

Early Microbiologic Markers of Pulmonary Tuberculosis Treatment Outcomes

Mandar Sudhir Paradkar^{1,2*}, Neeta Nitin Pradhan^{1,2*}, Subramanyam Balaji³, Sanjay Narayan Gaikwad⁴, Amol Chavan^{1,2}, Sujata Nagnath Dharmashale⁵, Tushar Sahasrabudhe⁶, Rahul Lokhande⁴, Sona Anil Deshmukh^{1,2}, Madhusudan Barthwal⁶, Sachin Atre^{2,6}, Swapnil Suresh Raskar^{1,2}, Trupti Uday Sawant⁶, Akshay N. Gupte^{8,9}, ArjunLal Kakrani⁷, Jonathan Golub⁸, Chandrasekaran Padmapriyadarsini³, Amita Gupta⁸, Nikhil Anil Gupte^{1,2,8}, and Vidya Mave^{1,2,8}

¹BJ Government Medical College-Johns Hopkins University Clinical Research Site, Pune, India; ²Johns Hopkins Center for Infectious Diseases in India, Pune, India; ³ICMR-National Institute for Research in Tuberculosis, Chennai, India; ⁴Department of Pulmonary Medicine and ⁵Department of Microbiology, BJ Government Medical College, Sassoon General Hospitals, Pune, India; ⁶Department of Respiratory Medicine and ⁷Department of Medicine, Dr. D.Y. Patil Medical College, Hospital & Research Centre, Pune, India; ⁸Division of Infectious Diseases, School of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland; ⁹School of Public Health, Boston University, Boston, Massachusetts

ORCID ID: 0000-0002-9696-3228 (N.N.P.).

Abstract

Rationale: Earlier biomarkers of pulmonary tuberculosis (PTB) treatment outcomes are critical to monitor shortened anti-TB treatment (ATT).

Objectives: To identify early microbiologic markers of unfavorable TB treatment outcomes.

Methods: We performed a subanalysis of 2 prospective TB cohort studies conducted from 2013 to 2019 in India. We included participants aged ≥ 18 years who initiated 6-month ATT for clinically or microbiologically diagnosed drug-sensitive PTB and completed at least one follow-up visit. Sputum specimens were subjected to a baseline Xpert *Mycobacterium tuberculosis*/rifampin (MTB/RIF) assay, acid-fast bacilli (AFB) microscopy and liquid and solid cultures, and serial AFB microscopy and liquid and solid cultures at weeks 2, 4, and 8. Poisson regression was used to assess the impact of available microbiologic markers (test positivity, smear grade, time to detection, and time to conversion) on a composite outcome of failure, recurrence, or death by 18 months after the end of treatment. Models were adjusted for age, sex, nutritional status, diabetes, smoking, alcohol consumption, and regimen type.

Results: Among 1,098 eligible cases, there were 251 (22%) adverse TB treatment outcomes: 127 (51%) treatment failures, 73 (29%) recurrences, and 51 (20%) deaths. The primary outcome was independently associated with the Xpert MTB/RIF assay (medium-positive adjusted incidence rate ratio [aIRR], 1.91; 95% confidence interval [CI], 1.07–3.40; high-positive aIRR, 2.51; 95% CI, 1.41–4.46), positive AFB smear (aIRR, 1.48; 95% CI, 1.06–2.06), and positive liquid culture (aIRR, 1.98; 95% CI, 1.21–3.23) at baseline; Week 2 positive liquid culture (aIRR, 1.47; 95% CI, 1.04–2.09); and Week 8 positive AFB smear (aIRR, 1.63; 95% CI, 1.06–2.50) and positive liquid culture (aIRR, 1.54; 95% CI, 1.07–2.22). There was no evidence of *Mycobacterium tuberculosis* growth in the *Mycobacterium* Growth Indicator Tube at Week 4 conferring a higher risk of adverse outcomes (aIRR, 1.25; 95% CI, 0.89–1.75).

Conclusions: Our analysis identifies Week 2 respiratory mycobacterial culture as the earliest microbiologic marker of unfavorable PTB treatment outcomes.

Keywords: PTB; treatment outcomes; microbiologic markers

(Received in original form February 15, 2023; accepted in final form September 26, 2023)

A complete list of Impact of Diabetes on TB Treatment Outcomes–Cohort for Tuberculosis Research by the Indo-U.S. Medical Partnership Regional Prospective Observational Research for Tuberculosis India study team members may be found in the online data supplement.

*Co-first authors

Ann Am Thorac Soc Vol 20, No 12, pp 1760–1768, Dec 2023

Copyright © 2023 by the American Thoracic Society

DOI: 10.1513/AnnalsATS.202302-144OC

Internet address: www.atsjournals.org

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (*Mtb*), affected an estimated 10.6 million people worldwide in 2021 and caused 1.6 million deaths (1). Optimizing prevention, early microbiologic detection, and prompt anti-TB treatment (ATT) are key components of the global TB control strategy (2–4). Despite these efforts, decreases in TB incidence and mortality have fallen short of global milestones to end the TB epidemic by 2030 (2, 4–7). The reasons are multifactorial. With respect to TB treatment, long and complicated traditional 6-month regimens are linked to poor patient compliance, which may contribute to increased adverse treatment outcomes, infectiousness, transmission, and development of drug resistance (8, 9).

To improve the real-world impact of ATT, multiple global clinical trials are investigating 3- to 4-month courses of potent antimycobacterial agents (10–14). If these are successful, shortened regimens could improve compliance and ease the logistics of directly observed therapy programs, which are inconsistently implemented (8, 9, 15). However, updated treatment monitoring strategies that reliably predict TB treatment outcomes are critical to advance this research (16).

For years, clinical trials and national TB programs have used acid-fast bacilli (AFB) microscopy and mycobacterial culture conversion at the end of the intensive phase

(Week 8 of ATT) to predict relapse-free TB cure (17, 18). These respiratory microbiologic markers leverage widely available technology and require minimally invasive specimens, and culture remains the gold standard for *Mtb* detection. However, a period of 6–8 weeks is required to confirm *Mtb* growth, rendering culture conversion at Week 8 an outdated marker for shortened regimens (18, 19). Furthermore, even though AFB microscopy provides rapid results, evidence suggests that neither marker may consistently predict TB treatment outcomes (20, 21).

Numerous blood-based biomarker discovery studies are under way, but none are ready for implementation, and data are lacking on respiratory microbiologic markers before Week 8. To address this data gap, we conducted a subanalysis among adult participants of 2 longitudinal TB cohort studies in India (22–24). The parent studies enrolled new clinically or microbiologically diagnosed drug-susceptible PTB cases and assessed factors associated with a composite unfavorable TB treatment outcome (failure, recurrence, or death). We aimed to evaluate all potential respiratory microbiologic markers (AFB smear, culture, and polymerase chain reaction–based tests assessed at baseline and serially during the intensive phase of ATT) of adverse treatment outcomes. Identifying an earlier marker of poor treatment outcome would define a primary endpoint for treatment-shortening

trials and provide a treatment monitoring strategy to potentially optimize outcomes (16, 19, 25).

