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Tuberculosis and non-tuberculous mycobacteria among HIV-infected individuals in Ghana

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Abstract

OBJECTIVES To assess the prevalence and clinical importance of previously unrecognised tuberculosis (TB) and isolation of non-tuberculous mycobacteria (NTM) among HIV-infected individuals in a teaching hospital in Ghana.

METHODS Intensified mycobacterial case finding was conducted among HIV-positive individuals before initiation of antiretroviral therapy (ART). Data were collected on socio-demographic characteristics, medical history and TB-related signs and symptoms, and participants were followed for six months to determine treatment and vital status. Two sputum samples were obtained and examined for mycobacteria with smear microscopy, culture and Xpert MTB/RIF assay. NTM species were identified with the GenoType Mycobacterium CM/AS or sequence analysis of 16S rRNA gene.

RESULTS Of 473 participants, 60 (12.7%) had confirmed pulmonary TB, and 38 (8.0%) had positive cultures for NTM. *Mycobacterium avium* complex was identified in 9/38 (23.7%) of NTM isolates. Participants with NTM isolated were more likely to have CD4 cell count < 100 cells/ μ L (aOR 2.37; 95% CI: 1.10–5.14), BMI < 18.5 kg/m² (aOR 2.51; 95% CI: 1.15–5.51) and fever \geq 2 weeks (aOR 2.76; 95% CI: 1.27–6.03) at baseline than participants with no mycobacteria. By six months, 76 (16.1%) participants had died; 20 (33.3%) with confirmed TB and 9 (23.7%) with NTM-positive culture. Mortality at six months was independently associated with TB diagnosis at enrolment (aHR 1.97; 95% CI 1.09–3.59), but not with NTM isolation after controlling for age, sex, CD4 cell count, BMI, prolonged fever and ART initiation.

CONCLUSIONS Intensified mycobacterial screening of HIV-infected individuals revealed a high burden of unrecognised pulmonary TB before ART initiation, which increased risk of death within six months. NTM were frequently isolated and associated with signs of poor clinical status but not with increased mortality.

keywords Tuberculosis, Non-tuberculous mycobacteria, HIV, Mortality, Ghana

Introduction

Tuberculosis (TB) continues to be a leading cause of morbidity and mortality among HIV-infected individuals, and low TB case detection rates are of major concern [1]. A recent review of autopsy studies among HIV-infected individuals found a mean TB prevalence of 43% (95% CI: 38–48) in sub-Saharan Africa with almost half of the fatal cases undiagnosed with TB in life [2]. Intensified TB case finding among HIV-infected individuals from South Africa and Ethiopia revealed a high prevalence of previously

unrecognised TB at initiation of antiretroviral therapy (ART) [3–5]. Individuals with advanced HIV disease are also more susceptible to non-tuberculous mycobacteria (NTM) and are at increased risk of disseminated NTM disease [6, 7]. Unlike *M. tuberculosis*, NTM are ubiquitous environmental organisms with a high diversity in pathogenicity and clinical relevance [7]. Among NTM, *Mycobacterium avium* complex (MAC) commonly causes disseminated disease in immunocompromised HIV-infected patients [8], and in developed countries, MAC has been identified as an independent predictor for mortality

[9]. Few data are available on the distribution and clinical importance of NTM from TB endemic and HIV prevalent settings due to inadequate laboratory capacity for mycobacterial culture and a primary focus on TB [10]. A study of NTM from South-East Asia found frequent NTM isolation (21%) among newly enrolled HIV-infected individuals and presented data suggesting that NTM contribute to clinical disease [11]. From sub-Saharan Africa, a prevalence survey in Zambia found that NTM were isolated in 913/6,123 (15.1%) patients considered presumptive TB cases [12], and NTM have previously been associated with TB-like disease [13].

In Ghana, the overall TB incidence is 165/100 000 population; the TB case detection rate is estimated as low as 33% by WHO [14]. A recent study reported HIV infection rates of up to 48% among newly diagnosed TB cases [15], but the burden of TB among HIV-infected individuals in Ghana is barely acknowledged. Moreover, the significance of NTM isolation in this context is largely unknown.

In this study, we carried out intensified mycobacterial investigations of HIV-infected individuals to assess the prevalence of pulmonary TB and NTM isolation and followed participants up to evaluate the clinical importance of these pathogens.

