A Time-to-Event Pharmacodynamic Model Describing Treatment Response in Patients with Pulmonary Tuberculosis Using Days to Positivity in Automated Liquid Mycobacterial Culture

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Days to positivity in automated liquid mycobacterial culture have been shown to correlate with mycobacterial load and have been proposed as a useful biomarker for treatment responses in tuberculosis. However, there is currently no quantitative method or model to analyze the change in days to positivity with time on treatment. The objectives of this study were to describe the decline in numbers of mycobacteria in sputum collected once weekly for 8 weeks from patients on treatment for tuberculosis using days to positivity in liquid culture. One hundred forty-four patients with smear-positive pulmonary tuberculosis were recruited from a tuberculosis clinic in Cape Town, South Africa. A nonlinear mixed-effects repeated-time-to-event modeling approach was used to analyze the time-to-positivity data. A biexponential model described the decline in the estimated number of bacteria in patients’ sputum samples, while a logistic model with a lag time described the growth of the bacteria in liquid culture. At baseline, the estimated number of rapidly killed bacteria is typically 41 times higher than that of those that are killed slowly. The time to kill half of the rapidly killed bacteria was about 1.8 days, while it was 39 days for slowly killed bacteria. Patients with lung cavitation had higher bacterial loads than patients without lung cavitation. The model successfully described the increase in days to positivity as treatment progressed, differentiating between bacteria that are killed rapidly and those that are killed slowly. Our model can be used to analyze similar data from studies testing new drug regimens.

Serial sputum CFU counts on solid media are an established method to investigate bactericidal activities of antitubercular drugs (1–5). However, CFU counting is expensive, labor-intensive, and technically challenging, with problems of bacterial clumping. In addition, this procedure is difficult to standardize across sites in multicenter studies. Another problem with CFU counting is that it does not take into account the metabolic activity of the different types of bacteria growing on solid media, i.e., actively replicating and slowly replicating bacteria (persisters). This distinction may be important when investigating antimycobacterial activities of drugs, since they act on different replicative states of the mycobacteria (6). Liquid culture systems may better represent the overall population of bacteria (7), since there are some mycobacterial populations that do not grow on solid media but grow in liquid media (8). Mycobacterial Growth Indicator Tube (MGIT; Becton Dickinson, Sparks, MD) systems have improved the detection of Mycobacterium tuberculosis in clinical samples and also shortened the time to obtain a positive result (9–11). The MGIT detection system is based on a silicon rubber disk impregnated with ruthenium pentahydrate, a fluorescent indicator whose natural fluorescence is quenched in the presence of oxygen. Bacterial growth utilizes oxygen in liquid medium, and the indicator then fluoresces, giving a positive result (10). It is possible that the lower the number of replicating bacteria inoculated into the MGIT, the longer it will take for the oxygen tension to fall below the threshold that would give a positive result. Indeed, days to a positive MGIT result have been found to be correlated with CFU counts (12), although one study found only a weak correlation between the two (13). Days to culture positivity at baseline have also been shown to predict sputum culture conversion at 8 weeks (14, 15), which is a widely accepted surrogate marker of tuberculosis cure (16).

To the best of our knowledge, there is no method or model available that quantitatively describes the change in days to positivity in automated liquid culture over time in patients receiving antituberculosis treatment. These kinds of data are becoming increasingly available as MGIT tests are becoming common in routine clinical settings because of the advantages described above. The aim of this work is to describe the decline in numbers of viable mycobacteria in the sputum during the 8-week intensive phase of standard short-course chemotherapy in patients with pulmonary tuberculosis using the quantitative measure of days to positivity in liquid culture by developing a nonlinear mixed-effects repeated-time-to-event modeling approach. A model that maximizes the use of the quantitative nature of MGIT days-to-positivity results to describe the mycobacterial response to treatment over time in sputum samples from patients, combined with the growth kinetics of the mycobacteria, could be useful for quantitatively analyzing similar data from studies investigating new drug combinations or testing the effects of various covariates on disease regression in patients.
MATERIALS AND METHODS

The study received ethical approval from the University of Cape Town research ethics committee and was carried out in accordance with the Helsinki Declaration of 1975 (revised in 2008).

**Study participants.** One hundred fifty-four patients participated in a randomized controlled trial of a micronutrient intervention (vitamin A and zinc) in Cape Town, South Africa. The micronutrient intervention had no effect on clinical or microbiological outcomes and was reported previously (17). The study participants had sputum smear-positive pulmonary tuberculosis and were all prescribed the same fixed-dose combination tablets (Rifafour; Aventis Pharma, Johannesburg, South Africa), each containing 150 mg rifampin, 75 mg isoniazid, 400 mg pyrazinamide, and 275 mg ethambutol. The administration of each dose was directly observed. Daily doses were administered 5 days a week according to body weight, in compliance with standard South African tuberculosis treatment guidelines (18): patients weighing 38 to 54 kg received 3 tablets, those weighing 55 to 70 kg received 4 tablets, and those weighing over 70 kg received 5 tablets. Patients were monitored up to 8 weeks after treatment only, which is the intensive phase of treatment for tuberculosis.

