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Evaluation of Time to Detection of *Mycobacterium tuberculosis* in Broth Culture as a Determinant for End Points in Treatment Trials▽†

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Time to detection of *Mycobacterium tuberculosis* in broth culture was examined for utility as a treatment efficacy end point. Of 146 patients in a phase IIB trial, a decreased mean time to detection was found in 5 with treatment failure. Time to detection in an analysis-of-covariance model was associated with lung cavities, less intensive treatment, and differences in the bactericidal effects of treatment regimens.

Development of new treatments for tuberculosis is hampered by the lack of an accurate surrogate end point and the high degree of efficacy of current 6-month regimens. Sputum culture status after 2 months of therapy, a binary test, is widely used for phase IIB trials but has only moderate accuracy for predicting failure/relapse (12) and requires large sample sizes (4, 8). Changes in the number of colonies found in dilutions of sputum applied to solid medium is an end point that has been used to assess activities of single drugs and doses in phase IIA (early-bactericidal-activity) studies (10) and has also been suggested as an end point for phase IIB trials (15). Though promising, quantitative culture on solid medium involves prolonged sputum collections and intensive laboratory techniques and has been difficult to standardize at multiple sites. Time to detection in broth culture (TTD) is a potential end point that has a good correlation with quantitative culture on solid medium (11, 13). An initial small study had suggested a correlation between a shorter time to detection (an indication of higher numbers of viable bacilli) and poor treatment outcomes (9). In this study, TTD was evaluated as a marker of regimen potency. Preliminary results have been reported elsewhere (16).

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MATERIALS AND METHODS

Experimental design. Patients for this study were drawn from Tuberculosis Trials Consortium (TBTC) Study 27, a randomized phase IIB trial that compared moxifloxacin to ethambutol and treatment 5 versus 3 times per week during the initial 2 months of treatment among patients with smear-positive, pulmonary tuberculosis (4). After completion of intensive-phase therapy, patients received standard continuation-phase treatment (3). The primary end point of the treatment trial was the dichotomous end point of 2-month culture status.

For this *post hoc* study, TTD data were available from two African sites. Of 176 African patients in study 27, TTD data were available for 163 (93%). Treatment failure occurred in six of these patients, and although the patients were not routinely followed after therapy, one with a relapse was identified 5 weeks after treatment.

Specimen collection and laboratory procedures. Spontaneously expectorated (spot) sputum samples were cultured every 2 weeks during the first 2 months and monthly thereafter. Standard culture methods were used but differed somewhat between sites. In Uganda, samples were processed using a final concentration of 1% sodium hydroxide, inoculated into Bactec 12B bottles, and monitored with the Bactec 460 system. In South Africa, a final concentration of 1.25% sodium hydroxide, MGIT broth, and the Bactec MGIT 960 instrument were used. Cultures were monitored daily at both sites, with the exception that after 2 weeks, the Bactec 460 protocol used daily monitoring for cultures with a Bactec growth index (GI) of ≥30 and once weekly for cultures with a GI of <30 (no. 444824; Bactec TB System Product & Procedure Manual; Becton Dickinson). TTD was calculated as the number of days between inoculation and detection of a positive culture by the Bactec instruments; the results for specimens not positive by the end of the monitored interval were recorded as 42 days. Most patients had two sputum specimens cultured at each time point. The result of the first culture for each patient and time point was used, with two exceptions. If the first culture was contaminated with bacteria or grew nontuberculosis mycobacteria (NTM), the second culture was used.

Data analysis. The primary objective of this analysis was to compare microbiological end points for predicting treatment failure by TTD and by 2-month culture status. We used test cutoffs for the TTD end point that identified all patients with treatment failure (sensitivity, 100%). The accuracy of the test was calculated as the number of true-positive patients plus the number of true-