Methods

The Cohort for TB Research by the Indo-U.S. Medical Partnership (CTRIUMPh) and the Impact of Diabetes on TB Treatment Outcomes (TBDM) study are prospective TB cohort studies conducted in western India between 2013 and 2019 at Byramjee Jeejeebhoy Government Medical College, Dr. D.Y. Patil University, and the National Institute for Research in Tuberculosis (22–24). The primary objective was to assess host and mycobacterial factors associated with a composite unfavorable TB treatment outcome (failure, recurrence, or death); this subanalysis aimed to assess all available respiratory microbiologic markers from baseline through Week 8 of ATT.

CTRIUMPh and TBDM followed similar protocols with respect to TB case recruitment, follow-up, and outcomes assessed. Both recruited patients with new clinically diagnosed (based on clinical or radiologic features) or microbiologically confirmed (Xpert MTB/RIF assay, AFB microscopy, or culture) drug-sensitive PTB; TBDM excluded people living with HIV. Participants initiated 6-month ATT (thrice-weekly directly observed therapy before 2017

Data in this manuscript were collected as part of the Regional Prospective Observational Research for Tuberculosis India Consortium. The Cohort for TB Research by the Indo-US Medical Partnership is supported by the Government of India Department of Biotechnology, Ministry of Science and Technology; the Indian Council of Medical Research; the National Institutes of Health; the National Institute of Allergy and Infectious Diseases; and the U.S. Office of AIDS Research; and distributed in part by CRDF Global (USB1-31147-XX-13). The Impact of Diabetes on TB Treatment Outcomes study was supported by National Institutes of Health grant 1R01A11097494-01A1. This work was also supported by the National Institutes of Health–funded Johns Hopkins Baltimore–Washington–India Clinical Trials Unit for National Institute of Allergy and Infectious Diseases Networks via CRDF Global (UM1A1069465 and DAA3-18-64774-1) and the Byramjee Jeejeebhoy Government Medical College/Johns Hopkins University HIV TB Program funded by the National Institutes of Health Fogarty International Center (D43TW009574; R.L.). The contents of this publication are solely the responsibility of the authors and do not represent the official views of the Government of India Department of Biotechnology, the Indian Council of Medical Research, the National Institutes of Health, or CRDF Global. Any mention of trade names or commercial projects or organizations does not imply endorsement by any of the sponsoring organizations. The sponsors had no role in the study design or writing of this report.

Author Contributions: M.S.P., N.N.P., N.A.G., and V.M. conceived the study. M.S.P. and N.N.P. contributed to study design, data collection, data analysis, data interpretation, and manuscript preparation. V.M. contributed to study design, data interpretation, and manuscript preparation. N.A.G. contributed to study design, data analysis and data interpretation. A.C. contributed to data analysis. A.G. and J.G. contributed to study design and data interpretation. C.P., S.N.D., S.N.G., M.B., T.S., A.K., R.L., S.A.D., S.A., S.S.R., and T.U.S. contributed to data collection and data interpretation. A.G., J.G., C.P., V.M., N.A.G., M.B., T.S., A.K., S.N.G., R.L., S.A.D., S.A., N.N.P., and M.S.P. are responsible for study oversight, management and co-ordination. All authors critically reviewed the manuscript for intellectual content and approved the final version of the report.

Correspondence and requests for reprints should be addressed to Neeta Nitin Pradhan, M.Sc., BJ Government Medical College–Johns Hopkins Clinical Research Site, c/o Mandar Sudhir Paradkar, 1st floor, Near ENT Department, Sassoon General Hospitals, Pune 411001, India. E-mail: neetapradhanpune@gmail.com.

This article has a data supplement, which is accessible from this issue's table of contents at www.atsjournals.org.

and daily regimens after 2017) within 1 week of enrollment. Follow-up during ATT was at Weeks 2, 4, 8, 20, and 24 (TBDM included additional visits at Weeks 6, 12, and 16) and continued for as long as 18 months after the end of treatment; a TB event visit was scheduled if treatment failure or recurrence was suspected. Relevant sociodemographic, epidemiologic, clinical, and laboratory data were collected at baseline and follow-up visits. The participant-reported adherence to ATT during the follow-up visits was evaluated in the CTRIUMPh cohort as the total number of doses missed among the total doses expected to be taken. At each visit, 2 sputum specimens were collected for microbiologic analysis.

This subanalysis was restricted to adult participants ≥ 18 years of age with at least one follow-up visit. We pooled data for all respiratory microbiologic markers assessed at baseline (Xpert MTB/RIF assay, AFB microscopy, and liquid and solid culture) and serially during the intensive phase of ATT (AFB microscopy and liquid and solid culture at Weeks 2, 4, and 8). These data included Xpert positivity grade (high, medium, or low positivity or negative) and cycle threshold; qualitative AFB smear, World Health Organization (WHO) smear grade (26), and time to conversion (TTC) from positive to negative AFB smear; and qualitative culture, colony count (solid culture only), time to detection of growth (TTD), and TTC from positive to negative culture.

Outcomes and Definitions

The primary outcome was a composite of treatment failure (*Mtb* growth on liquid or solid culture or clinical TB diagnosis from Week 20 to Week 24), recurrence (*Mtb* growth on liquid and/or solid culture or clinical TB diagnosis after successful ATT completion, defined as no TB symptoms and no microbiologic evidence of *Mtb* at Week 24), or all-cause mortality by 18 months after the end of treatment (23, 24, 27). If treatment failure or recurrence preceded death, the former was assigned as the treatment outcome. Mortality was further classified as TB-related or unlikely due to TB based on independent review by at least 2 clinician reviewers, and discordant cases were adjudicated by a third clinician. The risk factors known to be associated with the negative outcomes measured were baseline age, sex, body mass index (BMI) (28, 29), diabetes mellitus (DM), tobacco use, alcohol use, and treatment regimen type (daily or thrice weekly).

Laboratory Methods

One sputum specimen was directly analyzed using the Xpert MTB/RIF assay (Cepheid) to detect and quantify *Mtb* DNA (30) and Ziehl-Neelsen smear microscopy to detect and quantify AFB (26). The second specimen was decontaminated and homogenized using the N-acetyl-L-homocysteine sodium hydroxide method, and the sediment was inoculated on liquid *Mycobacterium* Growth Indicator Tube (MGIT) and solid Löwenstein-Jensen (LJ) culture media (the culture contamination rate did not exceed 6% at any study visit); the Global Laboratory Initiative protocol was followed for MGIT cultures, and LJ cultures were followed for 8 weeks before they were considered negative (26). Drug susceptibility testing for first-line anti-TB drugs was performed when a culture was positive at baseline or a TB event visit (31).

Statistical Analysis

The sample size for the study was adjusted for a 10% loss to follow-up at the design stage. The participants who were lost were considered as censored, and their follow-up data for as long as they participated in the study were used in the analysis. Participants lost to follow-up during TB treatment (incomplete treatment, two consecutive missed follow-up visits, or not reachable [22–24]) were censored for the duration of follow-up; those lost to follow-up after the end of treatment were included if a primary outcome was assigned. Covariates were summarized as proportions or medians with IQRs and compared by primary outcome status using the Fisher's exact test and Wilcoxon rank-sum test, respectively. The relative change in baseline TTD was calculated at Weeks 2, 4, and 8 as $(TTD_{\text{time point}} - TTD_{\text{baseline}}) / TTD_{\text{baseline}}$. TTC by AFB smear and culture was estimated using the Kaplan-Meier product limit estimator and compared using the log-rank test.

