DOI: 10.1111/hiv.12635
HIV Medicine (2018), **19**, 654–661

SHORT COMMUNICATION

Comparison of Löwenstein–Jensen and BACTEC MGIT 960 culture for *Mycobacterium tuberculosis* in people living with HIV

J Hongler,[1] J Musaazi,[2] B Ledergerber,[1] N Eberhard,[1,2] C Sekaggya-Wiltshire,[2] PM Keller,[3] J Fehr[1,4] and B Castelnuovo[2]

[1]*Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, University of Zurich, Zurich, Switzerland,* [2]*Infectious Diseases Institute, College of Health Sciences, Makerere University, Kampala, Uganda,* [3]*Institute of Medical Microbiology and National Centre for Mycobacteria, University of Zurich, Zurich, Switzerland and* [4]*Department of Public Health, Epidemiology, Biostatistics and Prevention Institute, University of Zurich, Zurich, Switzerland*

Objectives

The aim of the study was to clarify how HIV infection affects tuberculosis liquid and solid culture results in a resource-limited setting.

Methods

We used baseline data from the Study on Outcomes Related to Tuberculosis and HIV Drug Concentrations in Uganda (SOUTH), which included 268 HIV/tuberculosis (TB)-coinfected individuals. Culture results from Löwenstein–Jensen (LJ) solid culture and mycobacteria growth indicator tube (MGIT) liquid culture systems and culture-based correlates for bacillary density from the sputum of HIV/TB-coinfected individuals at baseline were analysed.

Results

Of 268 participants, 243 had a CD4 cell count available and were included in this analysis; 72.2% of cultures showed growth on solid culture and 82.2% in liquid culture systems ($P < 0.015$). A higher CD4 cell count was predictive of LJ positivity [adjusted odds ratio (OR) 1.14; 95% confidence interval (CI) 1.03–1.25 per 50 cells/μL increase; $P = 0.008$]. The same, but insignificant trend was observed for MGIT positivity (adjusted OR 1.09; 95% CI 0.99–1.211 per 50 cells/μL increase; $P = 0.094$). A higher CD4 cell count was associated with a higher LJ colony-forming unit grade (adjusted OR 1.14; 95% CI 1.05–1.25 per 50 cells/μL increase; $P = 0.011$) and a shorter time to MGIT positivity [adjusted hazard ratio (HR) 1.08; 95% CI 1.04–1.12 per 50 cells/μL increase; $P < 0.001$].

Conclusions

In a resource-limited setting, the MGIT liquid culture system outperformed LJ solid culture in terms of culture yield and dependence on CD4 cell counts in HIV/TB-coinfected individuals. We therefore suggest considering an adaptation of diagnostic algorithms: when resources allow only one culture method to be performed, we recommend that MGIT liquid culture should be used exclusively in HIV-positive individuals as a first-line culture method, to reduce costs and make TB culture results accessible to more patients in resource-limited settings.

Keywords: CD4, culture, HIV, resource-limited setting, tuberculosis

Accepted 25 April 2018

Introduction

HIV and tuberculosis (TB) coinfection is a major driving force of the global TB epidemic and has led to several challenges in the diagnosis of pulmonary TB (PTB). Clinical presentation, radiographic findings and case ascertainment are severely affected by HIV-related

Correspondence: Jan Hongler, Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, Rämistrasse 100, 8091 Zürich, Switzerland. Telephone +41 44 255 33 22; Fax +41 44 255 32 91; e-mail: jan.hongler@uzh.ch

immunosuppression [1,2]. A number of studies have shown that HIV coinfection leads to a reduced cellular immune response and consequentially to a change in pulmonary immunopathology, and more disseminated and less focally controlled disease [3–5].

Traditionally, acid-fast bacilli (AFB) sputum smear microscopy has been the initial diagnostic tool for more than 100 years, and it is still widely used in resource-limited settings (RLSs) [1]. AFB sputum smear microscopy, however, is a suboptimal test in the diagnosis of PTB, as it has low sensitivity, especially in HIV-positive individuals [6–8]. In recent years, the World Health Organization (WHO) has endorsed the use of the Xpert MTB/RIF (Cepheid, Sunnyvale, CA, USA) molecular test as an initial diagnostic tool for detection of TB in HIV-positive patients [9]. However, this novel diagnostic tool is not able to substitute for *Mycobacterium tuberculosis* (MTB) culture entirely, as it provides only information about rifampicin susceptibility and cannot be used for treatment monitoring [10]. Culture-based methods remain the gold standard in the diagnosis of TB, drug susceptibility testing (DST) and treatment monitoring [1].