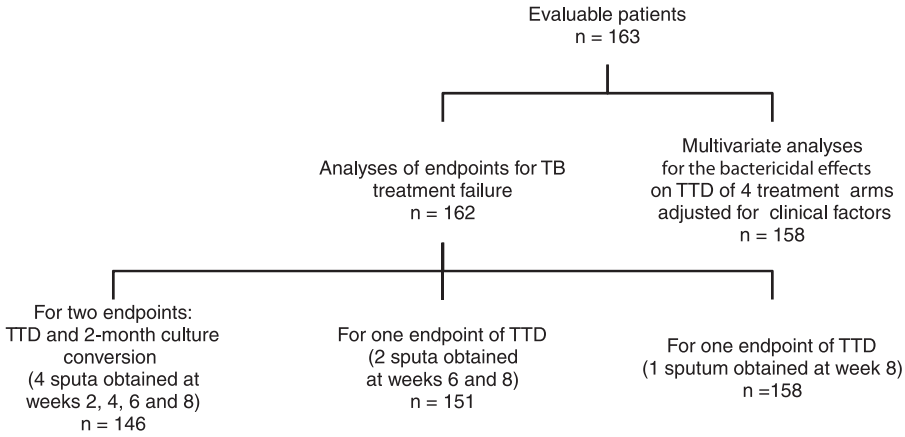

FIG. 1. Flow of patients into the end point study of TTD.

negative patients for treatment failure with tuberculosis divided by the number of true-positive, false-positive, true-negative, plus false-negative patients.

Data analyses were performed using the SAS program (version 9.1) for the mixed-model analysis of covariance (ANCOVA) and generalized estimating equations (GEE), the StatXact program for nonparametric tests, and the NCSS 2007 program for other tests. For binary data, differences between groups were determined using Fisher's exact test or Pearson's chi-square statistic, and for nonparametric analyses, the Kruskal-Wallis test by the Monte Carlo method was used. Arithmetic means (see Table 1 and Fig. 2) and adjusted means with the ANCOVA model (see Table 4 and Fig. 3) were used where applicable. Differences between groups were considered statistically significant at the level of a P value of <0.05.

We also evaluated the effect of TTD with clinical and radiographic factors and the randomized study arms. Initially, univariate analysis of each factor, adjusted for missing data (TTD at weeks 2, 4, 6, and 8), was performed. A mixed-model ANCOVA with repeated measures was then evaluated. In the final model, main effects were cavity (absent, <4 cm in total size, ≥ 4 cm in total size), HIV infection status (infected or not infected), drug (moxifloxacin versus ethambutol), frequency of study treatment (5 versus 3 times per week), site (Uganda or South Africa), baseline TTD, the repeated measure (2, 4, 6, and 8 weeks), and the two-way interactions of the main effects (with the exceptions of study site and the baseline culture). Because of the model's complexity, a parsimonious approach with elimination of nonsignificant higher-level interactions was adopted. Of the 163 patients, 158 patients had complete data for independent factors and baseline TTD (Fig. 1). Homogeneity of variance was supported by plotting of residuals versus predicted values. Adjusted means refers to means adjusted for all model effects, unequal sample sizes, and missing data among the repeated measures. The model takes into account the intrasubject correlation of repeated sampling; serial correlation was estimated to be small, with R^2 being <0.05. The significance of pairwise comparisons is reported using Fisher's least significant difference (LSD).

We also performed an alternate GEE analysis with cultures identified as

TABLE 1. Comparison of demographic, clinical, and radiographic characteristics of patients in study 27 who were part or not part of this substudy

Characteristic	Study 27		P value
	Patients in this study[a]	Patients not in this study[b]	
Median (IQR[c]) age (yr)	29 (23.3–36.0)	36 (26.3–49.8)	<0.001
No. of patients male/total no. of patients (% male)	99/163 (61)	87/115 (76)	0.01
Race/ethnicity[d]			
African	163/163 (100)	13/115 (11)	<0.001
Black	162/163 (99)	46/115 (49)	<0.001
Hispanic	0/163 (0)	39/115 (34)	<0.001
White	0/163 (0)	45/115 (39)	<0.001
Asian	1/163 (1)	19/115 (17)	<0.001
Median (IQR) wt (kg)	52.4 (47.1–57.1)	58.6 (53.9–66.5)	<0.001
Chest radiographic features[d]			
Cavitation	133/163 (82)	73/115 (64)	<0.001
Bilateral involvement	103/161 (64)	56/102 (55)	0.16
HIV infected[d]	43/163 (26)	18/114 (16)	0.04
Median (IQR) CD4 cell count (μl^{-1}) in HIV-infected patients	200 (110–286)	189 (85–335)	0.97
Patients for whom moxifloxacin was used as study drug[d]	81/163 (50)	58/115 (50)	1.00

[a] $n = 163$ (59%).
[b] $n = 115$ (41%).
[c] IQR, interquartile range.
[d] Data represent number of patients with the indicated characteristic/total number of patients in group (percent).