Poisson regression analysis was used to evaluate baseline and longitudinal markers associated with the primary outcome, and random-effects Poisson regression was used to evaluate the serial change in TTD. Models adjusted for measured confounders; Week 2 cultures included additional adjustment for initial bacterial burden (i.e., baseline AFB smear grade). Receiver operating characteristic (ROC) curves were used to assess the accuracy of baseline and early longitudinal markers (AFB smear and

culture at Weeks 2 and 4) as predictors of the primary outcome; areas under the ROC curve (AUCs) were evaluated for each marker. All adjusted models included variables that were known to have association with, or showed an increased risk of, unfavorable TB treatment outcomes.

The primary analysis was repeated with TB-related death substituted for all-cause mortality in the primary outcome definition. Additionally, we performed subgroup analyses restricted to: 1) MGIT culture-confirmed, isoniazid-sensitive *Mtb*; 2) microbiologically confirmed drug-sensitive PTB (positive AFB smear, Xpert MTB/RIF assay, or culture); and 3) MGIT culture-confirmed *Mtb* irrespective of other drug resistance status.

Statistical methods for these analyses are tabulated in Table E1 in the data supplement. All *P* values were 2-sided, and statistical significance was evaluated at the 0.05 α -level. Data were analyzed using Stata version 14.2 (StataCorp).

We performed predictive modeling at each predetermined follow-up time (Weeks 2, 4, 8, 12, 16, 20, and 24) after the initiation of treatment. In the modeling, each microbiological marker was examined independently to assess sensitivity and specificity of predicting an unfavorable TB treatment outcome. The original data of 1,098 participants were randomly divided into 2 parts: the first two thirds constituted the discovery cohort and the remaining third was the validation cohort. All models were adjusted for age, sex, alcohol use, BMI, daily versus thrice-weekly AKT, and lung cavitation.

Ethical Approval

The CTRIUMPh and TBDM studies were approved by the institutional review board or ethics committee at each participating institution: Johns Hopkins University (Baltimore, Maryland), Byramjee Jeejeebhoy Government Medical College (Pune, India), Dr. D.Y. Patil University (Pune, India), and the National Institute for Research in Tuberculosis (Chennai, India). All participants provided written informed consent.

Results

Study Population and Characteristics

Of 1,143 adult PTB cases enrolled in the parent studies, we excluded 45 for the following reasons: multidrug-resistant TB ($n = 24$), no follow-up data ($n = 20$), and no sputum

microbiology results available ($n = 1$). The remaining 1,098 (96%) were included in this analysis; 1,003 and 95 had microbiologically confirmed and clinically diagnosed PTB, respectively (Figure 1). Baseline median age was 35 years (IQR, 25–48 yr), the prevalence of DM was 32% (50% treated with metformin), the prevalence of HIV was 3%, and there were 251 (23%) primary outcomes, including 127 (51%) treatment failures, 73 (29%) TB recurrence events, and 51 (20%) deaths (35 considered TB-related). Compared with those with a favorable outcome, cases with the primary outcome were more likely to be in male participants (75% vs. 64%; $P = 0.002$), undernourished participants (69% vs. 52%; $P < 0.001$), ever-smokers (28% vs. 19%; $P = 0.004$), those who reported alcohol use (48% vs. 31%; $P < 0.0001$), and those who received intermittent ATT (90% vs. 80%; $P < 0.0001$), but were less likely in participants with DM (27% vs. 33%; $P = 0.01$) (Table 1). The overall participant-reported adherence assessed in one of our cohorts was $>91\%$ ($n = 433$).

Primary Outcome

Baseline respiratory microbiologic markers. Most participants had Xpert assay (93%), AFB

microscopy (99%), and liquid (95%) and solid (98%) culture data available at baseline (Figure 1). In multivariable analyses, each adjusted for baseline age, sex, BMI, DM, smoking, alcohol consumption, and regimen type (daily or thrice weekly), the primary outcome was associated separately with Xpert positivity grade (medium-positive adjusted incidence rate ratio [aIRR], 1.91; 95% confidence interval [CI], 1.07–3.40; high-positive aIRR, 2.51; 95% CI, 1.41–4.46), Xpert cycle threshold (aIRR, 0.96; 95% CI, 0.93–0.99), positive AFB smear (aIRR, 1.48; 95% CI, 1.06–2.06), and positive liquid culture (aIRR, 1.98, 95% CI, 1.21–3.23). Median TTD was 8.5 (IQR, 6.3–12.8) days on MGIT; a TTD <8 days was not associated with the primary outcome (Figure 2A and Table E2).

Longitudinal microbiologic markers.

Availability of AFB smear and culture data ranged from 86% to 95% at Week 2, from 79% to 87% at Week 4, and from 76% to 86% at Week 8 (Table E3). Multivariable analysis identified Week 2 MGIT culture as the only early marker associated with the primary outcome (aIRR, 1.47; 95% CI, 1.04–2.09). There was no evidence that *Mtb* growth at Week 4 conferred a higher risk (aIRR, 1.25; 95% CI, 0.89–1.75) (Figure 2B);

however, on univariate analysis, Week 4 MGIT culture was associated with adverse outcomes (adjusted IRR, 1.42; 95% CI, 1.06–1.89). At Week 8, the primary outcome was associated with a positive AFB smear result (aIRR, 1.61; 95% CI, 1.05–2.46), positive liquid culture (aIRR, 1.54; 95% CI, 1.07–2.22), and positive solid culture (aIRR, 1.75; 95% CI, 1.10–2.76) (Figure 2B).

Figure 3 shows serial TTD from baseline to Week 8 on liquid and solid culture media according to TB treatment outcome. The relative increase in baseline TTD on MGIT culture at Week 8 was associated with the primary outcome (aIRR, 0.71; 95% CI, 0.67–0.91) (Figure 2B). A 1-day increase in TTD reduced the risk of the primary outcome by 4% (aIRR, 0.96; 95% CI, 0.93–0.99) (see Table E3).

Overall, median TTCs were 3.3 weeks (IQR, 2.1–5.3) for AFB smear and 5.3 weeks (IQR, 3.3–8.4) and 3.7 weeks (IQR, 2.1–6) for liquid and solid culture, respectively. TTC was significantly longer in cases with the primary outcome than without for MGIT (5.7 wk vs. 5.3 wk; log-rank $P = 0.003$) and LJ culture (3.9 wk vs. 3.7 wk; log-rank $P = 0.03$). TTC was not associated with the primary outcome in multivariable models (see Table E3).

