIV clinic. We excluded patients who had (1) a history of antiretroviral therapy prior to enrollment at the JUTH HIV clinic, (2) those with no HIV RNA or CD4 T cell count results after 1 year of ART, or (3) a diagnosis of TB during the first year of ART.

Study setting

In 2002, the JUTH HIV treatment program commenced with support from the Government of Nigeria and subsequently from the United States President's Emergency Plan for AIDS Relief (PEPFAR). During the study period, ART was initiated by local providers using the WHO-based Nigerian National HIV treatment guidelines,[13] which recommended starting ART in patients with CD4 T cell count $<$200 cells/mm^3 or WHO stage IV disease irrespective of CD4 T cell count. First-line ART consisted of stavudine (d4T) or zidovudine (ZDV) or tenofovir (TDF) with lamivudine (3TC) or emtricitabine (FTC) and nevirapine (NVP) or efavirenz (EFV). Patients with CD4 T cell counts less than 350 cells/mm^3 received daily co-trimoxazole prophylaxis. None of the patients received isoniazid preventive therapy (IPT) despite recommendations in the National TB treatment guidelines.[14] All patients picked up their antiretroviral drugs at the pharmacy units within the clinic complex and pharmacy pick-ups were electronically captured. The pharmacy record was used to determine duration on cART, with the date of ART initiation used as a reference point. A year of ART use was defined as 365 days from the date of ART initiation.

Adherence to ART was expressed as a percentage and assessed using pharmacy pick-up dates. Adherence was reported as 100% if the patient picked up ART on or before the due date. Adherence was reduced proportionally after each missed day of the appointment.

HIV treatment and monitoring

Routine follow-up of patients on ART occurred monthly or every other month for drug pick-ups and included adherence counseling and a brief clinical review. Emergency visits as clinically indicated were allowed outside the usual schedule. Patients who missed three consecutive scheduled clinic visits were considered lost to follow-up. Antiretroviral treatment response was assessed using CD4 T cell count and HIV-1 RNA assays determined on site using Partec CyFlow Counter and Roche PCR Amplicor Monitor version 1.5, respectively. These tests were performed as per routine clinic care at baseline, 3 and 6 months after ART initiation, and every 6 months subsequently.

Data collection

Information on clinical parameters including WHO HIV clinical stage and antiretroviral therapy was routinely captured by providers at all patient encounters in an electronic data collection system. This was merged with databases for pharmacy dispensing and laboratory results (hematology, chemistry, CD4 T cell count, and HIV-1 RNA) into a central data repository managed by the Harvard School of Public Health, Boston, MA. For this study, we retrospectively analyzed deidentified data captured longitudinally in the merged database. Patients provided informed consent for their information to be used for analyses of treatment trends and outcomes upon entry into the clinic. The study was approved by the Institutional Review Boards of the Jos University Teaching Hospital and Harvard School of Public Health, and was ruled exempt at Northwestern University.

Incident TB diagnosis and treatment

Routine screening for TB (using symptoms, chest radiograph $\pm$ sputum smear microscopy) was the standard of care for all new patients engaging in care at the JUTH HIV clinic. Patients with symptoms suggestive of TB underwent chest radiograph and sputum smear microscopy for acid fast bacilli (AFB). Other available radiologic, microbiologic, and histologic evaluations were performed as appropriate. Mycobacterial culture was not routinely done. The treating physician made the diagnosis of TB using clinical, radiologic, and laboratory (including histology) tests. All AFB smear positive cases were diagnosed as TB. In patients with a negative AFB smear, TB could be clinically diagnosed based on a combination of symptoms including chronic cough, fever, night sweats, unexplained weight loss, or features of extrapulmonary TB, and appropriate radiograph and laboratory (including histology) tests.

New TB cases (WHO category 1) were treated with quadruple antitubercular therapy consisting of rifampicin (or rifabutin if on a protease inhibitor), isoniazid, ethambutol, and pyrazinamide for the first 2 months, then ethambutol and isoniazid for the remaining 6 months. Tuberculosis treatment-experienced patients (WHO category 2) received daily streptomycin injection for the first 2 months of TB treatment in addition to the drugs received by category 1 patients, as well as a longer treatment duration of 8 months, as recommended by the National Tuberculosis and Leprosy Control Program (NTBLCP) Nigeria guidelines.[14]

The primary outcome measure for this study was incident TB after at least 1 year of ART. TB was defined using electronic pharmacy records as the initiation of antitubercular therapy.