It has been shown that HIV coinfection and decreasing CD4 cell counts not only render TB diagnosis more difficult, but also increase the likelihood of more severe and disseminated disease [2,11]. Therefore, it is of utmost importance to better understand the impact of decreasing CD4 cell counts on the diagnostic gold standard. In this study, we aimed to clarify if there is an association between CD4 cell count and Löwenstein–Jensen (LJ) and mycobacterial growth indicator tube (MGIT) culture results.

Methods

Patients

Patients were enrolled in the Study on Outcomes Related to Tuberculosis and HIV Drug Concentrations in Uganda (SOUTH) (clinicaltrials.gov NCT01782950). Details of the study have been described elsewhere [12]. This was a single-centre prospective observational study in HIV/TB-coinfected individuals exploring the correlation between TB treatment outcomes and anti-TB drug exposure. The study was conducted in a large HIV-infected out-patient clinic with an integrated HIV-TB clinic at the Infectious Disease Institute at the Makerere University College of Health Sciences in Kampala, Uganda between 2013 and 2015.

The inclusion criteria of SOUTH were: confirmed HIV infection, age $\geq$ 18 years and having newly diagnosed PTB, either clinically diagnosed or confirmed by a positive sputum AFB smear, Xpert MTB/RIF assay or culture. Approval was given by the Joint Clinical Research Centre Ethical Committee, the Uganda National Council of Science and Technology and the National Drug Authority. Written informed consent was obtained from all participants.

Microbiological investigations

Microbiological investigations in SOUTH were performed by the mycobacteriology laboratory in the Department of Medical Microbiology of Makerere University in Kampala. Standard procedures were performed for mycobacterial culture and smear microscopy [13].

For sputum culture, two systems were used: LJ solid medium and MGIT liquid culture (BACTEC MGIT 960; Becton Dickinson, Franklin Lakes, NJ, USA). The mycobacterial colony count was performed manually according to WHO criteria using the LJ solid medium. However, in July 2014, WHO changed the LJ culture grading guidelines. To compare the two grading systems, we merged the groups according to WHO laboratory report stratification (Appendix 1).

Cultures on LJ solid medium were incubated at 37°C for up to 8 weeks in an incubator (Thermo Scientific Forma Reach CO_2 Incubator; Thermo Fisher Scientific, Waltham, MA, USA) and MGIT cultures were incubated for up to 6 weeks in a dedicated Becton Dickinson, Franklin Lakes, NJ, USA system. Cultures that showed growth were subcultured on sheep blood agar (BD) to exclude contamination and a Ziehl–Neelsen smear was microscopically examined. Specimens that tested positive for AFB had Capillia Neo TB™ (TAUN, Numazu, Japan) testing performed. Capillia-positive specimens were categorized as MTB and Capillia-negative specimens were categorized as non-tuberculous mycobacteria (NTM). AFB-negative specimens that showed growth on blood agar were classified as contaminated (with organisms other than mycobacteria) and those without growth in LJ medium or MGIT were classified as negative. For MGIT, we recorded culture status and days to positivity (DTP).

Statistical analysis

Demographic factors (age and sex), body mass index (BMI), antiretroviral therapy (ART) status (not on ART and on ART at baseline) and MTB microbiological findings (LJ and MGIT culture results) at baseline were described and stratified by CD4 count. LJ and MGIT positivity were compared in a 2 $\times$ 2 contingency table using a χ^2 test. The CD4 cell counts were categorized as $<$ 200, 200–350 and $>$ 350 cells/μL and treated as a continuous

variable (per 50 cells/μL increase). Pearson's χ^2 tests or Fisher's exact tests were used to compare categorical variables, while analysis of variance and Kruskall–Wallis tests were used to compare normally distributed variables and nonnormally distributed variables across CD4 cell count strata.

Logistic regression adjusted for age, gender, BMI and ART status (not on ART *vs*. on ART) was used to determine whether CD4 cell count was predictive of culture positivity using LJ and MGIT culture systems. Ordered logistic regression was used to determine whether CD4 cell count was predictive of LJ culture grade. The proportional odds assumption of the ordered logistic regression was checked by comparing it with the multinomial logistic regression using Akaike information criteria (AIC). Time to positivity (TTP) in the MGIT culture system was analysed using time-to-event analysis (Kaplan–Meier curves and Cox proportional hazards regression). Culture time in MGIT was measured up to 42 days and negative cultures were censored at 42 + 1 days.

