Subclinical tuberculosis among HIV-infected adults: Clinical features and outcomes in a South African cohort

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A thesis submitted in partial fulfillment of the requirements for the degree of
Master of Science

University of Washington

2017

Committee:
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Program Authorized to Offer Degree:
Department of Epidemiology

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Abstract

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OBJECTIVE: Describe the prevalence, clinical characteristics, and mortality risk for HIV-infected adults with subclinical tuberculosis.

DESIGN: Untreated HIV-infected adults presenting for outpatient care in Durban, South Africa were screened for tuberculosis-related symptoms and had sputum tested by acid-fast bacilli smear and tuberculosis culture. Active tuberculosis and subclinical tuberculosis were defined as having any tuberculosis symptom or no tuberculosis symptoms with either smear-positive or culture-positive sputum. We evaluated the association between tuberculosis disease category and 12-month survival.

RESULTS: Among 670 participants, 106 were diagnosed with active tuberculosis disease and 34 with subclinical disease. The mean CD4 count was 138 cells/mm^3 in patients with active tuberculosis, 200 cells/mm^3 in patients with subclinical disease, and 289 cells/mm^3 in those without tuberculosis disease (*P <0.001*). A greater proportion of individuals in the subclinical group were smear positive (41%) compared to the active tuberculosis group (22%, *P 0.04*). Risk of mortality was not increased in individuals with subclinical tuberculosis relative to no tuberculosis (adjusted hazard ratio 0.74, 95% CI 0.23-2.40).

CONCLUSION: Subclinical tuberculosis in HIV-infected adults was characterized by an intermediate degree of immunosuppression and greater sputum smear positivity. Although there was no significant difference in survival, these findings have important implications for preventing tuberculosis transmission.

INTRODUCTION

In 2015, over 10 million people worldwide developed active tuberculosis (TB) [1]. TB was the leading infectious disease cause of mortality as well as a leading cause of HIV-related mortality. Early diagnosis of highly contagious individuals as well as those at risk for progressing from latent infection to active disease may be critical for controlling the TB epidemic.

The traditional dichotomy of latent versus active TB has been more accurately modeled as a spectrum of infection whereby weakened host innate and acquired immune responses, as occur in HIV, allow bacterial replication [2-4]. In the setting of increasing bacillary burden and host damage, clinical symptoms may develop. The preceding asymptomatic period during which *M. tuberculosis* can be isolated from the host has been described as subclinical TB [5, 6].

The World Health Organization currently recommends all HIV-infected individuals be screened for TB infection by assessing four TB-related symptoms—current cough, fever, night sweats, or weight loss [7]. Those who screen negative are eligible for isoniazid preventive therapy, while those who screen positive require further investigation for active TB disease. The overall sensitivity of this screen among people with HIV is estimated at 79% [8]. Persons with subclinical TB will be missed by this approach, potentially placing others at risk for infection and allowing the infection to progress to active TB [8-10]. We describe the prevalence, clinical and laboratory findings, and risk of death in a cohort of untreated HIV-infected adults with subclinical TB in South Africa.

STUDY POPULATION AND METHODS

Study design

We enrolled untreated HIV-infected adults between October 2011 and January 2014 at four outpatient sites in KwaZulu-Natal, South Africa as previously described [11, 12]. Non-pregnant adults ≥ 18 years who had not received anti-tuberculous therapy (ATT) within three months were eligible for enrollment. The study was approved by McCord Hospital, St. Mary's Hospital, the University of KwaZulu-Natal, Partners HealthCare, and the University of Washington. All participants provided written informed consent.

Procedures

At enrollment, study nurses recorded demographic and clinical information including TB-related symptoms: cough, fever, night sweats, and weight loss. Participants underwent CD4 T-cell count testing and provided a single expectorated sputum specimen; those unable to provide a sample underwent sputum induction with 3% hypertonic saline delivered by nebulizer. Specimens were transported daily to the TB laboratory at the University of KwaZulu-Natal for concentrated AFB smear and mycobacterial culture. Acid-fast bacilli (AFB) smear was performed using both Ziehl-Neelsen and auramine staining with fluorescence microscopy and graded as negative, low, 1+, 2+, or 3+ according to standard criteria [13]. Culture was performed using both solid Middlebrook 7H11 agar and Bactec mycobacterial growth indicator tubes (MGIT) 960 system. Cases were considered culture positive based on identification of *M. tuberculosis* in either solid or liquid media. Participant urine samples were also tested for lipoarabinomannan (LAM) using the Determine™ TB LAM assay (Alere Inc.) [11]. LAM positive cases were designated as grade 1 or higher on using the manufacturer's 5-grade reference card. All participants were offered treatment for HIV and TB according to South African Department of Health and WHO guidelines [14, 15].