The extent and size of lung cavities at baseline were assessed independently by two pulmonologists experienced in the use of the Chest Radiograph Reading and Recording System (CRRS) (19). Disagreements on radiographic readings were resolved by consensus.

**Bacteriology.** A sputum specimen was collected in the early morning from each patient at baseline and then once weekly thereafter for 8 weeks. The specimens were processed for culture on liquid medium using the Bactec MGIT 960 system (Becton Dickinson, Sparks, MD). The time to obtain a positive culture result was recorded in days. A lack of growth after 42 days of culture was recorded as a negative result.

**Data analysis.** A nonlinear mixed-effects repeated-time-to-event model was implemented with NONMEM 7.2.0 software (Icon Inc., Verona, PA). An Intel Fortran compiler was used, and the runs were executed by using Perl-Speaks-NONMEM 3.4.10 (http://psn.sourceforge.net). Population parameter estimates and variability were obtained by using the Laplacian estimation method, which has been shown to have low parameter bias and imprecision when a high proportion of individuals in the data set have events (20). The objective function value (OFV), visual predictive checks (postprocessed and plotted using the statistical programming language R, version 2.8.1 [21]), and parameter precision were used for model building and evaluation. When comparing two hierarchical models, the OFV (−2 × log likelihood) was used. A decrease in the OFV of at least 3.84 points after the inclusion of one model parameter was regarded as statistically significant. A bootstrap of 300 replicates was carried out on the final model.

For the repeated-time-to-event model, a positive MGIT culture result was treated as an event, while a negative culture result was treated as a right-censored observation. Mono-, bi-, and trinomial exponential models were investigated in an attempt to describe the decline in bacillary loads in weekly sputum specimens from patients. The relative amount of bacteria in the bacterial growth compartment in the model (corresponding to the MGIT inoculum) was initialized to the value estimated from these models. We use the term relative amount because the amounts do not relate to actual numbers of bacteria but, upon integration according to the growth models described below, are an equivalent related to oxygen consumption, which is then directly related to the hazard of a positive test result. In other words, after the subsequent mathematical calculations and integration, the relative amount must translate to a reasonable hazard (or probability of obtaining a positive test result). Therefore, the relative amount in the MGIT inoculum for a particular week is driven by the hazard for that week. Therefore, had true amounts been measured (which would be higher by several orders of magnitude), they would need to be divided by a very large factor to translate to a reasonable probability, which would not always be 1. An exponential growth model, the Gompertz model (22), and the logistic model (23, 24) were investigated to describe the subsequent growth of the mycobacteria in the MGIT culture during the days of incubation. The differential equations for these models implemented in the ADVAN6 subroutine of NONMEM are as follows:

\[ N_t = N_i \times K_{\text{growth}} \]  
\[ N_t = N_i \times K_{\text{growth}} \times \log\left(\frac{N_{\text{max}}}{N_t}\right) \]  
\[ N_t = N_i \times K_{\text{growth}} \times \left( N_{\text{max}} - N_t \right) \]

where \( N_t \) is the amount of bacteria in the MGIT culture at time \( t \) after the start of incubation, \( N_{\text{max}} \) is the carrying capacity of the MGIT culture, and \( K_{\text{growth}} \) is a constant relating to the growth rate of the bacteria. Thus, at the beginning of the MGIT incubation, where \( t \) is zero, \( N_t \) was initialized as described above. The bacteria would then grow until a positive MGIT result was recorded or a negative result was found after 42 days. The bacterial growth models were investigated with and without a lag phase of the mycobacteria before growth commenced. The lag time in days (\( L \)) was estimated as a parameter in the model which delayed the commencement of bacterial growth by a period of time (\( L \) days) after the system starts running at time zero, such that bacterial growth commenced at time \( zero + L \) days. An attempt was made to model different growth rates and different lag times of actively replicating bacteria and the slowly replicating dormant bacilli in the MGIT culture.

The hazard of a positive MGIT result was directly related to the cumulative amount of bacteria in the tube by using the following equation:

\[ h(t)dt = Pr(t \leq T < (t + dt) | T > t) \]

where \( Pr \) is the probability of having an event within the very short time interval \( dt \), provided that one did not have an event before time \( t \).

The probability of not having a positive sputum MGIT culture event was a function of the cumulative hazard of a positive event (integral of the hazard with respect to time) for each day of incubation in the MGIT culture by using the following equation:

\[ S(t) = e^{-\int_0^t h(t) dt} \]

The probability density function (pdf), i.e., the likelihood of having an event at time \( t \) when an individual had a positive sputum culture event, was calculated as follows:

\[ pdf = h(t) \times S(t) \]

Thus, when an individual had a positive test result, the pdf was estimated, whereas for negative results, the survival to 42 days was estimated.