The analysis was performed using STATA version 13.0 (StataCorp LCC, College Station, TX, USA).

Results

A total of 268 TB/HIV-coinfected patients were identified and recruited within SOUTH. We included 243 individuals who had a CD4 cell count result and LJ or MGIT available in this analysis. Baseline characteristics of included individuals are described in Table 1 and patient selection is illustrated in Figure 1.

Of the 243 individuals included in this study, 214 (88%) had results for both LJ and MGIT available at the same time and were directly compared regarding culture conversion to positivity (Appendix 2). One hundred and fifty (72.4%) individuals had positive LJ and MGIT results, one (0.5%) exclusively showed growth in the LJ culture system and 22 (10.3%) showed growth exclusively in MGIT. Thirty-six (16.8%) specimens were negative in both the LJ and MGIT culture methods. MGIT showed a higher yield per patient (0.83) when compared with LJ (0.73) ($P < 0.015$; Appendix 2). Contamination occurred in five (2.28%) of the 219 specimens for each culture method.

Higher CD4 cell count category was associated with higher odds of LJ positivity and higher colony-forming unit (CFU) grade, with 3+ being considered the highest CFU grade (*P*-values for the test of lack of linear trend were 0.89 and 0.80, respectively). Patients with CD4 counts of 200–350 and > 350 cells/μL were 2.4 times [adjusted odds ratio (AOR) 2.44; 95% confidence interval (CI) 1.12–5.32; $P = 0.025$) and 3.5 times (AOR 3.52; 95% CI 1.54–8.03; $P = 0.002$), respectively, more likely to have a higher grade CFU count when compared with patients with CD4 counts < 200 cells/μL, after adjusting for BMI, age, sex and ART status. Similarly, when considering CD4

Table 1 Participants' ($n = 243$) baseline characteristics at tuberculosis (TB) diagnosis by CD4 count

Characteristic	Overall	CD4 count (cells/μL) strata			*P*-value*,λ
		< 200 ($n = 144$; 59.3%)	200–350 ($n = 53$; 21.8%)	> 350 ($n = 46$; 18.9%)	
Gender, male [*n* (%)]	147 (60.5)	90 (62.5)	31 (58.5)	26 (56.5)	0.728
Age (years) [mean (SD)]	34 (8)	33 (8)	34 (8)	35 (9)	0.657
Not on ART [*n* (%)]	196 (80.7)	123 (85.4)	39 (73.6)	34 (73.9)	0.077
BMI (kg/m^2) [median (IQR)]†	19.2 (17.5–21.1)	18.8 (17.3–20.7)	19.2 (17.7–20.9)	19.4 (18.2–22.3)	0.066
BMI < 18.5 [*n* (%)]	96 (39.8)	61 (43.0)	23 (43.4)	12 (26.1)	0.106
BMI ≥ 18.5 [*n* (%)]	145 (60.2)	81 (57.0)	30 (56.6)	34 (73.9)	
LJ results [*n* (%)]	219 (90.1)	124 (56.6)	49 (22.4)	46 (21)	
Positive [*n* (%)]	158 (72.2)	83 (66.9)	37 (75.5)	38 (82.6)	0.108
LJ CFU grade [*n* (%)]					
0+	16 (10.1)	15 (17.9)	1 (2.7)	0 (0.0)	0.015
1+	34 (21.5)	20 (23.8)	8 (21.6)	6 (16.2)	
2+	18 (11.4)	10 (11.9)	4 (10.8)	4 (10.8)	
3+	90 (57.0)	39 (46.4)	24 (64.9)	27 (73.0)	
MGIT results [*n* (%)]	219 (90.1)	124 (56.6)	49 (22.4)	46 (21)	
Positive [*n* (%)]	180 (82.2)	100 (80.6)	41 (83.7)	39 (84.8)	0.784
TTP (days) [median (IQR)]	9 (5–17)	11 (7–19)	9 (6–15)	8 (5–12)	0.031

λ = Fisher's exact test when expected cell count is < 5 cells/μL.
ART, antiretroviral therapy; BMI, body mass index; CFU, colony-forming units; IQR, interquartile range; LJ, Löwenstein–Jensen solid culture; MGIT, mycobacteria growth indicator tube liquid culture; SD, standard deviation; TTP, time to positivity.
*χ^2 *P*-values comparing CD4 count strata.
†Numbers of patients: overall, 241; < 200 cells/mL, 142; 200–350 cells/μL, 53; > 350 cells/μL, 46. The *P*-value for BMI was obtained from a Kruskall–Wallis test.