Outcomes were assessed 12 months after enrollment through review of clinical site TB registers and the Department of Health's TB Control Programme. Information was recorded on TB treatment initiation, completion, default, failure, and death according to the South African Population Register [16].

Statistical analysis

Participants were categorized as having active TB disease, subclinical TB disease, or no TB disease. Active TB cases were defined as having a positive *M. tuberculosis* culture or AFB smear in addition to the presence of at least one TB-related symptom. Subclinical TB cases were defined as having a positive *M. tuberculosis* culture or AFB smear but no TB-related symptoms. No TB disease was defined as having no microbiologic evidence of *M. tuberculosis* by smear or culture and further subcategorized into those who were and were not treated empirically with ATT at the discretion of the clinician.

Statistical methods used included Fisher's exact test and one-way ANOVA to compare baseline demographic, clinical, and laboratory characteristics between participants with active or subclinical TB and no TB disease. Kaplan-Meier curves were used to display survival across TB diagnosis groups. We also evaluated the association between TB category and 12-month survival outcomes using Cox regression and adjusting for age, sex, and categorical CD4 count. We calculated both unadjusted and adjusted hazard rates and 95% confidence intervals. The proportional hazards assumption was tested using log-log plots and time-varying predictors. Analyses were conducted in R [17].

RESULTS

We enrolled 727 untreated HIV-infected adults. After excluding 57 persons for whom mycobacterial culture and AFB smear data were missing, we included 670 participants in the analysis. Mean age was 34 years, 355 (53%) were men, 163 (24%) currently smoked tobacco, and 53 (8%) reported prior treatment for TB (Table 1). At baseline, 249 (37%) had no TB-related symptoms, 144 (21%) had one symptom, 119 (18%) had two symptoms, 85 (13%) had three symptoms, and 74 (11%) had four symptoms. Mean CD4 cell count was 247 cells/mm^3 (standard deviation 206). AFB smear was positive in 37 individuals (6%), mycobacterial culture was positive in 124 (19%), and urine LAM was positive in 101 (15%).

One hundred six (16%) participants were diagnosed with active TB disease, 34 (5%) with subclinical TB disease, and 530 did not have microbiologic evidence of TB. Of those without proven disease, 40 (8%) were empirically treated with ATT. We included as TB disease individuals with AFB smear positive sputum and missing or negative sputum culture. Of the 34 persons with subclinical TB, information on mycobacterial culture was missing in one case and negative in five others. Of the 106 persons with active TB, culture was negative in 10 cases. There were no significant differences in mean age, education, marital status, baseline tobacco consumption, or prior TB treatment between the four subgroups. The active TB group had the highest proportion of men (66%, *P 0.03*).

Mean CD4 count differed across groups: 138 cells/mm^3 in active TB, 200 cells/mm^3 in subclinical TB, 128 cells/mm^3 in no TB empirically treated, and 289 cells/mm^3 in no TB non-treated, (*P <0.001*). A greater proportion of persons in the subclinical group were AFB smear positive (41%) compared to the active TB group (22%, *P 0.04*). The proportion positive for urine LAM also differed across groups: 33% in active TB, 26% in subclinical TB, 23% in no TB empirically treated, and 10% in no TB non-treated (*P <0.001*). Of note, information on ATT

initiation was missing in a large number of subclinical and active TB cases. Among the 34 persons with subclinical TB, ATT was started in 19 while information on treatment initiation was missing in the rest. Among the 106 persons with active TB, ATT was started in 51 while information on treatment initiation was missing in the rest.