Covariates

The primary covariates of interest were immunologic and virologic responses to ART. Based on CD4 T cell count and HIV-1 RNA level attained at the end of the first year on ART, we defined four treatment response groups (TRGs): TRG 1:

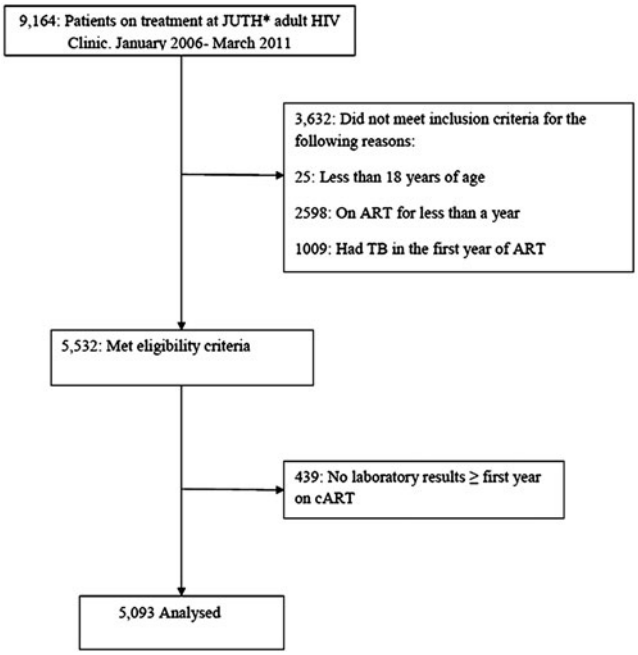

FIG. 1. Flow chart of patients in the study cohort based on inclusion/exclusion criteria This figure shows patient selection for the study based on predetermined inclusion and exclusion criteria. *JUTH, Jos University Teaching Hospital; HIV, human immunodeficiency virus; ART, antiretroviral therapy.

CD4 T cell count ≥350 cells/mm^3 and HIV-1 RNA level ≤400 copies/ml. TRG 2: CD4 T cell count ≥350 cells/mm^3 and HIV-1 RNA level >400 copies/ml. TRG 3: CD4 T cell counts <350 cells/mm^3 and HIV-1 RNA level ≤400 copies/ml. TRG 4: CD4 T cell counts <350 cells/mm^3 and HIV-1 RNA level >400 copies/ml.

For this study, CD4 T cell count ≥350 cells/mm^3 was considered a good immunologic response and an HIV-1 RNA level below the limit of assay detection (≤400 copies/ml) was considered a good virologic response. The CD4 T cell threshold of 350 cells/mm^3 was chosen based on more recent WHO recommendations to initiate ART in asymptomatic patients with a CD4 T cell count below 350 cells/mm^3,[15] instead of below 200 cells/mm^3 as recommended previously.[16] CD4 T cell count (in increments of 100 cells/ mm^3) and log change in HIV-1 RNA (log change) after the first year of ART were also independently modeled as linear variables.

As quantification of HIV-1 RNA is not readily available in most resource-limited settings, we also compared TB incidence in patients with a CD4 T cell count of 350 cells/mm^3 and above to those with a CD4 T cell count below 350 cells/mm^3, irrespective of HIV-1 RNA.

In adjusted analyses, all variables that were significant in the univariate analysis at $p<0.20$ or were considered a priori to be of clinical importance were included. Based on these criteria, variables included in the model were age groups (<30, 30–45, and >45 years), sex, previous TB (defined as TB before the commencement of ART), WHO stage at initiation of ART,[17] level of education (secondary or higher versus primary or less), marital status, and baseline CD4 T cell count at time of ART initiation.

Data analysis

We calculated overall TB incidence as the number of cases per 1,000 patient-years of follow-up (PYFU). Follow-up was determined as the time between a year of ART and the date of incident TB diagnosis, the last clinic visit before March 2011, or death/loss to follow-up. Time to incident TB for each of the four treatment response groups was analyzed using the Kaplan–Meier method to estimate cumulative TB-free survival probabilities. Univariate and multivariate Cox proportional hazards regression models were used to calculate hazard ratios (HRs) with 95% confidence intervals. We evaluated for confounding using stratification and for interactions by including interaction terms in the models. Due to the low incidence of TB in the study population, as a sensitivity analysis we ran the identical model estimated with Poisson regression with follow-up time after a year of treatment as the exposure variable. Because incidence rate ratio results were virtually identical to Cox model results, we present all results as hazards ratios derived from the Cox model. Statistical analyses were done using SPSS version 19.0 (Chicago, IL) and Stata version 11 (College Station, TX).