TABLE 2. Clinical, microbiologic, radiographic, and molecular data from six patients with treatment failure (cases 1 to 6) and another with early relapse (case 7)

Case no. (outcome)	Clinical status at failure or relapse[a]	HIV infection status at baseline	Culture medium	TTD (in days) at:				Status at:						CXR vs baseline[b]	DNA fingerprint of baseline vs failure isolate[c]
				Wk 2	Wk 4	Wk 6	Wk 8	Mo 3	Mo 4	Mo 5	Mo 6	Mo 7	Mo 8	Mo 9	
1 (failure)	Persistent cough	Positive	BACTEC	13	20	20	19	NG[d]	*M. tuberculosis*[e]	NG	NG	NG	NG		Δ mo 10	Match RFLP 13 band (IS*6110*)
2 (failure)	Persistent cough	Positive	BACTEC	9	14	18	19	NG	*M. tuberculosis*[e]	NG	NG	NG	NG	NG	Δ mo 10	Match RFLP 10 band (IS*6110*)
3 (failure)	Persistent cough	Positive	BACTEC	16	21	21	23	*M. tuberculosis*	*M. tuberculosis*[e]	NG	NG	NG	NG	NG	Δ mo 7	Match RFLP 17 band (IS*6110*)
4 (failure)	Persistent cough	Positive	BACTEC	10	11	16	23	*M. tuberculosis*	*M. tuberculosis*[e]	NG	NG	NG	NG	NG	None	Match RFLP 14 band (IS*6110*)
5 (failure)	Persistent cough	Negative	BACTEC	16	*M. tuberculosis*[h]	15	16	*M. tuberculosis*	*M. tuberculosis*[e]	NG	NG	NG	NG	NG	None	No isolate available
6 (failure)	Cough returned	Negative[f]	MGIT	10	14	14	14	NG	NG				*M. tuberculosis*[e]		▽ mo 9	Match DST and spoligotype
7 (relapse)	Fever for 3 wk[g]	Negative	BACTEC	14	14	20	27	NG	NG	NG	NG	NG	NG	*M. tuberculosis*[e]	▽ mo 12	Match DST result, spoligotype, and MIRU type

[a] The chest X ray at the baseline showed cavities in all patients.
[b] Δ, chest X-ray (CXR) improved compared to that at baseline; ▽, chest X ray worsened compared to that at baseline; mo, month of chest X ray.
[c] Three methods of strain typing were used: spoligotyping, mycobacterial interspersed repetitive-unit–variable-number tandem-repeat (MIRU) genotyping, and restriction fragment length polymorphism (RFLP) genotyping of IS*6110*, in addition to matching drug sensitivity testing (DST). Where all methods were tested and matched, only the restriction fragment length polymorphism result is indicated. Where not all methods were tested, results are given for the methods tested.
[d] NG, no growth.
[e] *M. tuberculosis* indicates which specimen shows treatment failure or relapse (case 7) and was compared with baseline specimen.
[f] HIV seroconversion found by viral load determination at month 7.
[g] Fever for 3 weeks, at late month 9 visit, 5 weeks after end of therapy.
[h] For case 5 at week 4, the culture report indicated that *M. tuberculosis* was identified, but no TTD for week 4 was reported.

TABLE 3. Range of sensitivity, specificity, positive and negative predictive values, and accuracy of mean TTD with different numbers and weeks of time point data and cutoff values in evaluable patients

Parameter and end-point label	Time point(s) (wk) used in analysis of surrogate end point[a]	No. of patients evaluated	No. of patients with treatment failure	Sensitivity (%)[b]	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)[c]	End point positive if mean TTD (days) was[d]:	*P* value of treatment failure vs others
TTD										
1	2, 4, 6, 8	146	5	100	74	12	100	75	<21	0.003[e]
2	6, 8	151	6	100	83	20	100	76	≤22	<0.001[e]
3	8	158	6	100	82	18	100	77	≤23	<0.001[e]
Two-month culture conversion, 4	8	146	5	100	49	6	100	47	—[f]	0.03[g]

[a] Five of six patients with tuberculosis failure had four TTD cultures results.
[b] Cutoff chosen to achieve sensitivity of 100%.
[c] (Number of true-positive cases + number of true negative cases)/all cases.
[d] The test was positive if the mean TTD was <21 days for the mean of four time points, ≤22 days for the mean for two time points at weeks 6 and 8, and ≤23 days if only the week 8 TTD was evaluated.
[e] *P* by *t* test of treatment failure versus others.
[f] —, test positive if 2-month culture was positive for *M. tuberculosis*.
[g] *P* value by Fisher's exact test.

positive or negative for *M. tuberculosis* and compared the results with those of the comparable ANCOVA model.