Vital status at 12 months was ascertained in all participants and death was documented in 25 (25%) active TB patients, 3 (9%) subclinical TB, 5 (13%) no TB empirically treated, and 44 (9%) no TB non-treated patients (Table 2, Figure 1). In univariate analysis, male sex, older age, and lower baseline CD4 count were associated with a higher hazard of death. Subclinical TB disease was associated with a similar hazard of death at 12 months compared to the reference no TB non-treated group (hazard ratio [HR] 0.99, 95% CI 0.31-3.20) while active TB disease was associated with a higher hazard of death compared to the reference group (HR 3.02, 95% CI 1.86-4.90). After adjusting for age, sex, and baseline CD4, there remained no evidence of a significant association between hazard of death comparing subclinical TB to reference (adjusted hazard ratio [aHR] 0.74, 95% CI 0.23-2.40), and the increased hazard of death comparing active TB to reference was attenuated and no longer significant (aHR 1.55, 95% CI 0.91-2.64). There was a two-fold non-significant difference in 12-month survival comparing active to subclinical TB (aHR 2.10, 95% CI 0.63-7.02).

DISCUSSION

The prevalence of subclinical TB in our population of untreated HIV-infected adults in South Africa was high, accounting for 24% of all TB cases and 5% of the entire screened population. Notably, a greater proportion of subclinical TB cases was smear positive compared to active TB cases. Subclinical TB cases presented with an intermediate degree of immunosuppression as

reflected by a mean CD4 count between those with active and non-treated no TB. We did not find evidence of reduced survival in adjusted models comparing subclinical or active TB to non-treated no TB individuals, but the numbers in each group were small.

The high prevalence of subclinical TB among all screened participants in our study is consistent with other untreated HIV-infected populations. Subclinical disease has been reported to account for 6 – 52% of TB cases diagnosed by sputum culture [9, 10, 18-29]. However, it remains an underrecognized entity among HIV-infected individuals.

Our finding of greater AFB smear positivity in the subclinical TB group compared to active TB has potential implications for onward transmission [30], particularly as studies have found that individuals with subclinical disease often progress to being symptomatic within several days to two months [10, 24, 25]. While one group described a similar pattern as seen in our study [9], others have reported findings suggestive of greater bacillary burden and shorter time to culture positivity in active TB disease relative to subclinical TB [10, 31]. The overall contribution of subclinical disease to TB transmission is not well understood.

The observed pattern of intermediate immunosuppression in subclinical TB is not consistently seen in all pre-ART cohorts. Some studies have reported similar CD4 values between groups [9] while others have described a notable difference, where average values are highest for those without disease, intermediate for those with subclinical TB, and lowest for those with active TB [10].

With regard to survival outcomes, the study was not powered to detect a difference in adjusted survival hazards across groups, even between active TB and non-treated no TB. Baseline CD4 was a significant confounder; lower counts were strongly associated with increased hazard of death. Other important potential confounders not fully measured included

ATT and adherence to ART. A number of individuals with subclinical TB were started on ATT which was likely influenced by knowledge of culture results; in routine clinical practice where cultures are not obtained in the absence of symptoms, survival among subclinical TB patients may differ.

Diagnostic limitations of our study include collection and testing of only a single sputum sample per patient. In addition, a number of adults without evidence of TB by sputum testing were urine LAM test positive; this finding could indicate extrapulmonary or undiagnosed pulmonary TB. Furthermore, we included as TB disease individuals with AFB smear positive sputum and missing or negative sputum culture. Though these could represent infection with non-tuberculous mycobacteria, they only accounted for a small portion of total TB cases. Despite these limitations, this is one of the largest series of subclinical TB to date.

We report a high prevalence of subclinical TB in our cohort of untreated HIV-infected adults in South Africa. These individuals were characterized by an intermediate degree of immunosuppression, and although no differences in mortality were observed, there was greater sputum smear positivity compared to active TB cases. Given the potential impact on TB transmission, particularly among HIV-infected individuals, early identification of subclinical TB is critical.

ACKNOWLEDGEMENTS

We thank Katherine Thomas and Ting Hong for their valuable input with regard to data analysis. KLB conducted the primary analysis and wrote the manuscript. IVB, SMC, DR, and KAG were involved in the design and conduction of the parent study as well as review of the manuscript. AW and PKD reviewed the data analysis and manuscript.

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TABLES AND FIGURES

Table 1: Characteristics of untreated HIV-infected adults stratified by tuberculosis status.