Results

Cohort characteristics and follow-up

A total of 9,164 HIV-infected patients received treatment at the adult HIV clinic of JUTH between January 2006 and March 2011. A total of 2,598 patients were excluded because they failed to reach 1 year on ART for unknown reasons. Therefore, 5,093 adults who were on ART for more than a year were included in our analyses. Study cohort selection using predefined inclusion/exclusion criteria is shown in Fig. 1.

A majority of the study patients were female (68.4%), with a mean age of 35.1 [standard deviation (SD) of 9.1] years and a median CD4 T cell count at ART initiation of 153 cells/mm^3 [interquartile range (IQR); 87, 172] (Table 1). Nonnucleoside reverse transcriptase inhibitors (NRTIs) were used by 99.8% of the study cohort, while the remaining patients received a triple NRTI-based or protease inhibitor (PI)-based regimen. Nevirapine was a component of first-line ART for 76.9% of the study population, while 22.9% received an efavirenz-containing first-line regimen. The mean ART drug adherence for the cohort was 93.15% (95% CI; 93.01–93.30). The mean adherence was 92.60% (95% CI; 91.58–93.61) for patients who developed TB and 93.16% (95% CI; 91.58–93.31) for patients who were TB free; $p=0.29$.

The median duration of follow-up was 26.5 months (IQR; 14.7, 38.2) with a total of 11,235 PYFU accrued. By March 2011, 10 (0.2%) deaths, 512 (10.1%) transfers to other HIV treatment centers, and 631 (12.4%) loss to follow-up cases were documented.

TB incidence

A total of 98 patients (1.9%) were diagnosed with incident TB over a follow-up period of 4 years and 3 months, with 64.29% smear positive TB, 12.24% smear negative, and 23.47% exclusively extrapulmonary disease. The overall TB incidence rate was 8.7 cases per 1,000 PYFU. The TB incidence in the four TRGs is shown in Table 2. Twenty patients (20.4%) had undetectable CD4 T cell counts at the time of TB diagnosis. Tuberculosis incidence rates (95% CI) were 12.5 (9.7–16.2), 7.2 (4.8–10.7), 5.2 (2.9–9.4), and 3.3 (1.1–10.1) per 1,000 PYFU in the first, second, third, and fourth year of follow-up, respectively.

Table 1. Baseline Characteristics of 5,093 Patients on Combination Antiretroviral Therapy for More Than 1 Year at Jos, Nigeria (2006–2011)

Characteristic	*No tuberculosis* N *(%)*	*Incident tuberculosis* N *(%)*	p-*value*
All	4,995	98	
Sex, male	1,563 (31.3)	33 (33.7)	0.66
Age groups (years)			0.73
<30	1,805 (36.1)	34 (34.7)	
≥30 to <45	2,512 (50.3)	48 (49.0)	
≥45	678 (13.6)	16 (16.3)	
Marital status, married	2,856 (57.2)	43 (43.9)	0.01
Secondary or higher level of education	3,089 (61.8)	55 (56.1)	0.25
WHO HIV clinical stage[a]			0.06
1	2,177 (43.8)	31 (31.6)	
2	1,433 (28.9)	33 (33.7)	
3	1,151 (23.0)	26 (26.5)	
4	224 (4.5)	8 (8.6)	
Treatment response group[b]			<0.01
1	1,740 (34.8)	17 (17.3)	
2	529 (10.6)	10 (10.2)	
3	1,745 (34.9)	37 (37.8)	
4	981 (19.6)	34 (34.7)	
Tuberculosis prior to HIV treatment	309 (6.2)	11 (11.2)	0.06

[a]World Health Organization HIV clinical stage.[17]
[b]Treatment response group (TRG) 1 = CD4 T cell count ≥350 cells/mm^3 and viral load ≤400 copies/ml; TRG 2 = CD4 T cell count ≥350 cells/mm^3 and viral load >400 copies/ml; TRG 3 = CD4 T cell count <350 cells/mm^3 and viral load ≤400 copies/ml; TRG 4 = CD4 T cell count <350 cells/mm^3 and viral load >400 copies/ml; results obtained after the first year of ART.
Obtained from χ^2 test.