In this model, TTDs of 1 to 41 days were scored positive and a TTD of 42 was scored negative. Because of the small sample size for one of the three cavity levels (≥4 cm), cavity was collapsed to a binomial response. Adjusted proportions refer to the proportions adjusted for all model effects, unequal sample sizes, and missing data among the repeated measures.

RESULTS

Patients. Of 163 patients, 147 (90%) were enrolled in Uganda and 16 (10%) were enrolled in South Africa. Compared to patients enrolled in the original clinical trial who were not included, patients in this substudy were significantly younger, more often male and of black race, weighed less, and were more likely to be HIV infected (Table 1). The median baseline $CD4^+$ cell count of HIV-infected subjects in the substudy was 200 μl^{-1} (range, 110 to 286 μl^{-1}).

Culture end points and treatment failure. Of 163 patients with TTD culture results, 146 patients, including 5 of the 6 patients with failure, had data for four cultures in the 2 months of study therapy. In these 146 patients, there was more rapid detection of growth (TTD of four cultures) in the five patients who failed treatment than in the others who did not fail treatment (mean ± standard deviation, 16.3 ± 2.9 days [range, 13.0 to 20.3 days] versus 25.4 ± 6.7 days [range, 6.0 to 42.0 days]; $P = 0.003$ by *t* test) (Tables 2 and 3 and Fig. 2). When TTD was assessed as a diagnostic end point for treatment failure, using a mean cutoff of less than 21 days (for specimens for culture obtained 2, 4, 6, and 8 weeks after the beginning of study treatment), the accuracy was 75%, the sensitivity was 100%, the specificity was 74%, the positive predictive value (PPV) was 12%, and the negative predictive value (NPV) was 100% (Table 3, end point label 1). Improved PPVs (18 to 20%) and similar sensitivities and specificities were obtained when fewer time points were used (two time points at week 6 and 8 cultures or one time point at week 8 culture) (Table 3, end point labels 2 and 3). By comparison, the alternative end point of culture conversion to negative after 2 months of treatment was less accurate and specific (accuracy, 47%; sensitivity, 100%; specificity, 49%; positive predictive value, 6%; negative predictive value, 100; Table 3, end point label 4).

Among 38 HIV-infected patients at initial testing, 7 (18%) had cavitary lung disease, received ethambutol, and were on intermittent therapy; 4 of the 7 (57%) had treatment failure. The accuracy of the TTD end point (four time points with a mean of <21 days) among the 24 HIV-infected patients with cavitary disease was 71% (sensitivity, 100%; specificity, 65%; PPV, 36%; NPV, 100%).

Correlation of TTD with known risk factors for combined end point of treatment failure and relapse. Significant effects of TTD in univariate analysis were also found in multivariate ANCOVA (Table 4). TTD was associated with cavitary lung disease; i.e., the greatest decrease (delayed response) was with large cavities, an intermediate decrease was with small cavities, and the lowest decrease was without cavities (Table 4; Fig. 3). The adjusted mean TTD was also significantly decreased among patients treated thrice weekly and with ethambutol (versus moxi-

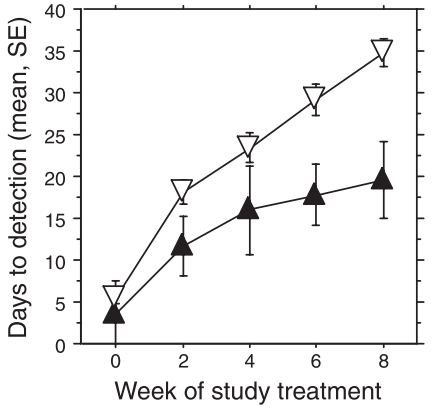

FIG. 2. Patients with (▲, $n = 5$) and without (▽, $n = 133$) treatment failure (all with four time points, relapse case excluded). Unadjusted means (bars, 95% confidence intervals) of time to detection are depicted on the *y* axis; the week of study treatment when sputum for was culture obtained is depicted on the *x* axis.