	All Individuals ***N=670***	**Active TB** ***N=106***	**Subclinical TB** ***N=34***	**No microbiologic TB: ATT**[1] ***N=40***	**No microbiologic TB: no ATT** ***N=490***	**P value**
Demographics						
Mean age, years (SD)	34 (9)	35 (9)	33 (9)	36 (11)	34 (10)	*0.48*[2]
Men	355 (53%)	70 (66%)	16 (47%)	21 (53%)	248 (51%)	*0.03*
Education: high school or higher	254 (38%)	37 (35%)	10 (29%)	12 (30%)	195 (40%)	*0.36*
Marital status						
Never married	555 (83%)	91 (87%)	27 (79%)	32 (80%)	405 (83%)	*0.61*
Currently married	91 (14%)	10 (10%)	7 (21%)	7 (18%)	67 (14%)	*0.29*
Clinical						
Current tobacco	163 (24%)	20 (19%)	9 (26%)	8 (20%)	126 (26%)	*0.44*
Prior TB treatment	53 (8%)	8 (8%)	0	3 (8%)	42 (9%)	*0.34*

TB-related symptoms						
None	249 (37%)	0	34 (100%)	7 (18%)	208 (42%)	--
Any 1 symptom	144 (21%)	28 (26%)	0	11 (28%)	105 (21%)	--
Any 2 symptoms	119 (18%)	78 (74%)	0	22 (55%)	177 (36%)	--
Any 3 symptoms	85 (13%)	55 (52%)	0	14 (35%)	89 (18%)	--
All 4 symptoms	74 (11%)	25 (24%)	0	6 (15%)	42 (9%)	--
Mean CD4 cell count (SD), cells/mm^3	247 (206)	138 (144)	200 (162)	128 (122)	289 (214)	*<0.001*
<100	172 (30%)	56 (57%)	11 (33%)	21 (54%)	84 (20%)	*<0.001*
100-200	112 (19%)	20 (20%)	10 (30%)	9 (23%)	73 (18%)	*0.28*
>200	298 (51%)	22 (22%)	12 (36%)	9 (23%)	255 (62%)	*<0.001*
TB test results						
AFB smear positive	37 (6%)	23 (22%)	14 (41%)	0	0	*0.04*
Low	6	4	2			
+	20	12	8			
++	4	2	2			
+++	7	5	2			
M. tuberculosis	124 (19%)	96 (91%)	28 (82%)	0	0	*0.35*

culture positive						
Urine LAM positive	101 (15%)	35 (33%)	9 (26%)	9 (23%)	48 (10%)	*<0.001*

Abbreviations: TB – tuberculosis; ATT – anti-tuberculous therapy; antituberculosis therapy; SD – standard deviation; AFB – acid fast bacillus; LAM - lipoarabinomannan

[1] Empiric ATT treatment.

[2] P-values compare all four subgroups with the exception of AFB smear positive and *M. tuberculosis* culture positive where active TB and subclinical TB only are compared.

Table 2: Cox proportional hazards stratified by tuberculosis status.

	Active TB ***N=106***	**Subclinical TB** ***N=34***	**No microbiologic TB: ATT**[1] ***N=40***	**No microbiologic TB: no ATT** ***N=490***
Death at 12 months	25 (25%)	3 (9%)	5 (13%)	44 (9%)
Unadjusted HR (95% CI)	3.02 (1.86, 4.90)	0.99 (0.31, 3.20)	1.40 (0.56, 3.54)	Reference
P value[2]	*<0.001*[3]	*0.99*	*0.47*	
Adjusted HR (95% CI)[3]	1.55 (0.91, 2.64)	0.74 (0.23, 2.40)	0.75 (0.29, 1.93)	--
P value[2]	*0.11*	*0.61*	*0.55*	
Unadjusted HR (95% CI)	3.04 (0.92, 10.05)	Reference		
P value[4]	*0.07*[4]			
Adjusted HR (95% CI)[3]	2.10 (0.63, 7.02)	--		
P value[4]	*0.23*			

Abbreviations: TB – tuberculosis; ATT – anti-tuberculous therapy; HR – hazard ratio; CI – confidence interval

[1]Empiric ATT treatment.