Materials and methods

Setting and study population

This was a prospective observational study of HIV-infected individuals enrolled between January 2013 and March 2014 with 6 months of follow-up. The study is based on data from a recently reported diagnostic accuracy study of the rapid urine lipoarabinomannan (LAM) test for TB diagnosis in Ghana [16]. In brief, the study was conducted at the Fevers Unit, Korle-Bu Teaching Hospital in the capital city of Accra. Fevers Unit provides HIV services comprising voluntary counselling and testing, medical care, laboratory services, ART and adherence counselling. As of 2014, more than 15 000 people living with HIV (PLHIV) were enrolled at the Unit and 9028 initiated on ART (Hospital records, data unpublished).

HIV-infected adults were consecutively enrolled if ≥ 18 years of age and eligible for lifelong ART according to the current programme criteria, that is a blood CD4 cell count ≤ 350 cells/ μ l, advanced HIV disease (WHO clinical stage 3 or 4) or pregnant [17, 18]. Patients with known TB diagnosis or who could not produce sputum samples were excluded from the study, as were patients treated for TB more than 2 days within the last 3 months before enrolment. Presence of TB-related symptoms was not used as an inclusion or exclusion criterion.

Ethics

Informed consent was obtained in writing from each participant before enrolment. The study protocol was approved by the Ethical and Protocol Review Committee, University of Ghana Medical School and evaluated by the Developing Country Committee of the Danish National Committee on Health Research Ethics.

Data collection and laboratory investigations

A standardised questionnaire was administered to collect data on socio-demographic characteristics and TB-relevant history, signs and symptoms. We performed a basic physical examination on all participants and obtained blood CD4 cell count from routine services performed prior to initiation of ART.

Participants were requested to provide one spot sputum specimen and an early-morning specimen. Sputum samples were decontaminated and centrifuged. The pellet was used to prepare smears that were examined microscopically and graded for acid-fast bacilli using both Ziehl–Neelsen staining method and fluorescence microscopy of auramine O stained smears. The pellet was cultured for mycobacteria using both solid Lowenstein–Jensen medium and the BACTEC mycobacteria growth indicator tube 960 system (BD Diagnostics, Sparks, MD, USA). Sputum samples were processed at designated laboratories for mycobacteria in Ghana according to standardised protocols for mycobacterial microscopy and culture [19–21]. The Xpert MTB/RIF assay (Cepheid Sunnyvale, CA, USA, ‘Xpert’) became available for study purposes in June 2013, that is after inclusion had started, and was performed on specimens from patients included after this date. The Xpert assay was performed on either fresh sputum sample or on sputum sediment according to the manufacturer’s specifications. Isolates of *M. tuberculosis* complex and NTM identified in Ghana were sent for further speciation at the German National Reference Centre for Mycobacteria in Borstel, a WHO-appointed supranational reference laboratory. We used the GenoType Mycobacterium CM/AS (Hain Lifescience, Nehren, Germany) assay to identify the most common mycobacteria species. Other species were identified by sequence analysis of 16S rRNA gene.

Diagnosis and treatment

Sputum samples were transported daily to the local laboratories for mycobacteria and processed within one week. All bacteriological test results were communicated to health staff at the Fevers Unit as soon as they became

available to guide subsequent patient management. In case of a positive test results, that is positive sputum microscopy for acid-fast bacilli, positive culture or Xpert result for *M. tuberculosis* complex, health staff contacted the patients to begin immediate antituberculous treatment. The study participants could also be initiated on presumptive antituberculous treatment based on clinical suspicion and positive radiological findings in accordance with national guidelines [22]. Health staff were equally notified in case of NTM isolation although NTM treatment is not part of routine care in Ghana. However, NTM speciation was performed after completion of the study and hence not used to guide treatment. With the exception of the intensified mycobacterial case finding by the research team, other components of patient care were at the discretion of the treating physician.

Participant outcome

All participants were followed up through review of their routine medical records from enrolment for at least 6 months. If medical records were unavailable or if the participant had not attended a follow-up visit as scheduled, we contacted the participant or contact person by phone. For all participants, we recorded the following information: initiation date of ART, TB diagnosis subsequent to enrolment, initiation date of TB treatment and ascertained retention in programme, loss to death, loss to follow-up and transfer out.