TABLE 4. Univariate and multivariate effects on TTD[a]

Factor or covariate	DF[b]	Univariate analysis of main effect		Multivariate analysis		
		TTD mean (days) not adjusted for other factors[c]	*P* value	*F* value	TTD adjusted mean (days)	*P* value
Drug						
Moxifloxacin	1	27.4	0.01	5.1	24.5	0.026
Ethambutol		24.5			21.9	
Cavity						
None	2	28.4	0.015	6.2	27.1	0.003
<4 cm		26.0			23.7	
≥4 cm		22.7			18.6	
Frequency of treatment per wk						
3 times	1	25.4	0.26	4.2	22.0	0.042
5 times		26.3			24.3	
Site						
Uganda (Bactec)	1	26.3	0.038	7.28	25.5	0.008
South Africa (MGIT)		22.3			20.8	
HIV infection status						
Infected	1	25.8	0.88	0.0	23.1	0.97
Not infected		26.0			23.2	
Duration of study treatment (wk)						
2	3	17.8	<0.001	51.4	15.7	<0.001
4		23.2			20.7	
6		28.3			25.4	
8		34.6			30.9	
Baseline culture (covariate of TTD)	1			19.1		<.001
Interaction						
Cavity by HIV infection status	2			5.3		0.006
Cavity by duration of treatment	6			2.8		0.013
HIV infection status by frequency of treatment per wk	1			4.5		0.036

[a] In the multivariate mixed-effect ANCOVA model, all the main effects and interactions with a *P* value of <0.05 are shown.
[b] DF, numerator degrees of freedom; $n = 158$.
[c] Not adjusted for other main effects or interaction of effects from the final model but adjusted for missing data on the repeated measure (TTD at weeks 2, 4, 6, and 8).

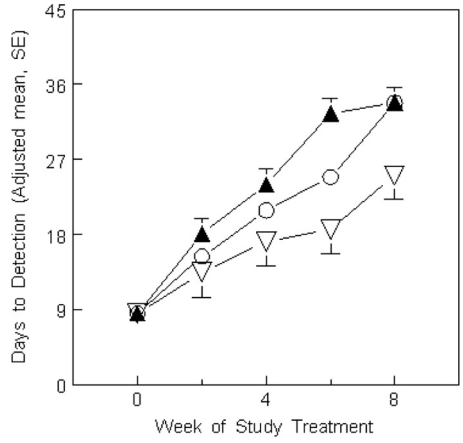

FIG. 3. Growth was detected sooner in patients with large cavities and was intermediate in those with small cavities than in patients without cavities (▽, with cavity of ≥4 cm; ○, with cavity of <4 cm) or no cavity (▲), with the adjusted mean (standard error [SE], bars) TTD (*y* axis) being plotted versus week of treatment (*x* axis).

floxacin). By comparison, 2-month culture conversion rates were similar among 158 patients treated thrice weekly versus 5 days/week (46% versus 51% culture conversion; $P = 0.5$, chi-square test) or treated with moxifloxacin versus ethambutol (culture conversion, 54% versus 46%; $P = 0.42$). Although not significant, the direction of proportions with 2-month culture conversion corresponded to the significant differences observed with TTD.

Other analyses of TTD. The adjusted mean TTD differed between study sites (Uganda versus South Africa, 25.5 days versus 20.8 days; $P = 0.008$), reflecting in part differences among patient groups and in the culture processing methods, the broth media, and the monitoring methodologies used. However, differences in the adjusted mean TTD of other cofactors were similar between models of Ugandan patients alone and all patients (Uganda and South Africa). Further, there were no significant differences by site between drugs or treatment frequency; i.e., there were no site interactions. These findings are consistent with an increase in power from a larger sample size with all patients.

From 161 patients, 591 duplicate sputum cultures (of specimens obtained from the baseline to week 8 of treatment) with

TABLE 5. Comparison between TTD data by ANCOVA and binary data (culture positive or negative) in a generalized model employing estimation equations[a]

Factor or covariate	ANCOVA		GEE	
	TTD adjusted mean (days)	*P* value	Probability of a negative culture (adjusted)[b]	*P* value
Drug				
Moxifloxacin	26.1	0.004	0.33	0.06
Ethambutol	24.1		0.22	
Cavity				
Present	23.5	<0.001	0.18	0.02
None	27.3		0.38	
Frequency of treatment per wk				
3 times	24.0	0.002	0.20	0.02
5 times	26.8		0.36	
Site				
Uganda	27.4	0.006	0.24	0.57
South Africa	23.4		0.30	
HIV infection status				
Infected	26.7	0.02	0.36	0.03
Uninfected	24.1		0.20	
Duration of study treatment (wk)				
2	17.0		0.05	
4	22.5		0.18	
6	28.6		0.39	
8	33.4		0.71	
Baseline culture (covariate of TTD)		<0.001		<0.001
Interaction				
Frequency of treatment per wk by HIV infections status		0.004		0.03
3 times for HIV-positive patients	24.0		0.21	
5 times for HIV-positive patients	29.4		0.54	
3 times for HIV-negative patients	24.2		0.19	
5 times for HIV-negative patients	24.2		0.21	
Cavity by HIV infection status		<0.001		<0.001
Positive cavity for HIV-positive patients	22.7		0.16	
Negative cavity for HIV-positive patients	30.6		0.61	
Positive cavity for HIV-negative patients	24.2		0.21	
Negative cavity for HIV-negative patients	24.0		0.19	
Cavity by duration of study treatment		0.06		0.35