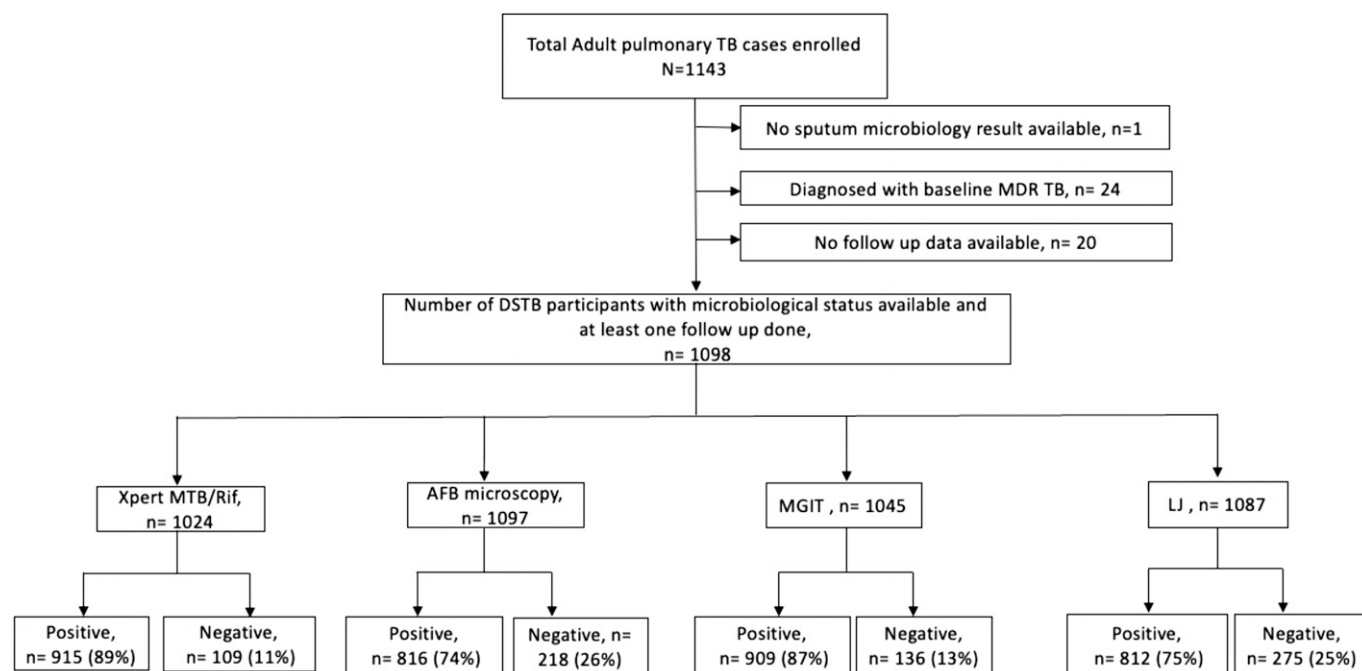


Figure 1. Study flow of adults with pulmonary tuberculosis enrolled in the parent cohort studies conducted in India from 2013 to 2019. The subanalysis included 1,098 participants with drug-sensitive microbiologically confirmed or clinically diagnosed pulmonary tuberculosis who completed at least one follow-up visit. Microbiologic confirmation was defined by an Xpert MTB/RIF assay, acid-fast bacilli microscopy, liquid *Mycobacterium* Growth Indicator Tube culture, or solid Löwenstein-Jensen culture positivity. AFB = acid-fast bacilli; DSTB = drug-sensitive tuberculosis; LJ = Löwenstein-Jensen; MDR = multidrug-resistant; MGIT = *Mycobacterium* Growth Indicator Tube; MTB = *Mycobacterium tuberculosis*; RIF = rifampicin; TB = tuberculosis.

Table 1. Baseline characteristics of adult pulmonary TB cases enrolled in 2 prospective cohort studies from 2013 to 2019 in India

Characteristic	Overall (N = 1,098)	Unfavorable Treatment Outcome*	
		Yes (n = 251)	No (n = 847)
Median age, yr (IQR)	35 (25–48)	36 (25–49)	34 (25–48)
Age group			
18–44 yr	730 (66)	164 (22)	566 (78)
≥45 yr	368 (33)	87 (24)	281 (76)
Sex			
Male	732 (67)	188 (75)	544 (64)
Female	366 (33)	63 (25)	303 (36)
Body mass index†			
<18.5 kg/m ²	615 (56)	172 (69)	443 (52)
18.5–25 kg/m ²	410 (37)	68 (27)	342 (40)
>25 kg/m ²	70 (6)	10 (14)	60 (7)
HIV status†			
Negative	1,050 (97)	239 (97)	811 (97)
Positive	29 (3)	8 (3)	21 (3)
Diabetes mellitus‡			
No	748 (68)	184 (73)	564 (67)
Yes	350 (32)	67 (27)	273 (33)
Ever smoker†			
No	829 (79)	175 (72)	654 (81)
Yes	218 (21)	67 (28)	151 (9)
Alcohol consumption†			
No	713 (65)	130 (52)	583 (69)
Yes	383 (35)	121 (48)	262 (31)
TB regimen			
Thrice weekly	902 (82)	227 (90)	675 (80)
Daily	196 (18)	24 (10)	172 (20)

Definition of abbreviation: TB = tuberculosis.

Data presented as n (%) unless otherwise indicated.

*Composite of treatment failure, recurrence or all-cause mortality by 18 mo after the end of treatment.

†Summarized among cases for which data were available: body mass index (n = 1,095), HIV status (n = 1,079), ever smoker (n = 1,047), and alcohol consumption (n = 1,096).

‡Defined as prior diagnosis, glycated hemoglobin ≥6.5%, fasting blood glucose ≥126 mg/dl, or random blood glucose ≥200 mg/dl.

ROC curves plotted for early longitudinal markers are shown in Figure 4. AUC values were similar between AFB smear and liquid culture at Week 2 (AFB smear, 0.68; 95% CI, 0.64–0.72; MGIT, 0.67; 95% CI, 0.63–0.71) and Week 4 (AFB smear, 0.68; 95% CI, 0.64–0.72; MGIT, 0.67; 95% CI, 0.63–0.71), and these were similar to the Week 8 AUC values. The sensitivity values of MGIT culture were 40.2% (95% CI, 33.9–46.7%) and 55.3% (95% CI, 47.9–62.6%) at Weeks 2 and 4, respectively (Table E4). Additionally, the ROC analysis for baseline covariates (including age, sex, BMI, alcohol consumption, DM, smoking, ATT regimen type, and cavitation on chest radiography) was performed without baseline AFB smear and MGIT (AUC, 0.596), with baseline smear (AUC, 0.627), and with baseline MGIT (AUC, 0.631), which demonstrated the added value of markers over and above baseline covariates.