Classification and statistical methods

Participants were classified according to mycobacterial status into one of the following three categories: ‘confirmed TB’, ‘NTM culture-positive’ and ‘no mycobacteria’. Confirmed TB was defined as a positive culture or Xpert result for *M. tuberculosis* complex in ≥ 1 sputum sample. If both *M. tuberculosis* complex and NTM were identified, we classified the participant as confirmed TB. Participants were classified as NTM culture-positive when NTM was isolated in ≥ 1 sputum samples. The remaining participants were classified as having no mycobacteria. This classification was used to enable analysis of clinical impact of NTM isolation in patients where pulmonary TB could be excluded. We thus assessed clinical characteristics and outcomes separately for cases with confirmed TB or with NTM-positive cultures and compared with those of the participants classified into the no mycobacteria group.

We present descriptive statistics as frequencies for categorical data and median with interquartile range (IQR) for numeric data. Difference in variables was tested using

Wilcoxon rank-sum test, chi-square test and Fisher’s exact tests to compare medians and proportions as appropriate. Logistic regression was used for univariate and multivariable analysis of risk factors’ association with NTM isolation in sputum. Risk factors included in analysis were age, sex, low baseline CD4 cell count ($CD4 < 100$ cells/ μ l), low body mass index ($BMI < 18.5$ kg/ m^2), cough ≥ 2 weeks and fever ≥ 2 weeks.

To assess the effect of mycobacterial status at baseline on mortality at 6 months, we used Cox proportional hazard regression analysis. In addition to mycobacterial status, we included other potential risk factors of early mortality identified in previous studies [1]: sex, age, low baseline CD4 cell count, low BMI, cough ≥ 2 weeks, fever ≥ 2 weeks and we adjusted for initiation of ART after enrolment. Factors associated with mortality in univariate analysis ($P < 0.05$) were included in the multivariate Cox proportional hazard analysis retaining also age and sex. For all factors included in the Cox model, the proportional hazard assumption was validated using a test of Schoenfeld residuals. Based on a global test value > 0.05 , we implied that the model fulfilled the proportional hazards assumption.

Statistical significance was defined as a two-sided P -value < 0.05 , and all analyses were conducted using STATA™ version 13.1 software.

Results

Study population

We screened 571 adults, of whom 473 were eligible for ART and able to provide 1–2 sputum samples (Figure 1). Of the eligible participants, 304 (64.3%) were females. Median age was 38 years (IQR 31–44.5) and median CD4 cell count was 126 cells/ μ l (IQR 34–255) (Table 1). Twenty-nine (6.1%) participants reported a prior history of TB treatment. Smoking was not common in the study population; only 13 (2.7%) participants were smokers at the time and 19 (4.0%) had a history of smoking.

Prevalence of pulmonary TB and isolation of NTM

We collected 845 sputum samples; 1 sample from 101 (21.4%) participants and 2 samples from 372 (78.7%) participants. All participants had at least one culture result available. Xpert results were available for 195/473 (41.2%) participants.

Pulmonary TB was confirmed in 60 participants (12.7%; 95% CI 10.0–16.0) with 12 participants having mixed infection with NTM. NTM alone were cultured in sputum from 38 (8.0%; 95% CI 5.9–10.9) participants.