[a] All main effects and interactions with a *P* value of <0.05 are shown.
[b] Probability of a negative culture adjusted for all other effects in the model.

results of *Mycobacterium tuberculosis* or no growth were evaluable. TTDs in the duplicate cultures were significantly correlated ($P < 0.0001$, $R^2 = 0.69$).

Other analyses of the TTD data were performed to explore the operating characteristics of the ANCOVA model. A frequency distribution of TTD data from 1 to 41 days demonstrated a middle peak and two tails with very low frequencies at days 1 and 2 and days 39, 40, and 41. Because about 30% of cultures did not demonstrate growth by the end of the monitored interval, we performed a sensitivity analysis of the effect of censoring at 42 days. Model term estimates and the significance of terms were insensitive to imputed censoring at 39 or at 45 days compared to 42 days.

We also performed GEE analysis to evaluate the effect of scoring all cultures as either positive or negative and compared the results to those obtained with a parallel TTD ANCOVA model. The significance of model terms and the direction of change between model terms were comparable between the different models, with one substantive exception (Table 5). The adjusted mean TTD of site differed significantly in the ANCOVA model but not in the GEE model. This demonstrates the similarity of data from the Bactec system, used at the Uganda site, and the MGIT system, used in South Africa, when culture results are analyzed as binary positive or negative results. However, a difference is distinguished between the monitoring systems with TTD data, likely reflecting the greater sensitivity of MGIT medium and monitoring to detect *M. tuberculosis.*

DISCUSSION

Identification of an accurate surrogate end point of treatment efficacy will advance trial design and development of new

antituberculosis drugs and regimens. In this study, TTD of growth appeared to be a promising end point for phase IIB trials because it identified patients who went on to treatment failure more accurately than 2-month culture status. Moreover, TTD was correlated with the most consistent risk factor for treatment failure or relapse: the presence and extent of pulmonary cavitation (1, 2, 4, 6, 7, 8). Finally, TTD appeared to be a more sensitive measure of differences between the randomized treatment arms than 2-month culture status, suggesting the greater activity of more frequent dosing and of moxifloxacin-based regimens than of ethambutol-based regimens. The efficacies of these treatment interventions have been demonstrated in prior studies (5, 7, 14). Notably, in our study, these differences were shown with a relatively small sample size (about 40 patients for each of four treatment arms) and with spot sputum sample collection (rather than prolonged sputum sample collection). Thus, the TTD end point may be valuable in exploring the pharmacodynamics of moxifloxacin and rifampin therapy (17).

Our study has several limitations. Broth cultures were performed according to the manufacturer's specifications and TTD was adjusted between sites, but we did not standardize laboratory processing or monitoring techniques. That we were able to identify cases of treatment failure and detect differences among the four treatment groups is, therefore, all the more notable. Residual confounding by other (unmeasured) differences among sites is possible. However, we found no interaction of site with study drug, frequency of treatment, or other clinical factors. The effect of censoring cultures at 42 days appeared to be small because differences in adjusted mean estimates of TTD and significance (*P*) values were similar between the final model with censoring set at 42 days and other models with censoring set at 39 or 45 days. Further, results were comparable in GEE analysis when all culture results were scored as either positive or negative. Finally, because the original trial was a phase II study, patients were not followed for relapse. However, a disease continuum between treatment failure and relapse was suggested, in that five of six cases of treatment failure in this study were detected in (more sensitive) liquid broth culture after 4 months of therapy but not in concurrent cultures on solid media.

In summary, TTD identified treatment failure more accurately than 2-month culture status and differentiated among treatment groups, suggesting superior bactericidal activity with more frequent dosing and with moxifloxacin. These findings support further evaluation of the utility of the TTD end point in treatment trials.

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None of us reports a conflict of interest.

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