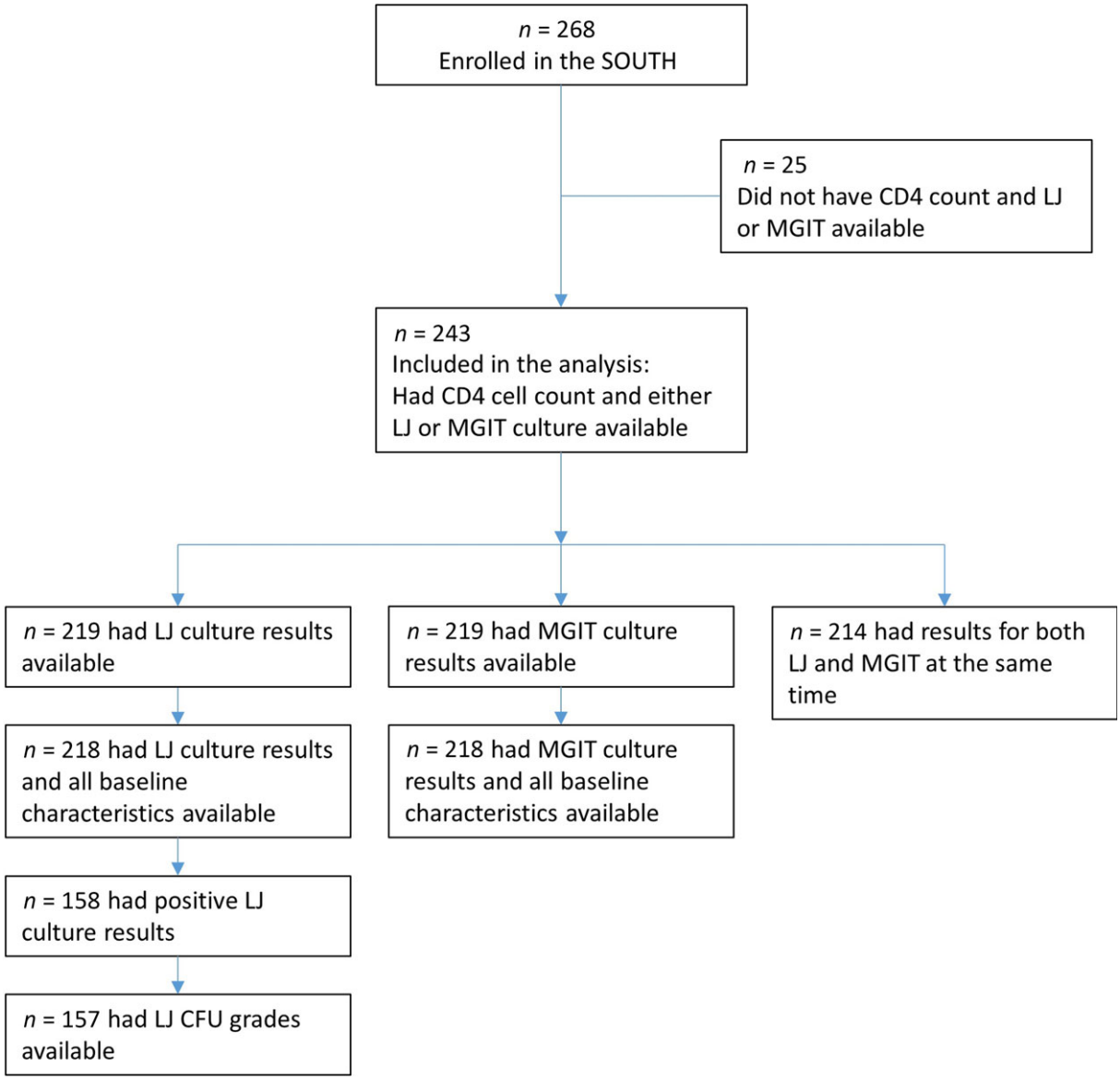

Fig. 1 Patient selection flow chart. CFU, colony-forming units; LJ, Löwenstein–Jensen solid culture; MGIT, mycobacteria growth indicator tube liquid culture; SOUTH, Study on Outcomes Related to Tuberculosis and HIV Drug Concentrations in Uganda.