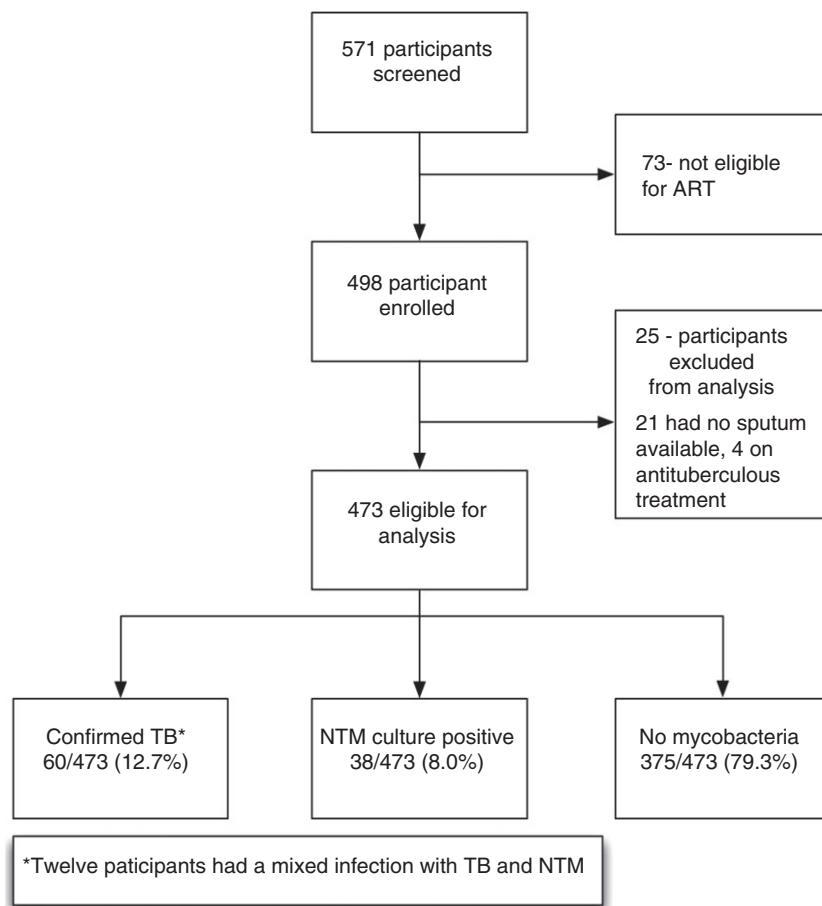


Figure 1 Flowchart of study participants.

Of these, NTM were cultured in two sputum specimens for 7/38 (18.4%) and in a single sputum sample for the remaining cases.

Comparative characteristics of participants across mycobacteria status

Respiratory and non-respiratory symptoms were common, and cough ≥ 2 weeks was reported by 36.2% and fever ≥ 2 weeks by 39.5% of participants (Table 1). Confirmed TB cases had lower median BMI, more often temperature ≥ 38 °C, increased respiration rate and reported more symptoms than participants with no mycobacteria (Table 1). While median CD4 cell count was lower for TB patients than for participants with no mycobacteria, it did not reach statistical significance.

In univariate analyses, NTM culture positivity was associated with CD4 cell count < 100 cells/ μ l, BMI < 18.5 kg/m², cough ≥ 2 weeks or fever ≥ 2 (Table 2). In the adjusted analysis, the association remained significant for low CD4 cell count, low BMI and fever ≥ 2 weeks.

Laboratory investigation for mycobacteria

Of the 60 participants with confirmed TB 55 were TB culture-positive and 5 were Xpert-positive, but TB culture-negative. Sputum smear microscopy identified TB in 32/60 (53.3%) cases. For participants with Xpert results available and a positive culture for TB Xpert correctly identified 27/35 (sensitivity 77.1%) of culture-confirmed TB cases, and Xpert was specific in 155/160 (96.9%) of culture-negative TB cases.

Two (5.3%) of the 38 participants with NTM-positive cultures were microscopy positive, and none was Xpert-positive. Several different NTM species were identified, the most frequent being MAC ($n = 9$) (Table 3).

Treatment status

ART was initiated for 340/473 (71.9%) participants after a median of 14 days (IQR 7–30) from enrolment (Table 4). TB treatment was prescribed for 39/60 (65.0%) of confirmed TB cases after a median of 14 days

S. Bjerrum *et al.* **TB and NTM among HIV-infected individuals in Ghana****Table 1** Baseline characteristics of HIV-infected individuals eligible to start ART and who were screened for mycobacteria at Korle-Bu Teaching Hospital, Accra, Ghana