One hundred and thirty-one patient-years were accrued in the last 3 months of observation (fifth year) with no documented case of incident TB.

HIV treatment response and risk for TB

Kaplan–Meier cumulative TB-free survival curves categorized by patient HIV treatment response group after the first year of ART are illustrated in Fig. 2 (log rank test $p = 0.005$).

Unadjusted and adjusted hazard ratios for incident TB are show in Table 3. Compared to patients in TRG 1 (CD4 T cell count ≥350 with viral suppression), hazard ratios (95%CI) for incident TB were 2.11 (0.97–4.61), 2.04 (1.10–3.79), and 3.65 (1.95–6.83) in TRG 2 (CD4 T cell count <350 with viral suppression), TRG 3 (CD4 T cell count ≥350 without viral suppression), and TRG 4 (CD4 T cell count <350 without viral suppression), respectively, after adjusting for age, sex, level of education, and marital status, as well as WHO HIV clinical stage, prior TB, and CD4 T cell count at ART initiation.

In multivariate Cox regression analysis utilizing CD4 T cell count instead of TRGs (irrespective of HIV RNA levels), the hazard ratio for incident TB was 0.47 (95% CI 0.28–0.77) in patients with CD4 T cell ≥350 cells/mm^3 compared to those with CD4 T cell counts below 350 cells/mm^3 after the first year on ART.

Table 2. Tuberculosis Incidence Rates per 1,000 Person-Years (95% CI) in 5,093 Patients on Combination Antiretroviral Therapy for More Than 1 Year at Jos, Nigeria (2006–2011)

	Rate per 1,000 person years	*95% CI*
Overall	8.72	7.15–10.63
Treatment response group[a]		
1	4.31	2.68–6.93
2	9.20	4.95–17.08
3	8.84	6.41–12.20
4	16.60	12.03–23.56
Sex		
Male	9.29	6.61–13.07
Female	8.46	6.63–10.79
Age groups (years)		
<30	8.41	6.01–11.77
≥30 to <45	8.54	6.44–11.33
>45	10.19	6.25–11.63
Marital status		
Married	6.66	4.94–8.98
Unmarried	11.51	8.84–15.00
Educational level		
Less than secondary	10.33	7.66–13.93
Secondary and above	7.77	5.97–13.93
WHO HIV clinical stage[b]		
1	6.67	4.70–9.49
2	8.83	6.27–12.41
3	11.31	7.70–16.60
4	14.51	7.26–29.01
TB prior to HIV treatment		
Yes	15.30	8.47–27.62
No	8.27	6.67–10.21

[a]Treatment response group (TRG) 1 = CD4 T cell count ≥350 cells/mm^3 and viral load ≤400 copies/ml; TRG 2 = CD4 T cell count ≥350 cells/mm^3 and viral load >400 copies/ml; TRG 3 = CD4 T cell count <350 cells/mm^3 and viral load ≤400 copies/ml; TRG 4 = CD4 T cell count <350 cells/mm^3 and viral load >400 copies/ml; results obtained after the first year of ART.
[b]World Health Organization HIV clinical stage.[17]

When modeled as linear variables, adjusted hazards for incident TB decreased by 12% (95% C1 0.78–0.99) per 100 cell increment in CD4 T cell count and increased by 41% (95% CI 1.16–1.71) for each log rise in VL (model not shown).

Other risk factors for TB

Age, sex, CD4 T cell count at time of ART initiation, TB treatment prior to ART initiation, and level of education were not independent risk factors for incident TB, but being unmarried was an independent risk factor for incident TB (adjusted HR = 1.74, 95% CI 1.16–2.58). In subgroup analysis based on sex, being unmarried was associated with increased hazards for incident TB only in men (HR 2.39, 95% CI 1.16–4.85), but not in women (HR 1.58, 95% CI 0.95–2.62). Although TB risk increased with WHO HIV clinical stage at initiation of ART, the differences were not statistically significant.