Sensitivity Analysis

In repeat analysis restricted to MGIT culture-confirmed PTB cases (n = 909) (Tables E5 and E6), the aIRR between early MGIT culture (Weeks 2 and 4) and the primary outcome still showed an increased risk, but this did not reach statistical significance in this subset (aIRRs, 1.35 and 1.20; 95% CIs, 0.93–1.95 and 0.86–1.68, respectively) (see Table E6). Additionally, Week 2 liquid culture remained independently associated with the primary outcome after excluding 95 cases without microbiologically confirmed PTB (adjusted odds ratio, 1.57; 95% CI, 1.13–2.18) and 71 cases with isoniazid-resistant *Mtb* (adjusted odds ratio, 1.49; 95% CI, 1.07–2.08). All results were similar when TB-related death was substituted for all-cause mortality in the primary outcome definition (data not shown). The predictive modeling showed poor sensitivity, specificity, and AUC for all

baseline and early microbiological markers of adverse treatment outcomes. The Week 2 MGIT culture showed 45% (95% CI, 26–64%) sensitivity and 32% (95% CI, 23–42%) specificity, with an AUC of 61% (95% CI, 51–72%). The detailed prediction analysis is shown in Table E7.

Discussion

This subanalysis of respiratory microbiological markers from 2 prospective PTB cohorts in a setting of a high TB burden identified that early mycobacterial liquid culture is independently associated with unfavorable TB treatment outcomes. A positive culture identified as early as 2 weeks after ATT initiation was associated with a 1.5-fold increased risk of treatment failure, recurrence, or death. Week 4 culture showed a 25% higher relative association with the primary outcome, but this was not statistically significant in the multivariable analysis. However, the univariate analysis shows an association of Week 4 cultures with adverse treatment outcomes and is therefore likely to be a clinically relevant finding. Overall, our study adds to the evidence describing pretreatment respiratory microbiological markers as strong predictors of TB treatment outcomes and provides new data to support early respiratory culture during treatment as a potential marker of poor outcomes.

To our knowledge, our analysis is among the first to demonstrate the ability of early respiratory culture, as early as 2 weeks after treatment initiation, as a marker of TB treatment outcomes. Another study by Phillips and coworkers evaluated smear and culture results during treatment, but this assessment was performed for markers from Week 6 and onward (32, 33).

Key longitudinal observations support our main findings, including the consistent association between culture positivity and the primary outcome across the intensive phase of ATT and the inverse relationship between TTD and the primary outcome (34). Importantly, Week 2 and Week 4 liquid culture demonstrate high AUCs in ROC analysis, indicating their potential as independent markers of WHO-defined unfavorable outcomes. Moreover, the risk of adverse outcomes remained unchanged in the sensitivity analysis. However, the prediction modeling analysis showed poor sensitivity and specificity for all models,

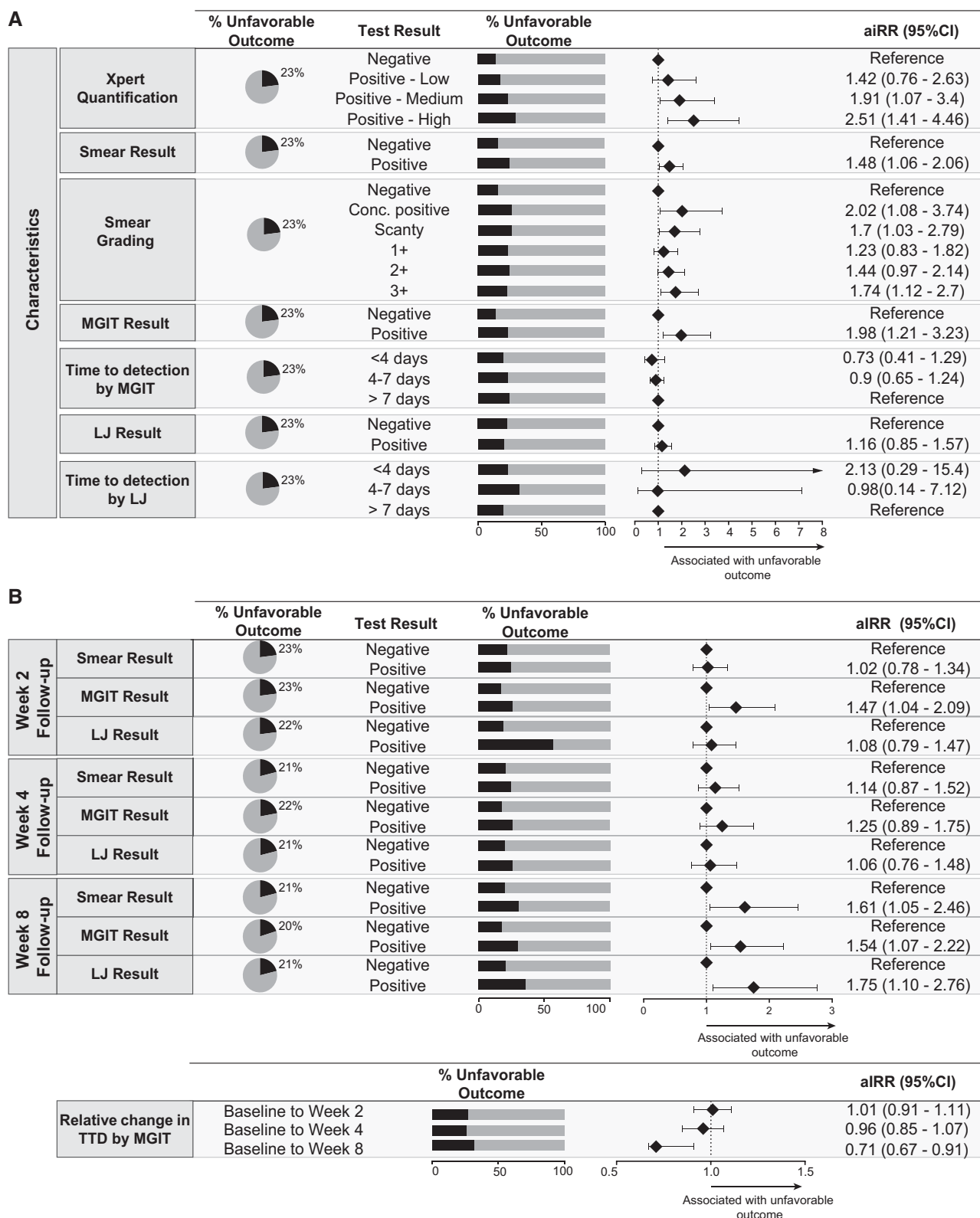


Figure 2. (A) Forest plot summarizing associations between baseline respiratory microbiologic markers and the composite unfavorable tuberculosis (TB) treatment outcome. Independent predictors were baseline Xpert MTB/RIF assay, acid-fast bacilli smear, and liquid MGIT culture positivity. Results are presented as adjusted incidence rate ratios from Poisson regression models adjusted for baseline age, sex, body mass index, diabetes, smoking, alcohol consumption, and regimen type (daily or thrice-weekly anti-TB treatment). (B) Forest plot summarizing