count as a continuous variable, we found a higher CD4 count to be predictive of LJ positivity [OR 1.14 (95% CI 1.03–1.25) per 50 cells/μL increase; $P = 0.008$] and higher colony grading [OR 1.14 (95% CI 1.05–1.25) per 50 cells/μL increase; $P = 0.011$] (Appendices 3 and 4).

When looking at predictive factors of MGIT culture positivity and TTP, respectively, after adjusting for gender, age, BMI and ART status, we did not find any significant association between CD4 count and MGIT culture positivity. When considering CD4 count as a continuous variable, we did not find any significant association between CD4 count and MGIT positivity [OR 1.09 (95% CI 0.99–1.211) per 50 cells/μL increase; $P = 0.094$], but a higher CD4 cell count was associated with a shorter time to MGIT positivity (hazard ratio 1.08; 95% CI 1.04–1.12; $P < 0.001$) (Appendices 5 and 6).

Discussion

This study is one of few studies to compare LJ solid and MGIT liquid culture in HIV/TB-coinfected individuals. We demonstrated [1] significantly lower yield of LJ culture

compared with MGIT, [2] an association between CD4 count and LJ culture positivity, and [3] that higher CD4 cell count was associated with higher LJ CFU grade and shorter MGIT TTP.

When we compared LJ solid culture to MGIT liquid culture, we were able to reproduce findings from previous studies showing lower yield of LJ solid culture, when compared with MGIT liquid culture [14,15].

Culture positivity only showed a significant association with CD4 cell count in LJ solid culture, with higher CD4 cell count being associated with higher culture positivity. This may be attributed to the reduced pulmonary immunopathology as a result of HIV-related immunosuppression which leads to fewer culturable bacilli being released to the airways [4,16]. Consequentially, this observation potentially reflects a higher detection threshold for LJ solid culture when compared with MGIT liquid culture. This association was not significant for MGIT liquid culture, although we observed the same trend. The margin between the two methods tended to be minor (AOR 1.14 *vs.* 1.09).

Additionally, we observed that higher CD4 cell count was associated with higher LJ CFU grade and shorter MGIT TTP. A South African study analysing the pretreatment composition of sputa made a similar observation: lower CD4 cell counts resulted in fewer routinely culturable MTB [17]; the study also observed that, compared with HIV-negative patients, HIV-positive patients harboured fewer differentially culturable MTB, which are responsive to the addition of resuscitation-promoting factors to liquid culture systems in order to enhance growth. Differences were also observed among the HIV-positive population, with lower differentially culturable MTB in patients with lower CD4 count, suggesting that reduced host immunity is associated with a lower prevalence of bacteria, which are responsive to resuscitation-promoting factors.

Contrary to our findings, a study investigating MTB load in the pleural tissue or granulomas of HIV/TB-coinfected individuals found an increased abundance of MTB with decreasing CD4 cell count [18]. This could be because LJ CFU grades and MGIT TTP from sputa samples in conventional culture methods do not entirely represent the bacillary load in the pulmonary tissue of coinfected patients.

Contamination has been reported to be a main limitation in the use of MGIT culture, with rates as low as 5.5% in high-income settings [14] and as high as 29.6% in RLSs [19]. We found low contamination rates of 2.3% for both LJ and MGIT, respectively. The reason for this low contamination rate is unclear, but it may be explained by the proximity of the mycobacterial laboratory and HIV clinic, which was likely to have led to a short specimen-processing time. A long specimen-processing time has been associated with higher contamination rates [20]. Additional limitations of MGIT compared with LJ include higher costs per additional TB case detected when compared with LJ in a clinical setting [15]. However, based on our data showing superiority of MGIT, the diagnostic laboratory procedure for TB could be streamlined, emphasizing MGIT. This approach might be cost-effective as it helps to identify TB cases more reliably and hence lower the burden and costs of TB on a public health level. Furthermore, Increased use of MGIT could be a good argument to negotiate for lower prices, especially in RLSs.