Discussion

We sought to estimate the incidence of TB after a year of ART, and determine how TB risk was influenced by immunologic

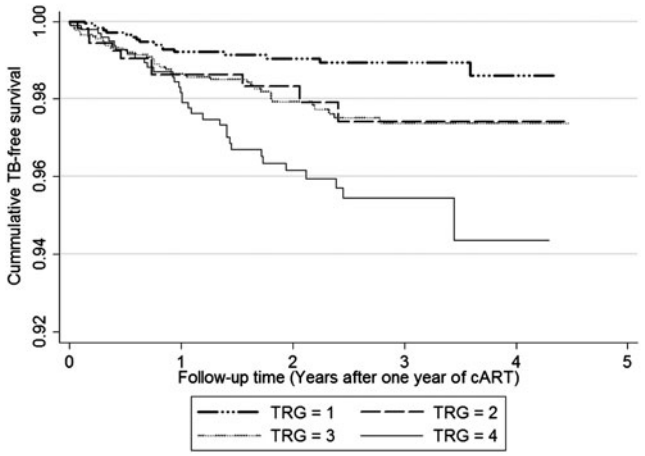

FIG. 2. Kaplan–Meier plots of tuberculosis free survival proportions among 5,093 patients after 1 year of combination antiretroviral therapy (cART) based on risk groups based on CD4 count and viral loads (log rank $p=0.05$). Treatment response group (TRG) 1 = CD4 T cell count ≥350 cells/mm^3 and viral load ≤400 copies/ml; TRG 2 = CD4 T cell count ≥350 cells/mm^3 and viral load >400 copies/ ml; TRG 3 = CD4 T cell count <350 cells/mm^3 and viral load ≤400 copies/ml; TRG 4 = CD4 T cell count <350 cells/mm^3 and viral load >400 copies/ ml; results were obtained after the first year of cART.

recovery and virologic suppression with HIV treatment. We employed a novel approach of risk stratification using a combination of CD4 T cell count (<350; ≥350 cells/mm^3) and HIV RNA levels [≤400 (undetectable); >400 copies/ml (detectable)] attained after a year of ART. We found that the overall TB incidence rate was 8.72 per 1,000 PYFU, much higher than the estimated case notification rate in Nigeria (3.11 per 1,000 person years).[18] This was largely driven by the high incidence of TB in persons with poor immunologic and/ or virologic responses to ART. The TB incidence in persons with CD4 T cell count ≥350 cells/mm^3 and undetectable HIV RNA levels after a year of cART was 4.31 per 1,000 PYFU, which remains higher than the estimated TB incidence in the general population. Similarly, results from a recently published South African cohort showed that TB incidence remained significantly higher among HIV-infected persons on long-term ART with a CD4 T cell count above 700 cells/mm^3 compared to HIV-seronegative community members.[19] We have demonstrated in our study that despite attaining a high CD4 T cell count, viral load over 400 copies/ml is also associated with increased TB risk.

The TB incidence rates in this study are lower than rates reported in studies from other countries with high TB and HIV burden. Reported incidence rates of TB among HIV-infected persons were 104, 73, 31, 28, 23, and 4.6 per 1,000 PYFU in Thailand,[20] South Africa,[9] Uganda,[21] India,[22] Senegal,[10] and Europe and North America,[23] respectively. A major contributor to this difference in TB incidence is our exclusion of TB cases occurring during the first year of ART, which is frequently very high and attributable in part to unmasking of subclinical disease.[9] Variations in TB incidence may also be due to differences in background HIV and TB disease burden in the various countries.

We found that exposure to ART was associated with a 73% reduction in incidence of TB between the first and fourth year after ART initiation. Similar reductions in risk of TB in HIV cohorts on ART have been reported in prospective studies in South Africa by Lawn *et al.*[24] (71.1%). This study, however,

Table 3. Cox Proportional Hazard Model Results for Risk Factors of Incident Tuberculosis in 5,093 Patients After the First Year of Combination Antiretroviral Therapy at Jos, Nigeria (2006–2011)

	Unadjusted analysis		*Adjusted analysis*	
	Hazard ratio	*(95% CI)*	*Hazard ratio*	*(95% CI)*
Treatment response group[a]				
1	1	Ref	1	Ref
2	2.08	(0.95–4.45)	2.11	(0.97–4.61)
3	2.11	(1.19–3.74)	2.04	(1.10–3.79)
4	3.84	(2.15–6.87)	3.65	(1.95–6.83)
Sex, male	1.10	(0.73–1.68)	1.12	(0.70–1.77)
Age group (years)				
<30	1	Ref	1	Ref
≥30 to <45	1.01	0.65–1.57	0.97	0.62–1.53
>45	1.22	0.67–2.20	1.11	0.59–2.10
Marital status, married	0.59	0.39–0.86	0.57	0.38–0.86
Secondary and above level of education	0.76	0.52–1.14	0.74	0.49–1.12
WHO HIV clinical stage[b]				
1	1	Ref	1	Ref
2	1.43	0.87–2.33	1.28	0.78–2.10
3	1.64	0.97–2.77	1.24	0.71–2.18
4	2.30	1.06–5.01	1.75	0.79–3.85
TB prior to HIV treatment	1.88	1.01–3.52	1.63	0.82–3.22
Baseline CD4 T cell count	—	—	1.00	0.99–1.00