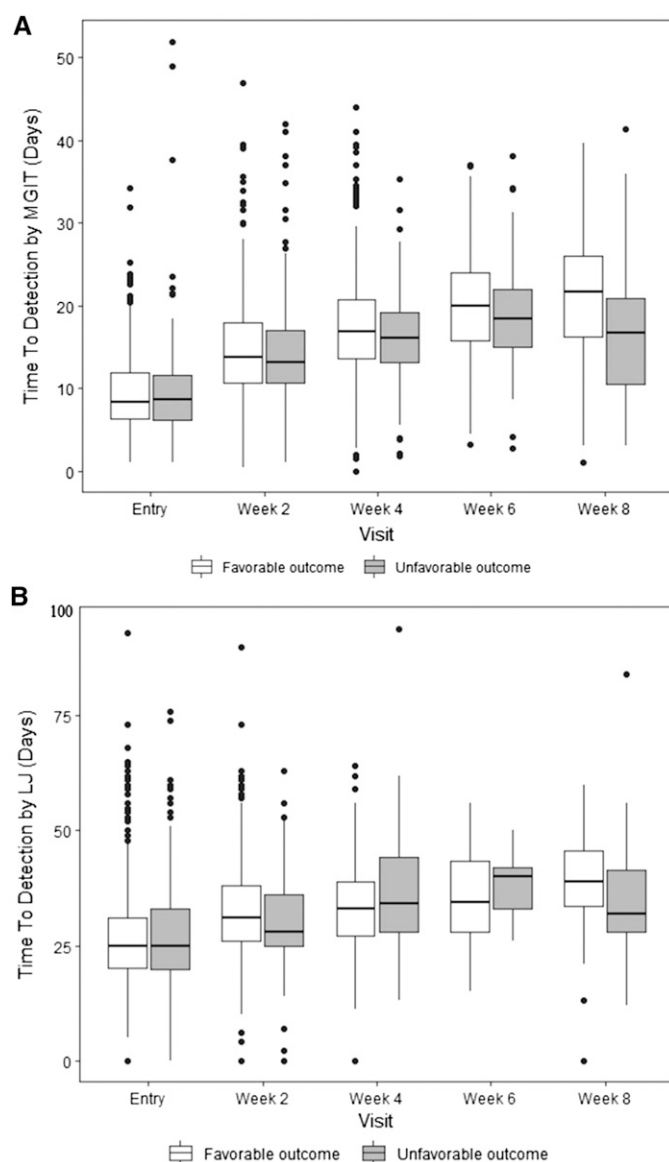


Figure 3. Longitudinal time to detection of growth of *Mycobacterium tuberculosis* on (A) liquid MGIT and (B) solid LJ culture media. Box plots summarize time to detection of growth at each time point from baseline to the end of the intensive phase of treatment according to tuberculosis treatment outcome. LJ = Löwenstein-Jensen; MGIT = *Mycobacterium* Growth Indicator Tube.

particularly for the early markers of interest. Taken together, these analyses imply that the presence of certain markers indicates an increased risk of adverse treatment outcomes; however, these

markers cannot be used as predictors of those outcomes.

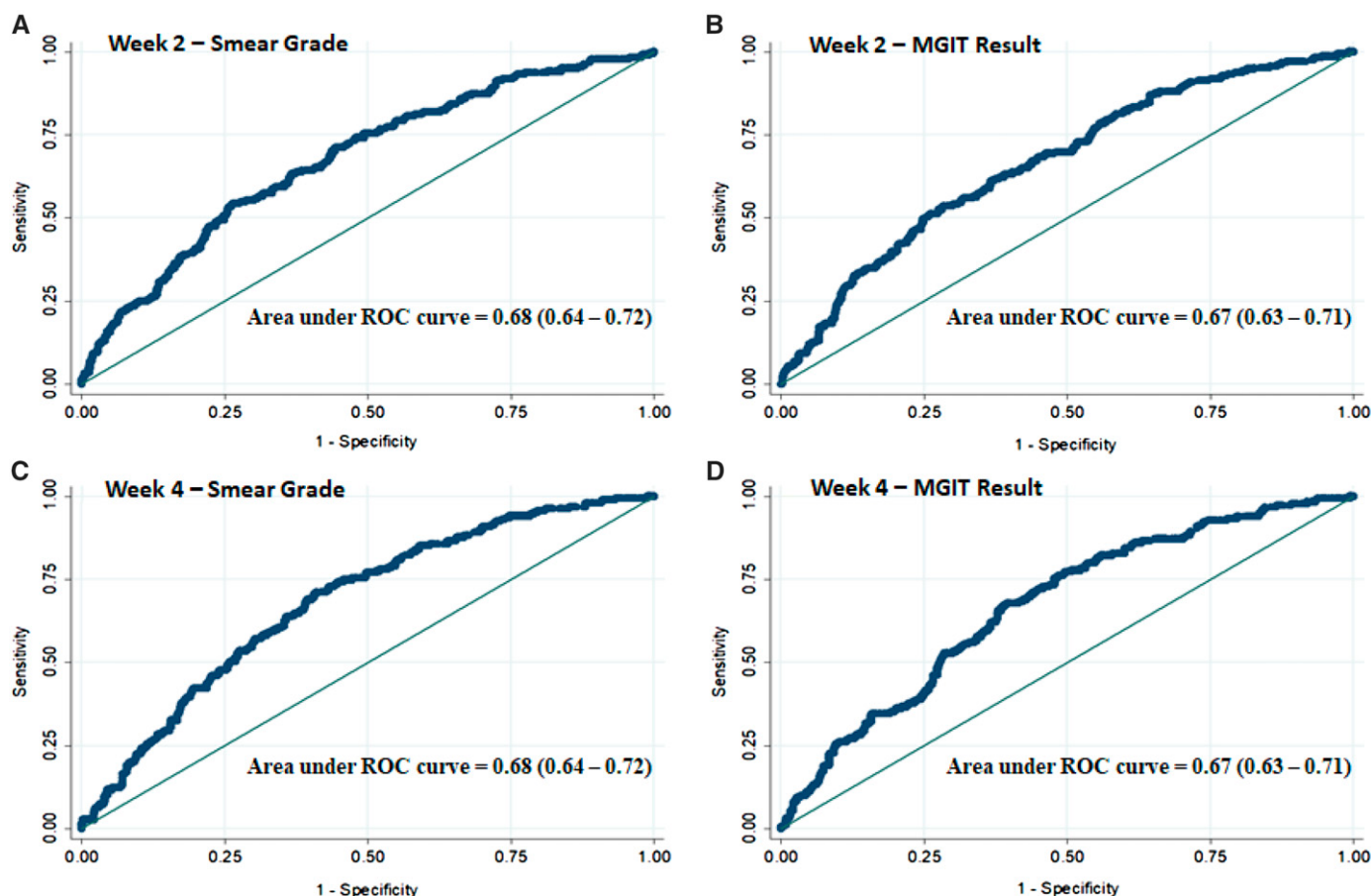
Finally, our study corroborates existing evidence that baseline respiratory microbiologic markers are strong predictors

of TB treatment outcomes (17, 35, 36). Consistent with previous reports, the Xpert MTB/RIF cycle threshold was associated with TB treatment outcome in our study. Consequently, we also found that moderate and high Xpert positivity independently predicted the primary outcome, reflecting the significance of a high initial bacterial load. These findings provide evidence that pretreatment Xpert positivity grading may be useful to identify patients at high risk in whom closer monitoring is warranted during TB treatment, particularly in settings in which mycobacterial culture analysis is not routinely available (17, 37).