	All	Confirmed TB*	NTM culture positive	No mycobacteria	<i>P</i> -value†	
Participants	473	60	38	375		Confirmed TB vs No mycobacteria
						NTM culture positive vs No mycobacteria
Age, median in years (IQR)‡	38 (31–44.5)	37 (29–43)	39 (33–45)	38 (32–45)	0.200	0.887
Sex, female	304 (64.3)	35 (58.3)	26 (68.4)	243 (64.8)	0.333	0.655
CD4, Median (IQR)‡	126 (34–255)	93 (41–183)	47 (11–187)	136 (38–269)	0.175	0.011
CD4 < 100	198 (43.3)	30 (52.6)	24 (64.9)	144 (39.7)	0.065	0.003
CD4 ≥ 100	259 (56.7)	27 (47.4)	13 (35.1)	219 (60.3)		
BMI, median (IQR)‡	19.8 (17.6–22.5)	17.8 (16.5–19.6)	17.9 (16.6–21.1)	20.6 (18.0–23.1)	<0.001	<0.001
Physical examination						
Temperature ≥ 38°C	47 (9.9)	22 (36.7)	3 (7.9)	22 (5.9)	<0.001	0.492
Respiration rate ≥ 20	245 (51.8)	51 (85.0)	20 (52.6)	174 (46.4)	<0.001	0.463
Lymphadenopathy	84 (17.8)	17 (28.3)	6 (15.8)	61 (16.3)	<0.001	0.939
Abnormal lung sounds (ronchic, crackles, wheezes)	149 (31.5)	37 (61.7)	13 (34.2)	99 (26.4)	<0.001	0.302
Symptoms presented						
Cough ≥ 2 weeks	171 (36.2)	35 (58.3)	21 (55.3)	115 (30.7)	<0.001	0.002
Productive cough‡	262 (55.6)	45 (75.0)	23 (60.5)	194 (52.0)	0.001	0.316
Dyspnoea‡	188 (40.2)	37 (62.7)	19 (50.0)	132 (35.6)	<0.001	0.079
Chest pain‡	201 (43.0)	33 (56.9)	21 (55.3)	147 (39.5)	0.013	0.060
Haemoptysis‡	47 (10.0)	7 (11.7)	5 (13.2)	35 (9.4)	0.584	0.399
Fever ≥ 2 weeks	187 (39.5)	40 (66.7)	23 (60.5)	124 (33.1)	<0.001	0.001
Significant weight loss (>10%)	283 (59.8)	48 (80.0)	23 (60.5)	212 (56.5)	0.001	0.636
Night Sweats	163 (34.5)	27 (45.0)	14 (36.8)	122 (32.5)	0.059	0.590
TB History						
Treated for TB	29 (6.1)	4 (6.7)	1 (2.6)	24 (6.4)	1.000	0.715
Exposure to person known with TB	21 (4.5)	5 (8.5)	0	16 (4.3)	0.172	–
Enrolment site						
In patients	72 (15.2)	17 (28.3)	7 (18.4)	48 (12.8)	0.002	0.331
Out patients	401 (84.8)	43 (71.7)	31 (81.6)	327 (87.2)		

Data are presented as *n* (%) or median (interquartile range), unless stated otherwise.

TB, Tuberculosis; NTM, Nontuberculous mycobacteria; BMI, Body Mass Index; IQR, Interquartile Range.

*Of TB cases 12 participants had a mixed infection with NTM.

†*P*-values in bold indicate values less than 0.05.

‡Missing values were excluded from analysis. Of participants; 5 had missing age; 16 had missing CD4 cell count; 16 had missing values to calculate BMI; 2 did not have information of productive cough; 5 did not have information on dyspnoea or chest pain; 3 did not have information on haemoptysis.

from enrolment (IQR 6–29). TB treatment was started before ART for 34 TB cases, while 5 patients started ART before TB treatment. Two months after enrolment, presumptive TB treatment had been started in 39 of TB-negative participants based on clinical presentation, x-ray abnormalities or a positive microscopy result. Prescription of presumptive TB treatment was more frequent in the NTM culture-positive than the no mycobacteria group (18.4% *vs.* 8.5%, *P* = 0.047). Of the NTM

culture-positive participants that were started on TB treatment one participant completed treatment, three died and three were lost to follow up (LTFU).

Participant outcomes at follow-up

By 6 months, 80/473 (16.9%) participants were LTFU with similar rates of LTFU across mycobacterial status (Table 4). Vital status at six months from enrolment was

S. Bjerrum *et al.* **TB and NTM among HIV-infected individuals in Ghana****Table 2** Risk factors for NTM isolation in sputum among HIV-infected individuals based on clinical characteristics at enrolment for individuals with NTM culture positive and no mycobacteria