[a]Treatment response group (TRG) 1 = CD4 T cell count ≥350 cells/mm^3 and viral load ≤400 copies/ml; TRG 2 = CD4 T cell count ≥350 cells/mm^3 and viral load >400 copies/ml; TRG 3 = CD4 T cell count <350 cells/mm^3 and viral load ≤400 copies/ml; TRG 4 = CD4 T cell count <350 cells/mm^3 and viral load >400 copies/ml; results obtained after the first year of ART.
[b]World Health Organization HIV clinical stage.[17]

differs from our study because of the inclusion of incident TB cases in the first year of ART and smaller sample sizes.

The role of CD4 T cell depletion in increasing the risk for TB has long been established[25]; however, the influence of HIV viral replication, independent of $CD4^+$ T cell depletion, on risk for TB is yet to be fully elucidated. Mechanistically, HIV has been shown to alter the function of innate immune cells, particularly alveolar macrophages. Phagocytosis of *Mycobacterium tuberculosis* (MTb) by alveolar macrophages appears to be enhanced in HIV-infected persons,[26] possibly due to chronic activation of the macrophages.[27] When compared to alveolar macrophages from non-HIV-infected persons, HIV-infected macrophages have decreased release of cytokines and chemokines,[28] impaired MTb phagosomal maturation,[29] as well as decreased apoptosis in response to MTb[28]: factors that may impair host defence against TB. These reasons may explain the association we found between high HIV RNA levels, low CD4 T cell counts, and increased risk for TB. Adjusted hazards for incident TB in our study population were highest in persons with detectable HIV RNA and CD4 T cells counts <350 cells/mm^3 (TRG 4), with hazards for incident TB in this group being 3.65 higher than the reference group (TRG 1). Persons with high CD4 T cell counts and detectable HIV RNA levels, however, had a risk profile comparable to those with low CD4 T cell counts and undetectable HIV RNA levels, suggesting that HIV viral replication plays a role in increasing TB risk independent of CD4 T cell depletion. This is supported by the independent association between HIV RNA levels and TB risk for TB, with a log increment in HIV RNA being associated with a 41% increase in hazards for incident TB.

An incidental finding was the increased risk of TB among unmarried men. Male sex and being unmarried have been previously described as independent risk factors for TB among Africans,[30] with a genome-wide study suggesting that an X-chromosome susceptibility gene may contribute to the excess TB risk reported in males[31]; however, this finding requires further study. Single males may also indulge in social habits such as cigarette smoking, which may increase the risk for TB.

The strengths of our study include the large cohort size and quality of prospectively captured data from a center with integrated TB and HIV services. Our large cohort provided adequate power to evaluate TB risk in subgroups of patient stratified by response to treatment. Among the limitations of this study were the limited availability of culture facilities, the gold standard for TB diagnosis. Although this had limited potential to introduce bias to this study since the same set of criteria was used for TB diagnosis in all patient groups, overestimation or underestimation of TB incidence cannot be excluded conclusively. Another limitation was the high attrition rate. We handled this through the use of the Cox regression models that robustly handle variable follow-up periods. Finally, CD4 T cell counts and HIV-1 RNA levels were not modeled as time varying covariates. We considered the 1-year immunologic and virologic responses to ART to be adequately predictive of subsequent risk for incident TB.

Conclusions

Our findings have implications for HIV treatment programs in regions of high TB and HIV burden. To effectively reduce the rate of TB in HIV-infected patients on ART, treatment programs must optimize long-term immunologic and virologic responses to HIV treatment. High rates of first-line and second-line ART failure need to be addressed, with strategies established for early identification and switching of patients failing therapy.[32,33] Implementation of isoniazid preventive therapy in HIV-infected person should also be prioritized. In addition to universal screening of all HIV-infected patients for TB at the initiation of HIV care, routine TB screening in patients failing ART in countries with high TB burdens should also be advocated. We also need to invest in innovative point of care TB diagnostic tools at HIV treatment facilities with high TB burdens.