A major strength of our study was the availability of biweekly AFB smear and culture data across 2 multisite PTB cohorts in the country with the world's largest absolute TB burden. The parent studies did have minor differences with respect to sample size and schedule of evaluations (23, 24). In particular, Week 6 AFB smear and culture data were available for only the TBDM cohort. Additionally, some risk of bias may exist as a result of the longitudinal cohort study design, as data were censored for loss to follow-up and/or unavailable microbiological data. Despite these limitations, CTRIUMPH and TBDM had large sample sizes; apart from Week 6, all evaluations of microbiologic markers during the intensive phase were similar, and both studies defined endpoints according to WHO guidelines.

In conclusion, this comprehensive analysis of respiratory microbiologic markers during the intensive phase of ATT suggests that liquid mycobacterial culture at Week 2 may be the earliest biomarker of PTB treatment outcomes. Although several weeks may be required to detect the growth of *Mtb*, collectively, these findings suggest that early respiratory culture may warrant consideration as a marker of adverse TB treatment outcomes until a more optimal biomarker emerges. Defining an early microbiologic marker of adverse treatment outcomes may help advance scientific efforts to optimize ATT by shortening TB treatment duration (38–40). In addition, our findings may inform

Figure 2. (Continued). associations between longitudinal respiratory microbiologic markers and the composite unfavorable TB treatment outcome. Independent predictors were Week 2 liquid culture positivity, Week 8 acid-fast bacilli smear and liquid and solid Löwenstein-Jensen culture positivity, and change in baseline time to detection on liquid culture at Week 8. Results are presented as adjusted incidence rate ratios from Poisson regression models adjusted for baseline age, sex, body mass index, diabetes, smoking, alcohol consumption, and regimen type (daily or thrice-weekly anti-TB treatment). aIRR = adjusted incidence rate ratio; CI = confidence interval; LJ = Löwenstein-Jensen; MGIT = *Mycobacterium* Growth Indicator Tube; MTB = *Mycobacterium tuberculosis*; RIF = rifampicin.



Each model adjusted for - Baseline MGIT TTD, Baseline Smear grade, Age, Sex, BMI, Diabetics, Ever-smoker, Alcohol and Regimen type.

Figure 4. ROC curves illustrate similar performance among Week 2 (A and B) and Week 4 (C and D) acid-fast bacilli smear microscopy and liquid MGIT cultures as markers of the composite unfavorable tuberculosis treatment outcome. MGIT = *Mycobacterium* Growth Indicator Tube; ROC = receiver operating characteristic.

national and international TB treatment monitoring strategies to identify individuals at risk for unfavorable outcomes and design appropriate intervention (4, 5). Further studies are warranted to confirm our findings, including evaluation among patients receiving shortened TB treatment regimens. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

Acknowledgment: The authors thank the study participants and the National TB Elimination Programme staff and authorities, including the state TB officer (Dr. Sunita Golhait, Maharashtra), city TB officers

(Dr. Balasaheb Hodgar, Pimpri Chinchwad Municipal Corporation; Dr. Prashant Bothe, Pune Municipal Corporation), and district TB officers (Dr. Sanjay Darade, Dr. Sunil Pote, Pune Rural). For a full list of the TBDM-Cohort for Tuberculosis Research by the Indo-US Medical Partnership Regional Prospective Observational Research for Tuberculosis India study team, see online data supplement.

References

- 1 Bagchi S. WHO's global tuberculosis report 2022. *Lancet Microbe* 2023; 4:e20.
- 2 World Health Organization. Global tuberculosis report 2021. Geneva, Switzerland: World Health Organization; 2021.
- 3 World Health Organization. WHO consolidated guidelines on tuberculosis. Module 3: diagnosis: rapid diagnostics for tuberculosis detection. Geneva, Switzerland: World Health Organization; 2020.
- 4 Central TB Division, Directorate General of Health Services. Revised National Tuberculosis Control Programme: national strategic plan for TB elimination, 2017–2025. New Delhi, India: Ministry of Health with Family Welfare; 2017 [accessed 2021 Nov 23]. Available from: <https://tbcindia.gov.in/WriteReadData/NSP%20Draft%2020.02.2017%201.pdf>.
- 5 Central Tuberculosis Division, Government of India. National Strategic Plan to End TB in India 2020-25 v6 26.05.2020 (draft copy). 2017 [accessed 2021 Nov 23]. Available from: <https://tbcindia.gov.in/index1.php?lang=1&level=2&sublinkid=5507&lid=3528>.
- 6 Stop TB Initiative. The paradigm shift: global plan to end TB: 2018–2022. Geneva, Switzerland: Stop TB Initiative; 2019 [accessed 2022 Jan 5]. Available from: <https://www.stoptb.org/about/global-plan-to-end-tb-2018-2022>.
- 7 Zumla A, Chakaya J, Khan M, Fatima R, Wejse C, Al-Abri S, *et al*. World Tuberculosis Day 2021 theme—"the clock is ticking"—and the world is