The study had some limitations. The reference standard for PTB diagnosis in this study was positive sputum AFB smear, Xpert MTB/RIF assay, culture or clinical diagnosis alone. Therefore, it is possible that patients who did not have TB were included. This could have lead to an underestimation of sensitivity and the analysis of relationship between CD4 cell count and culture positivity, as patients with lower CD4 counts may have been more likely to have other opportunistic infections that were clinically misdiagnosed as TB.

Conclusions

We were able to demonstrate the superiority of MGIT liquid culture when compared with LJ solid culture in an HIV/TB-coinfected population in an RLS. The MGIT liquid culture system showed a higher yield when compared with LJ solid culture and was, in contrast to LJ solid culture, not significantly affected by CD4 cell count with regard to culture positivity. When resources allow only one culture method to be performed, we therefore suggest an adaptation of the diagnostic algorithm to include only MGIT liquid culture, especially in those individuals with known low CD4 cell counts.

Appendix 1: Löwenstein–Jensen (LJ) solid medium culture colony-forming unit (CFU) grading

WHO grade	Before 31 July 2014	After 31 July 2014
0+	1–49 LJ colonies	1–9 LJ colonies
1+	50–100 LJ colonies	10–100 LJ colonies
2+	101–200 LJ colonies	101–200 LJ colonies
3+	> 200 LJ colonies	> 200 LJ colonies

Appendix 2: Cross-tabulation of the culture recovery for Löwenstein–Jensen (LJ) solid culture and mycobacteria growth indicator tube (MGIT) liquid culture (n = 214)

	MGIT results		
	Negative	Positive	Total
LJ results			
Negative	36	22	58
Positive	1	155	156
Total	37	177	214

Pearson χ^2: $P = 0.015$.

Appendix 3: Unadjusted and adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for predictive factors of Löwenstein–Jensen (LJ) culture positivity using logistic regression (n = 218)*

Variable	Unadjusted OR (95% CI)	*P*-value	Adjusted OR (95% CI)	*P*-value
Sex				
Female	Reference		Reference	
Male	1.05 (0.58–1.91)	0.870	1.07 (0.57–2.03)	0.825
Age				
< 34 years	Reference		Reference	
≥ 34 years	0.63 (0.35–1.15)	0.130	0.73 (0.38–1.37)	0.324
BMI				
< 18.5 kg/m^2	Reference		Reference	
≥ 18.5 kg/m^2	1.53 (0.84–2.79)	0.165	1.43 (0.76–2.71)	0.270
ART status at baseline				
On ART	Reference		Reference	
Not on ART	3.34 (1.64–6.81)	0.001	3.75 (1.74–8.07)	0.001
CD4 count strata				
< 200 cells/μL	Reference		Reference	
200–350 cells/μL	1.54 (0.73–3.27)	0.259	1.79 (0.81–3.93)	0.147
> 350 cells/μL	2.38 (1.02–5.55)	0.046	2.86 (1.15–7.11)	0.023
CD4 count increase by 50 cells/μL	1.01 (1.01–1.20)	0.014	1.14 (1.03–1.25)	0.008

ART, antiretroviral therapy; BMI, body mass index.
*n = 218, because of adjusting: missing BMI value for one of the 219 LJ culture specimens.

Appendix 4: Unadjusted and adjusted odds ratios (ORs) and their 95% confidence intervals (CIs) for predictive factors of higher colony counts [colony-forming unit (CFU) grades] using ordinal logistic regression (n = 157)*

	Unadjusted OR (95% CI)	*P*-value	Adjusted OR (95% CI)	*P*-value
Sex				
Female	Reference		Reference	
Male	0.88 (0.47–1.64)	0.688	0.94 (0.50–1.78)	0.848
Age group				
< 34 years	Reference		Reference	
≥ 34 years	1.07 (0.58–1.98)	0.831	1.03 (0.54–1.95)	0.324
BMI				
< 18.5 kg/m^2	Reference		Reference	
≥ 18.5 kg/m^2	1.36 (0.73–2.54)	0.337	1.32 (0.69–2.51)	0.402
ART status at baseline				
On ART	Reference		Reference	
Not on ART	0.64 (0.25–1.65)	0.354	0.71 (0.26–1.91)	0.497
CD4 count strata				
< 200 cells/μL	Reference		Reference	
200–350 cells/μL	2.45 (1.14–5.30)	0.022	2.44 (1.12–5.32)	0.025
> 350 cells/μL	3.66 (1.61–8.32)	0.002	3.52 (1.54–8.03)	0.003
CD4 count increase by 50 cells/μL	1.14 (1.05–1.24)	0.002	1.14 (1.05–1.25)	0.011

ART, antiretroviral therapy; BMI, body mass index; CFU, colony-forming units; LJ, Löwenstein-Jensen solid culture.
*n = 157, because of adjusting: missing LJ CFU value for one of 158 positive LJ culture specimens.