[3]P-values correspond to hazard ratios comparing survival to no microbiologic TB not treated reference group.

[3]Adjusted for age, sex, CD4.

[4]P-values correspond to hazard ratios comparing survival to subclinical TB group.

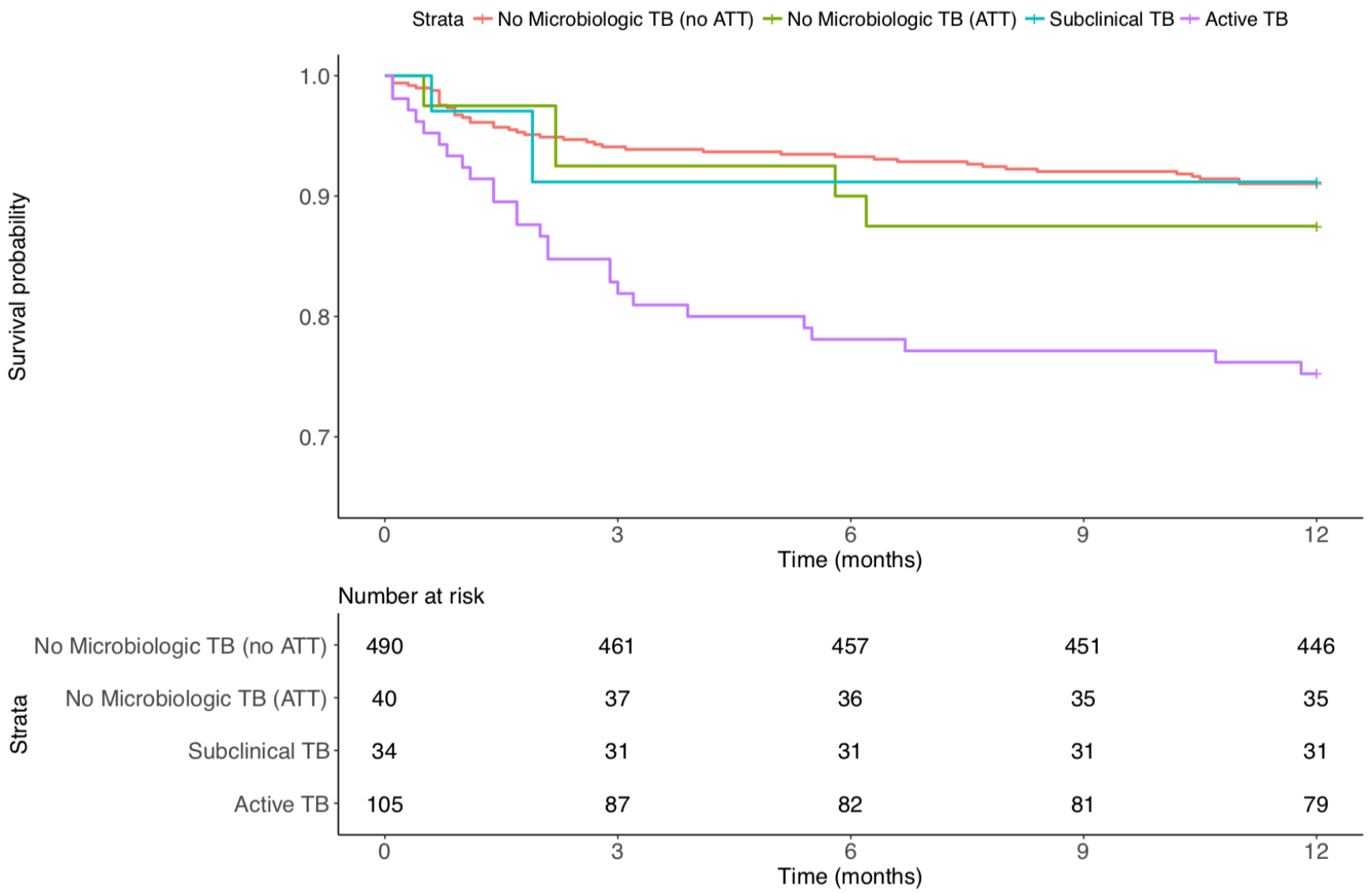

Figure 1. Kaplan Meier survival curves of untreated HIV-infected adults stratified by tuberculosis status.