	Odds ratio (95%CI)	P-value	AOR*	P-value‡
Age (years)†				
18–34	REF		REF	
35–54	1.04 (0.51–2.13)	0.920	1.02 (0.47–2.21)	0.966
55–	0.69 (0.15–3.22)	0.637	0.65 (0.13–3.29)	0.603
Sex				
Female	REF		REF	
Male	0.85 (0.42–1.74)	0.656	0.74 (0.33–1.66)	0.465
CD4 count (cells/ μ)†				
CD4 \geq 100			REF	
CD4 <100	2.81 (1.38–5.69)	0.004	2.37 (1.10–5.14)	0.028
BMI, (kg/m ²)†				
18.5–24.9	REF		REF	
<18.5	2.72 (1.33–5.55)	0.006	2.51 (1.15–5.51)	0.021
>25	0.68 (0.19–2.46)	0.560	1.09 (0.28–4.21)	0.898
Cough \geq 2 weeks				
No	REF		REF	
Yes	2.79 (1.42–5.49)	0.003	1.67 (0.77–3.60)	0.194
Fever \geq 2 weeks				
No	REF		REF	
Yes	3.10 (1.56–6.16)	0.001	2.76 (1.27–6.03)	0.011

AOR, Adjusted odds ratio; BMI, Body Mass Index.

*AOR adjusted for all variables included in table.

†Missing values were excluded from analysis. Of participants; 5 had missing age; 16 had missing CD4 cell count; 16 had missing values to calculate BMI.

‡P-values in bold indicate values less than 0.05

known for 382/473 (80.8%) participants; 76 (16.1%) had died in this group after a median of 21.5 days (IQR 10–65.5). Mortality among inpatients was 48.6% (35/72) vs. 10.2% (41/401) among outpatients ($P < 0.001$). Six-month mortality was 33.3% (20/60) among participants with TB vs. 12.5% (47/375) among participants with no mycobacteria. Nine (23.7%) of the participants with NTM had died within the six months. In univariate analysis, TB diagnosis at enrolment was strongly associated with increased risk of death at six months (HR 3.04, 95% CI 1.80–5.13) (Table 5), and this risk remained significant after controlling for sex, age, baseline CD4, BMI, presence of prolonged fever and initiation of ART (Adjusted HR 1.97; 95% CI 1.09–3.59). For participants

Table 3 Identification of NTM species isolated in sputum of HIV-infected individuals

NTM culture positive	38
<i>M. avium complex</i>	9 (23.7)
<i>M. chelonae complex</i>	3 (7.9)
<i>M. simiae</i>	3 (7.9)
<i>M. fortuitum complex</i>	2 (5.3)
<i>M. kansasii</i>	1 (2.6)
<i>M. flavescens</i>	1 (2.6)
<i>M. terrae</i>	1 (2.6)
<i>M. arupense</i>	1 (2.6)
Mycobacteria of unknown origin	6 (15.8)
Species not identified*	11 (29.0)

Data are presented as n (%). NTM, Nontuberculous mycobacteria.

*For 11 of the NTM culture positive participants the species of mycobacteria could not be identified; 3 isolates got contaminated before speciation was performed, 8 isolates were unavailable for further speciation.

with NTM, we saw a trend towards an increased risk of death at six months (HR 1.85; 95% CI 0.91–3.77), but it did not reach statistical significance in univariate analysis and was not seen in multivariable analysis. NTM species reported for those participants that subsequently died were MAC ($n = 2$), *M. chelonae complex* ($n = 2$), *M. kansasii* ($n = 1$), *M. flavescens* ($n = 1$), *M. simia* ($n = 1$), unknown mycobacteria ($n = 2$).

Discussion

In this study, intensified screening for mycobacteria revealed a substantial prevalence of unrecognised pulmonary TB (12.7%) and frequent NTM isolation (8%) in a population of HIV-infected individuals starting ART at a teaching hospital in Ghana. MAC was the most commonly identified NTM species and severe clinical signs and symptoms predicted NTM isolation. All-cause mortality at 6 months was high (16%) in our study population. TB diagnosis at baseline, but not NTM isolation, was independently associated with almost doubled risk of death.