Appendix 5: Unadjusted and adjusted odds ratios (ORs) and their 95% confidence intervals (CIs) for predictive factors of mycobacteria growth indicator tube (MGIT) culture positivity using logistic regression (n = 218)*

Variable	Unadjusted OR (95% CI)	*P*-value	Adjusted OR (95% CI)	*P*-value
Sex				
Female	Reference		Reference	
Male	1.12 (0.56–2.26)	0.747	1.13 (0.54–2.34)	0.745
Age groups				
< 34 years	Reference		Reference	
≥ 34 years	0.64 (0.32–1.28)	0.207	0.73 (0.35–1.52)	0.324
BMI				
< 18.5 kg/m^2	Reference		Reference	
≥ 18.5 kg/m^2	1.14 (0.56–2.30)	0.724	1.13 (0.54–2.37)	0.749
ART status at baseline				
On ART	Reference		Reference	
Not on ART	3.62 (1.65–7.91)	0.001	3.75 (1.66–8.49)	0.002
CD4 count strata				
< 200 cells/μL	Reference		Reference	
200–350 cells/μL	1.24 (0.52–3.00)	0.628	1.46 (0.58–3.63)	0.421
> 350 cells/μL	1.35 (0.54–3.39)	0.522	1.68 (0.63–4.47)	0.299
CD4 count increase by 50 cells/μL	1.06 (0.97–1.16)	0.217	1.09 (0.99–1.211)	0.094

ART, antiretroviral therapy; BMI, body mass index; MGIT, mycobacteria growth indicator tube liquid culture.
*n = 218, because of adjusting: missing BMI value for one of the 219 LJ culture specimens.

Appendix 6: Unadjusted and adjusted hazard ratios (HRs) and their confidence intervals (CIs) for predictive factors of mycobacteria growth indicator tube (MGIT) culture time to culture positivity using Cox regression models (n = 218)*

	Unadjusted HR (95% CI)	*P*-value	Adjusted HR (95% CI)	*P*-value
Sex				
Female	Reference		Reference	
Male	1.10 (0.82–1.48)	0.525	1.12 (0.83–1.52)	0.452
Age groups				
< 34 years	Reference		Reference	
≥ 34 years	0.85 (0.63–1.14)	0.281	0.87 (0.64–1.18)	0.650
BMI				
< 18.5 kg/m^2	Reference		Reference	
≥ 18.5 kg/m^2	1.07 (0.79–1.45)	0.653	1.03 (0.76–1.40)	0.749
ART status at baseline				
On ART	Reference		Reference	
Not on ART	1.56 (1.01–2.41)	0.043	1.73 (1.10–2.70)	0.017
CD4 count strata				
< 200 cells/μL	Reference		Reference	
200–350 cells/μL	1.19 (0.83–1.72)	0.340	1.24 (0.86–1.79)	0.244
> 350 cells/μL	1.45 (1.00–2.11)	0.048	1.63 (1.11–2.40)	0.013
CD4 count increase by 50 cells/mL	1.07 (1.03–1.10)	< 0.001	1.08 (1.04–1.12)	< 0.001

ART, antiretroviral therapy; BMI, body mass index; MGIT, mycobacteria growth indicator tube liquid culture.
*n = 218, because of adjusting: missing BMI valuefor one of the 219 LJ culture specimens.

References

1 World Health Organisation. Global tuberculosis report 2016, 2016.

2 Getahun H, Harrington M, O'Brien R, Nunn P. Diagnosis of smear-negative pulmonary tuberculosis in people with HIV infection or AIDS in resource-constrained settings: informing urgent policy changes. *Lancet* 2007; **369**: 2042–2049.

3 Diedrich CR, Mattila JT, Klein E *et al.* Reactivation of latent tuberculosis in cynomolgus macaques infected with SIV is associated with early peripheral T cell depletion and not virus load. *PLoS ONE* 2010; **5**: e9611.