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Author Disclosure Statement

No competing financial interests exist.

References

1. WHO: Global Tuberculosis Control: A short update to the 2009 report. World Health Organization, Geneva, 2009.
2. Straetemans M, Bierrenbach AL, Nagelkerke N, Glaziou P, and van der Werf MJ: The effect of tuberculosis on mortality in HIV positive people: A meta-analysis. PLoS One 2010;5(12):e15241.
3. Corbett EL, Watt CJ, Walker N, Maher D, Williams BG, Raviglione MC, and Dye C: The growing burden of tuberculosis: Global trends and interactions with the HIV epidemic. Arch Intern Med 2003;163(9):1009–1021.
4. van der Sande M, Schim van der Loeff M, Bennett R, Dowling M, Aveika A, Togun T, Sabally S, Jeffries D, Adegbola R, and Sarge-Njie R: Incidence of tuberculosis and survival after its diagnosis in patients infected with HIV-1 and HIV-2. AIDS 2004;18:1933–1941.
5. Lucas S, De Cock K, Hounnou A, Peacock C, Diomande M, Honde M, Beaumel A, Kestens L, and Kadio A: Contribution of tuberculosis to slim disease in Africa. Bmj 1994;308:1531–1533.
6. Lawn SD and Wood R: Incidence of tuberculosis during highly active antiretroviral therapy in high-income and low-income countries. Clin Infect Dis 2005;41(12):1783–1786.
7. Badri M, Wilson D, and Wood R: Effect of highly active antiretroviral therapy on incidence of tuberculosis in South Africa: A cohort study. Lancet 2002;359(9323):2059–2064.
8. Seyler C, Toure S, Messou E, Bonard D, Gabillard D, and Anglaret X: Risk factors for active tuberculosis after antiretroviral treatment initiation in Abidjan. Am J Respir Crit Care Med 2005;172(1):123–127.