- running out of time to deliver the United Nations General Assembly commitments to end TB due to the COVID-19 pandemic. *Int J Infect Dis* 2021;113(suppl 1):S1–S6.
- 8 Kendall EA, Malhotra S, Cook-Scalise S, Denkiner CM, Dowdy DW. Estimating the impact of a novel drug regimen for treatment of tuberculosis: a modeling analysis of projected patient outcomes and epidemiological considerations. *BMC Infect Dis* 2019;19:794.
 - 9 Dorman SE, Nahid P, Kurbatova EV, Phillips PPJ, Bryant K, Dooley KE, *et al.*; AIDS Clinical Trials Group; Tuberculosis Trials Consortium. Four-month rifapentine regimens with or without moxifloxacin for tuberculosis. *N Engl J Med* 2021;384:1705–1718.
 - 10 Silva DR, Mello FCQ, Migliori GB. Shortened tuberculosis treatment regimens: what is new? *J Bras Pneumol* 2020;46:e20200009.
 - 11 Dorman SE, Nahid P, Kurbatova EV, Goldberg SV, Bozeman L, Burman WJ, *et al.* High-dose rifapentine with or without moxifloxacin for shortening treatment of pulmonary tuberculosis: study protocol for TBTC study 31/ACTG A5349 phase 3 clinical trial. *Contemp Clin Trials* 2020;90:105938.
 - 12 Imperial MZ, Nahid P, Phillips PPJ, Davies GR, Fielding K, Hanna D, *et al.* A patient-level pooled analysis of treatment-shortening regimens for drug-susceptible pulmonary tuberculosis. *Nat Med* 2018;24:1708–1715.
 - 13 Lee M, Mok J, Kim DK, Shim TS, Koh WJ, Jeon D, *et al.* Delamanid, linezolid, levofloxacin, and pyrazinamide for the treatment of patients with fluoroquinolone-sensitive multidrug-resistant tuberculosis (Treatment Shortening of MDR-TB Using Existing and New Drugs, MDR-END): study protocol for a phase II/III, multicenter, randomized, open-label clinical trial. *Trials* 2019;20:57.
 - 14 Chabala CAO, Turkova A, Thomason MJ, Wobudeya E, Hissar S, Mave V, *et al.* Shorter treatment for minimal tuberculosis (TB) in children (SHINE): a study protocol for a randomized controlled trial. *Trials* 2018;19:23.
 - 15 Out AA. Is the directly observed therapy short course (DOTS) an effective strategy for tuberculosis control in a developing country? *Asian Pac J Trop Dis* 2013;3:227–231.
 - 16 Günther G, Heyckendorf J, Zellweger JP, Reimann M, Claassens M, Chesov D, *et al.* Defining outcomes of tuberculosis (treatment): from the past to the future. *Respiration* 2021;100:843–852.
 - 17 Shenai S, Ronacher K, Malherbe S, Stanley K, Kriel M, Winter J, *et al.* Bacterial loads measured by the Xpert MTB/RIF assay as markers of culture conversion and bacteriological cure in pulmonary TB. *PLoS One* 2016;11:e0160062.
 - 18 Phillips PPJ, Fielding K, Nunn AJ. An evaluation of culture results during treatment for tuberculosis as surrogate endpoints for treatment failure and relapse. *PLoS One* 2013;8:e63840.
 - 19 Goletti D, Lindestam Arlehamn CS, Scriba TJ, Anthony R, Cirillo DM, Alonzi T, *et al.* Can we predict tuberculosis cure? What tools are available? *Eur Respir J* 2018;52:1801089.
 - 20 Su WJ, Feng JY, Chiu YC, Huang SF, Lee YC. Role of 2-month sputum smears in predicting culture conversion in pulmonary tuberculosis. *Eur Respir J* 2011;37:376–383.
 - 21 Olaru ID, Heyckendorf J, Grossmann S, Lange C. Time to culture positivity and sputum smear microscopy during tuberculosis therapy. *PLoS One* 2014;9:e106075.
 - 22 Deshmukh S, Atre S, Chavan A, Raskar S, Sawant T, Mave V, *et al.* Assessment of the Xpert assay among adult pulmonary tuberculosis suspects with and without diabetes mellitus. *Int J Tuberc Lung Dis* 2020;24:113–117.
 - 23 Gupte A, Padmapriyadarsini C, Mave V, Kadam D, Suryavanshi N, Shivakumar SV, *et al.*; CTRIUMPH Study Team. Cohort for Tuberculosis Research by the Indo-US Medical Partnership (CTRIUMPH): protocol for a multicentric prospective observational study. *BMJ Open* 2016;6:e010542.
 - 24 Mave VAO, Gaikwad S, Barthwal M, Chandanwale A, Lokhande R, Kadam D, *et al.* Diabetes mellitus and tuberculosis treatment outcomes in Pune, India. *Open Forum Infect Dis* 2021;8:ofab097.
 - 25 Sivakumaran D, Jennum S, Vaz M, Selvam S, Ottenhoff THM, Haks MC, *et al.* Combining host-derived biomarkers with patient characteristics improves signature performance in predicting tuberculosis treatment outcomes. *Commun Biol* 2020;3:359.
 - 26 Lumb R, Van Deun A, Bastian I, Fitzgerald M. Laboratory diagnosis of tuberculosis by sputum microscopy. In: Lumb R, Van Deun A, Bastian I, Fitzgerald M, editors. The handbook. IAdelaide, South Australia: SA Pathology; 2013 [accessed 2023 Oct 8]. Available from: <https://theunion.org/technical-publications/laboratory-diagnosis-of-tuberculosis-by-sputum-microscopy-the-handbook>.
 - 27 World Health Organization. Standards for TB care in India. Geneva, Switzerland: World Health Organization, Country Office for India; 2014 [accessed 2019 Feb 22]. Available from: <https://tbcindia.gov.in/showfile.php?lid=3061>.
 - 28 U.S. Centers for Disease Control and Prevention. Body mass index. Atlanta, GA: Centers for Disease Control and Prevention; 2020 [accessed 2020 Jun 14]. Available from: https://www.cdc.gov/healthyweight/assessing/bmi/adult_bmi/index.html.
 - 29 Nuttall FQ. Body mass index: obesity, BMI, and health: a critical review. *Nutr Today* 2015;50:117–128.
 - 30 Steingart KR, Schiller I, Home DJ, Pai M, Boehme CC, Dendukuri N. Xpert MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults. *Cochrane Database Syst Rev* 2014;2014:CD009593.
 - 31 Bergmann JS, Fish G, Woods GL. Evaluation of the BBL MGIT (mycobacterial growth indicator tube) AST SIRE system for antimycobacterial susceptibility testing of *Mycobacterium tuberculosis* to 4 primary antituberculous drugs. *Arch Pathol Lab Med* 2000;124:82–86.
 - 32 Calderwood CJ, Wilson JP, Fielding KL, Harris RC, Karat AS, Mansukhani R, *et al.* Dynamics of sputum conversion during effective tuberculosis treatment: a systematic review and meta-analysis. *PLoS Med* 2021;18:e1003566.
 - 33 Phillips PPJ, Mendel CM, Burger DA, Crook AM, Nunn AJ, Dawson R, *et al.* Limited role of culture conversion for decision-making in individual patient care and for advancing novel regimens to confirmatory clinical trials. *BMC Med* 2016;14:19. [Published correction appears in *BMC Med* 14:36.]
 - 34 Vongthilath-Moeung R, Poncet A, Renzi G, Schrenzel J, Janssens JP. Time to detection of growth for *Mycobacterium tuberculosis* in a low incidence area. *Front Cell Infect Microbiol* 2021;11:704169.
 - 35 Pires MM, Pereira GR, Barbosa MS, Dias NJD, Secchi C, Hoff JS, *et al.* Association of Xpert MTB/RIF cycle threshold values with tuberculosis treatment outcomes. *Lung* 2020;198:985–989.
 - 36 Namugenyi J, Musaaizi J, Katamba A, Kalyango J, Sendaula E, Kambugu A, *et al.* Baseline Xpert MTB/RIF CT values predict sputum conversion during the intensive phase of anti-TB treatment in HIV infected patients in Kampala, Uganda: a retrospective study. *BMC Infect Dis* 2021;21:513.
 - 37 Miotto P, Bigoni S, Migliori GB, Matteelli A, Cirillo DM. Early tuberculosis treatment monitoring by Xpert(R) MTB/RIF. *Eur Respir J* 2012;39:1269–1271.
 - 38 Chen RY, Via LE, Dodd LE, Walz G, Malherbe ST, Loxton AG, *et al.* Using biomarkers to predict TB treatment duration (Predict TB): a prospective, randomized, noninferiority, treatment shortening clinical trial. *Gates Open Res* 2017;1:9.
 - 39 Lienhardt C, Nahid P. Advances in clinical trial design for development of new TB treatments: a call for innovation. *PLoS Med*. 2019;16:e1002769.
 - 40 Dorman SE, Nahid PAO, Kurbatova EV, Phillips PPJ, Bryant K, Dooley KE, *et al.*; AIDS Clinical Trials Group; Tuberculosis Trials Consortium. Four-month rifapentine regimens with or without moxifloxacin for tuberculosis. *N Engl J Med* 2021;384:1705–1718.