4 Lawn SD, Butera ST, Shinnick TM. Tuberculosis unleashed: the impact of human immunodeficiency virus infection on the host granulomatous response to *Mycobacterium tuberculosis*. *Microbes Infect* 2002; **4**: 635–646.

5 Geldmacher C, Ngwenyama N, Schuetz A *et al.* Preferential infection and depletion of *Mycobacterium tuberculosis*-specific CD4 T cells after HIV-1 infection. *J Exp Med* 2010; **207**: 2869–2881.

6 Klein NC, Duncanson FP, Lenox 3rd TH, Pitta A, Cohen SC, Wormser GP. Use of mycobacterial smears in the diagnosis of pulmonary tuberculosis in AIDS/ARC patients. *Chest* 1989; **95**: 1190–1192.

7 Hassim S, Shaw PA, Sangweni P *et al.* Detection of a substantial rate of multidrug-resistant tuberculosis in an HIV-infected population in South Africa by active monitoring of sputum samples. *Clin Infect Dis* 2010; **50**: 1053–1059.

8 Elliott AM, Namaambo K, Allen BW *et al.* Negative sputum smear results in HIV-positive patients with pulmonary tuberculosis in Lusaka, Zambia. *Tuber Lung Dis* 1993; **74**: 191–194.

9 *Policy Update: Xpert MTB/RIF Assay for the Diagnosis of Pulmonary and Extrapulmonary TB in Adults and Children*. Geneva, World Health Organisation, 2011.

10 Friedrich SO, Rachow A, Saathoff E *et al.* Assessment of the sensitivity and specificity of Xpert MTB/RIF assay as an early sputum biomarker of response to tuberculosis treatment. *Lancet Respir Med* 2013; **1**: 462–470.

11 Reid MJ, Shah NS. Approaches to tuberculosis screening and diagnosis in people with HIV in resource-limited settings. *Lancet Infect Dis* 2009; **9**: 173–184.

12 Sekaggya-Wiltshire C, Castelnuovo B, von Braun A *et al.* Cohort profile of a study on outcomes related to tuberculosis and antiretroviral drug concentrations in Uganda: design, methods and patient characteristics of the SOUTH study. *BMJ Open* 2017; **7**: e014679.

13 Diagnostic Standards and Classification of Tuberculosis in Adults and Children. This official statement of the American Thoracic Society and the Centers for Disease Control and Prevention was adopted by the ATS Board of Directors, July 1999. This statement was endorsed by the Council of the Infectious Disease Society of America, September 1999. *Am J Respir Crit Care Med* 2000;**161**(4 Pt 1):1376–1395.

14 Chien HP, Yu MC, Wu MH, Lin TP, Luh KT. Comparison of the BACTEC MGIT 960 with Löwenstein-Jensen medium for recovery of mycobacteria from clinical specimens. *Int J Tuberc Lung Dis* 2000; **4**: 866–870.

15 Chihota VN, Grant AD, Fielding K *et al.* Liquid vs. solid culture for tuberculosis: performance and cost in a resource-constrained setting. *Int J Tuberc Lung Dis* 2010; **14**: 1024–1031.

16 Post FA, Wood R, Pillay GP. Pulmonary tuberculosis in HIV infection: radiographic appearance is related to CD4+ T-lymphocyte count. *Tuber Lung Dis* 1995; **76**: 518–521.

17 Chengalroyen MD, Beukes GM, Gordhan BG *et al.* Detection and quantification of differentially culturable tubercle bacteria in sputum from patients with tuberculosis. *Am J Respir Crit Care Med* 2016; **194**: 1532–1540.

18 Diedrich CR, O'Hern J, Wilkinson RJ. HIV-1 and the *Mycobacterium tuberculosis* granuloma: a systematic review and meta-analysis. *Tuberculosis* 2016; **98**: 62–76.

19 Muyoyeta M, Schaap JA, De Haas P *et al.* Comparison of four culture systems for *Mycobacterium tuberculosis* in the Zambian National Reference Laboratory. *Int J Tuberc Lung Dis* 2009; **13**: 460–465.

20 Pfyffer GE, Welscher HM, Kissling P *et al.* Comparison of the Mycobacteria growth indicator tube (MGIT) with radiometric and solid culture for recovery of acid-fast bacilli. *J Clin Microbiol* 1997; **35**: 364–368.