To our knowledge, this is the first prospective study from Ghana describing the yield of intensified TB case finding among HIV-infected individuals. A retrospective cohort study of PLHIV described TB as the major AIDS defining event, but reported a quite modest event rate of 4.4% in the cohort with a median follow-up time of 30 months (IQR; 12–54) [23]. A review of intensified TB-screening in other low- and middle-income countries found a prevalence of confirmed TB between 6.3% and 25.7% in populations attending HIV care; the highest

Table 4 Treatment status and clinical outcomes of HIV-infected individuals at 6-months after enrolment into ART services by mycobacteria identified upon enrolment

	All	Confirmed TB	NTM culture positive	No mycobacteria
Participants	473	60	38	375
ART status				
Started ART	340 (71.9)	42 (70)	25 (65.8)	273 (72.8)
Median time to ART (IQR)*	14 (7–30)	26 (12–42)	19 (9–39)	12 (6–25)
Tuberculosis treatment				
TB treatment started	78 (16.5)	39 (65.0)	7 (18.4)†,‡	32 (8.5)†
Median time to TB treatment (IQR)	10 (2–24)	14 (6–29)	11 (2–30)	7 (1–12)
Outcome at 6 months				
Alive and retained in programme	306 (64.7)	30 (50.0)	23 (60.5)	253 (67.5)
Died	76 (16.1)	20 (33.3)	9 (23.7)§	47 (12.5)
Lost to follow-up	80 (16.9)	9 (15.0)	6 (15.8)	65 (17.3)
Transfer out	11 (2.3)	1 (1.7)	0	10 (2.7)

Data are presented as *n* (%) or median (interquartile range), unless stated otherwise.

ART, Antiretroviral treatment; TB, Tuberculosis; NTM, Nontuberculous mycobacteria.

*Time to treatment defined as the time interval in days between date of inclusion and start date.

†TB treatment was started within two months of follow-up based on a positive microscopy result, clinical presentation of the participants or chest x-ray abnormalities.

‡Pairwise comparison between participants with NTM culture positive and no mycobacteria ($P = 0.047$).

§Pairwise comparison between participants with NTM culture positive and no mycobacteria ($P = 0.056$).

rates were found among HIV-positive patients starting ART in South Africa [24].

In our cohort, individuals were typically initiated on ART at advanced stage of HIV disease as indicated by the low median CD4 cell count (126 cells/ μ l). This has also been observed in other HIV cohorts from sub-Saharan African settings [4, 5, 25] and is known to be an important risk factor for TB and mortality among PLHIV entering ART services [1, 26]. The high mortality in our study population is comparable to mortality rates previously reported for other sub-Saharan African countries within the first year after starting ART [1, 27]. We further found that TB diagnosed upon enrolment was strongly associated with increased mortality also after controlling for other known risk factors for mortality, that is age, sex, CD4 cell count and BMI at baseline. This confirms similar findings from Uganda [28] and South Africa [26, 29].

Intensified TB case finding in our study gave opportunity to start TB treatment early, but still more than one-third never started treatment and one-third of the TB patients died within the first 6-month period. We did not include any control group and will not know how intensified TB case finding possibly impacted on morbidity and mortality compared to the standard of care. However, in the context of routine care, we anticipate that far fewer TB cases would have been identified or the TB diagnosis delayed. Less sensitive diagnostic tools

including symptom screening followed by direct sputum smear microscopy and chest x-ray are commonly used for routine TB diagnosis in Ghana [15] as in other resource limited settings [30], although the Xpert assay is becoming increasingly available.

The frequent NTM isolation in our study population was associated with characteristics of clinical disease upon diagnosis and start of presumptive treatment for TB. This indicates that NTM are of clinical relevance and present with TB-like disease. The challenge is to differentiate between NTM disease, NTM colonisation or sample contamination. The American Thoracic Society and the Infectious Disease Society of America provide guidelines for the diagnosis and treatment of NTM disease that rely on three components, that is presence of symptoms, radiological abnormalities and positive cultures for NTM [6]. However, these guidelines do not specifically target NTM disease among HIV-infected individuals and are not applicable for routine use in low-resource settings with limited access to culture-based diagnostics. Specific guidelines for diagnosis of MAC diseases among HIV-infected are available but require laboratory-intensive investigations with culture of sterile tissue or body fluids in addition to compatible signs and symptoms [31]. After the introduction of ART, the rate of disseminated MAC reduced substantially in high-income countries, although severely immunocompromised individuals remained at risk [8]. Globally, isolation of

Table 5 Cox proportional hazards analysis for the outcome of death within 6 months based on mycobacteria status and clinical characteristics at enrolment among HIV-infected individuals attending HIV care at Korle-Bu Teaching Hospital in Ghana