9. Lawn SD, Myer L, Edwards D, Bekker LG, and Wood R: Short-term and long-term risk of tuberculosis associated with CD4 cell recovery during antiretroviral therapy in South Africa. AIDS 2009;23(13):1717–1725.
10. Etard JF, Diouf A, De Beaudrap P, Akoi K, Ngom-Gueye NF, Ndiaye I, Ecochard R, Sow PS, and Eric D: Short and long-term incidence of tuberculosis and CD4-cell count dynamic on HAART in Senegal. Open AIDS J 2009;3:63–70.
11. Braitstein P, Brinkhof MW, Dabis F, Schechter M, Boulle A, Miotti P, Wood R, Laurent C, Sprinz E, Seyler C, *et al.:* Mortality of HIV-1-infected patients in the first year of antiretroviral therapy: Comparison between low-income and high-income countries. Lancet 2006;367(9513):817–824.
12. Date AA and Miller B: Antiretroviral therapy and tuberculosis: What's the connection and what's the way forward? J Acquir Immune Defic Syndr 2011;57(4):255–257.
13. World Health Organization. Department of HIV/AIDS: Scaling up antiretroviral therapy in resource-limited settings: Guidelines for a public health approach: Executive summary. World Health Organization, Geneva, 2002.
14. National Tuberculosis and Leprosy Control Programme (NTBLCP): Workers Manual, 5th ed. Federal Ministry of Health Nigeria, Abuja, 2008.
15. World Health Organization: Antiretroviral therapy for HIV infection in adults and adolescents: Recommendations for a public health approach—2010 revision, 2010 rev. ed. World Health Organization, Geneva, 2010.
16. Manabe YC, Breen R, Perti T, Girardi E, and Sterling TR: Unmasked tuberculosis and tuberculosis immune reconstitution inflammatory disease: A disease spectrum after initiation of antiretroviral therapy. J Infect Dis 2009;199(3): 437–444.
17. World Health Organization: WHO case definitions of HIV for surveillance and revised clinical staging and immunological classification of HIV-related disease in adults and children. World Health Organization, Geneva, 2007.
18. World Health Organization: Global tuberculosis control: Surveillance, planning, financing: WHO report 2008. World Health Organization, Geneva, 2008.
19. Gupta A, Wood R, Kaplan R, Bekker LG, and Lawn SD: Tuberculosis incidence rates during 8 years of follow-up of an antiretroviral treatment cohort in South Africa: Comparison with rates in the community. PLoS One 2012;7(3):e34156.
20. Bonnet MM, Pinoges LL, Varaine FF, Oberhauser BB, O'Brien DD, Kebede YY, Hewison CC, Zachariah RR, and Ferradini LL: Tuberculosis after HAART initiation in HIV-positive patients from five countries with a high tuberculosis burden. AIDS 2006;20(9):1275–1279.
21. Hermans SM, Kiragga AN, Schaefer P, Kambugu A, Hoepelman AI, and Manabe YC: Incident tuberculosis during antiretroviral therapy contributes to suboptimal immune reconstitution in a large urban HIV clinic in sub-Saharan Africa. PLoS One 2010;5(5):e10527.
22. Rajasekaran S, Raja K, Jeyaseelan L, Vijilat S, Priya K, Mohan K, Parvez A, Mahilmaran A, and Chandrasekar C: Post-HAART tuberculosis in adults and adolescents with HIV in India: Incidence, clinical and immunological profile. Indian J Tuberc 2009;56(2):69–76.
23. Girardi E, Sabin CA, d'Arminio Monforte A, Hogg B, Phillips AN, Gill MJ, Dabis F, Reiss P, Kirk O, Bernasconi E, *et al.:* Incidence of tuberculosis among HIV-infected patients receiving highly active antiretroviral therapy in Europe and North America. Clin Infect Dis 2005;41(12):1772–1782.
24. Lawn SD, Badri M, and Wood R: Tuberculosis among HIV-infected patients receiving HAART: Long term incidence and risk factors in a South African cohort. AIDS 2005;19(18):2109–2116.
25. Williams BG and Dye C: Antiretroviral drugs for tuberculosis control in the era of HIV/AIDS. Science 2003;301(5639): 1535–1537.
26. Day RB, Wang Y, Knox KS, Pasula R, Martin WJ 2nd, and Twigg HL 3rd: Alveolar macrophages from HIV-infected subjects are resistant to Mycobacterium tuberculosis in vitro. Am J Respir Cell Mol Biol 2004;30(3):403–410.
27. Buhl R, Jaffe H, Holroyd K, Borok Z, Roum J, Mastrangeli A, Wells F, Kirby M, Saltini C, and Crystal R: Activation of alveolar macrophages in asymptomatic HIV-infected individuals. J Immunol 1993;150(3):1019–1028.
28. Patel NR, Zhu J, Tachado SD, Zhang J, Wan Z, Saukkonen J, and Koziel H: HIV impairs TNF-alpha mediated macrophage apoptotic response to Mycobacterium tuberculosis. J Immunol 2007;179(10):6973–6980.
29. Mwandumba HC, Russell DG, Nyirenda MH, Anderson J, White SA, Molyneux ME, and Squire SB: Mycobacterium tuberculosis resides in nonacidified vacuoles in endocytically competent alveolar macrophages from patients with tuberculosis and HIV infection. J Immunol 2004;172(7): 4592–4598.
30. Lienhardt C, Fielding K, Sillah J, Bah B, Gustafson P, Warndorff D, Palayew M, Lisse I, Donkor S, Diallo S, *et al.:* Investigation of the risk factors for tuberculosis: A case–control study in three countries in West Africa. Int J Epidemiol 2005;34(4):914–923.
31. Bellamy R, Beyers N, McAdam KPWJ, Ruwende C, Gie R, Samaai P, Bester D, Meyer M, Corrah T, Collin M, *et al.:* Genetic susceptibility to tuberculosis in Africans: A genome-wide scan. Proc Natl Acad Sci USA 2000;97(14):8005–8009.
32. Sigaloff KC, Hamers RL, Wallis CL, Kityo C, Siwale M, Ive P, Botes ME, Mandaliya K, Wellington M, Osibogun A, *et al.:* Unnecessary antiretroviral treatment switches and accumulation of HIV resistance mutations; two arguments for viral load monitoring in Africa. J Acquir Immune Defic Syndr 2011;58(1):23–31.
33. Keiser O, Chi BH, Gsponer T, Boulle A, Orrell C, Phiri S, Maxwell N, Maskew M, Prozesky H, Fox MP, *et al.:* Outcomes of antiretroviral treatment in programmes with and without routine viral load monitoring in Southern Africa. AIDS 2011;25(14):1761–1769.

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