	Unadjusted HR (95%CI)	<i>P</i> -value	Adjusted HR (95%CI)*	<i>P</i> -value‡
Mycobacteria status				
No mycobacteria	REF		REF	
Confirmed TB	3.04 (1.80–5.13)	<0.001	1.97 (1.09–3.59)	0.026
NTM culture positive	1.85 (0.91–3.77)	0.091	0.56 (0.24–1.28)	0.168
Age (years)†	1.01 (0.98–1.03)	0.548	1.01 (0.98–1.03)	0.620
Sex				
Female	REF		REF	
Male	0.99 (0.62–1.58)	0.955	1.23 (0.73–2.08)	0.438
CD4 Cell count†				
CD4 ≥100	REF		REF	
CD4 <100	5.55 (3.13–9.82)	<0.001	6.06 (3.31–11.08)	<0.001
BMI, kg/m ² †				
≥18.5	REF		REF	
<18.5	2.27 (1.44–3.56)	<0.001	2.05 (1.22–3.45)	0.007
Cough ≥2 weeks				
No	REF		–	–
Yes	1.52 (0.97–32.39)	0.069	–	–
Fever ≥2 weeks				
No	REF		REF	
Yes	2.25 (1.43–3.54)	<0.001	1.80 (1.07–3.03)	0.028
ART status				
No ART	REF		REF	
ART initiated	0.13 (0.08–0.21)	<0.001	0.08 (0.05–0.14)	<0.001

ART, Antiretroviral treatment; CI, Confidence Interval; BMI, Body Mass Index; HR, Hazard Ratio; NTM, Nontuberculous mycobacteria; TB, Tuberculosis;

*Hazard ratio adjusted for sex, age, and all variables significantly associated with mortality in univariate analysis ($P < 0.05$).

†Missing values were excluded from analysis. Of participants; 5 had missing age; 16 had missing CD4 cell count; 16 had missing values to calculate BMI.

‡*P*-values in bold indicate values less than 0.05

NTM and NTM disease is reported more frequently [7] and encountered with wide geographic diversity [32]. From sub-Saharan Africa, we came across studies and case reports from Zambia [12, 33], Tanzania [13], Nigeria [34], and Côte d'Ivoire [35] that confirm great variation of NTM species and a notable contribution of NTM to TB-like disease [11, 13, 33, 34]. The most frequently reported pathogenic species among HIV-infected include MAC, *M. kansasii* and *M. fortuitum* [6–8] that are also among the species identified in our study population. Although there is no doubt that TB is the main cause of mycobacterial disease among HIV-infected, our findings contribute to a growing concern that the clinical impact of NTM in TB endemic area is underestimated and that misclassification of NTM as TB occurs [7, 10, 12, 34].

Our study was based on a rigorous strategy to identify mycobacteria among HIV-infected individuals regardless of presentation at enrolment. Still, we may have missed some cases of TB as even culture and Xpert-based diagnosis have imperfect diagnostic sensitivity among patients

with progressive immunodeficiency [36]. We focused on pulmonary samples that did not allow us to identify patients presenting with only extrapulmonary TB. Furthermore, our diagnosis relied on collection of spontaneous sputum samples from, at times, sputum-scarce individuals. This might have compromised the diagnostic yield of TB and NTM, but we did not find the capacity to make use of techniques for sputum induction under safe conditions. Excluding patients not able to produce samples or consent due to very severe clinical status or cerebral impairment might further have introduced a potential selection bias as it is likely that TB disproportionately affect these patients. Another limitation is the high rate of LTFU in our study despite of various approaches to ascertain clinical outcomes for patients who did not continue ART services. Although a high proportion of LTFU is not unusual for this kind of study setting [37], we may have underestimated mortality, as mortality is a common cause of LTFU [38, 39]. We did not find an association between NTM at baseline and

increased risk of death, but this is based on a limited number of cases. Larger studies including more extensive evaluation of the patients, consistent use of radiological and serial diagnostic workout are required to further elucidate the clinical relevance of NTM among HIV-positive individuals and determine the prevalence of NTM disease in patients with HIV in TB endemic settings.

Conclusion

Intensified screening for mycobacteria revealed a high prevalence of previously unrecognised pulmonary TB and frequent isolation of NTM among HIV-infected patients eligible for ART in Ghana. The six-month mortality rate was high in our study population, and TB diagnosis was associated with increased risk of death. Isolation of NTM was not associated with mortality at 6 months, but data presented indicate that NTM could be of clinical relevance warranting increased attention and more research.

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