Although the primary aim of the work was to develop a method utilizing “days-to-positivity” data to describe the treatment response in patients with tuberculosis, we investigated the effect of covariates, including
the presence of lung cavitation and HIV infection, on disease progression. For the patients who did not have lung cavitation data available, a mixture model was used to assign an individual to one group or another. The proportion of patients who had no cavitation was fixed to that calculated from those who did have cavitation results.

**RESULTS**

Ten of the 154 patients recruited in the study were omitted from the analysis because their baseline MGIT culture was negative. Sixteen of the remaining 144 patients (11%) were HIV infected. Ninety-seven (67%) patients had lung cavities visible on chest X-ray, 20 (14%) did not have lung cavitation, and 27 (19%) patients had no available lung cavitation data. Baseline drug susceptibility testing results were available for 118 participants (82%). Of these, 6 (5%) patients had isoniazid monoresistance, and 1 (0.1%) had rifampin monoresistance, while resistance to both rifampin and isoniazid was found for 4 (3%) patients. Therefore, the vast majority of patients had drug-susceptible tuberculosis. Only 53% of patients had a negative sputum culture result after 8 weeks of treatment. As patients were monitored only up to 8 weeks after treatment initiation, there are no further data on the final patient outcomes upon the completion of the additional 4-month continuation phase. Figure 1 shows the progression of days to positivity with weeks on treatment. Table 1 shows the increasing percentages of patients with negative MGIT culture results as treatment

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**TABLE 1** Percentages of patients ($n = 144$) with negative sputum culture results and with missing culture results for each week of treatment for tuberculosis

<table>
<thead>
<tr>
<th>Wk of treatment</th>
<th>% negative results</th>
<th>% missing data</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>13</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>19</td>
<td>9</td>
</tr>
<tr>
<td>6</td>
<td>29</td>
<td>11</td>
</tr>
<tr>
<td>7</td>
<td>33</td>
<td>13</td>
</tr>
<tr>
<td>8</td>
<td>48</td>
<td>10</td>
</tr>
</tbody>
</table>

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**FIG 2** Visual predictive check from 100 simulations using the final model stratified into each week of treatment. The continuous line is a Kaplan–Meier plot for the real data. A positive sputum result was regarded as an event and will result in a step on the staircase plot. The shaded area is a 90% prediction interval based on the simulated data from the model.
progressed as well as the percentages of patients with no MGIT culture results at each week, mainly due to contamination of the culture. We sought to develop a quantitative method to analyze the days-to-positivity data and test its potential use by investigating HIV infection and the presence of lung cavitation as covariates.

Model for decline in bacillary load in sputum specimens from patients. A biexponential model best described the decline in the number of bacteria in sputum samples over time on treatment. The model was of the form described in the following equation:

\[ \text{Amount of bacteria} = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t} \]  

(7)

where \( A \) is the baseline amount of bacteria that are killed rapidly, with the rate of kill being equal to \( \alpha \), and \( B \) is the baseline amount of bacteria that are killed more slowly, with their rate of kill being equal to \( \beta \).

A visual predictive check from 100 simulations is shown in Fig. 2, which shows that the model described the data well. Table 2 shows the final parameter estimates from the model. Deriving from alpha, the typical time to kill half the amount of bacteria killed rapidly is 1.8 days. This means that after about 1 week, most of these bacteria are no longer present in the patients’ sputum. Similarly, for beta, the time to kill half the amount of bacteria killed slowly is 39 days, and it can take more than 5 months to kill most of these bacteria.

Figure 3 shows the decline in bacillary burden with time based on the parameter estimates from the final model for a patient with no lung cavitation. At baseline, the model predicts that the more rapidly killed mycobacteria are typically 41 times more prevalent in the MGIT inoculum, as calculated from the ratio of the baseline amount of rapidly killed bacteria to that of those that are killed slowly. Patients with lung cavitation present were found to have 1.73 times the bacterial load of patients with no lung cavitation. The ratio of rapidly killed bacteria to slowly killed bacteria at base-

**TABLE 2 Population parameter estimates**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimated value (95% CI)</th>
</tr>
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<tbody>
<tr>
<td>Baseline amt of rapidly killed bacteria</td>
<td>0.0573 (0.00387, 0.119)</td>
</tr>
<tr>
<td>Rate of kill for rapidly killed bacteria (wk (^{-1}))</td>
<td>2.68 (1.07, 4.32)</td>
</tr>
<tr>
<td>Baseline amt of slowly killed bacteria</td>
<td>0.00141 (0.0003, 0.00248)</td>
</tr>
<tr>
<td>Fractional increase in baseline amt of slowly killed bacteria for presence of lung cavitation</td>
<td>0.728 (0.0782, 4.04)</td>
</tr>
<tr>
<td>Rate of kill for slowly killed bacteria (wk (^{-1}))</td>
<td>0.124 (0.0126, 0.173)</td>
</tr>
<tr>
<td>Bacterial lag time before growth in MGIT culture commences (days)</td>
<td>4.00 (2.96, 4.00)</td>
</tr>
<tr>
<td>Baseline maximum growth rate of bacteria in MGIT culture (day (^{-1}))</td>
<td>8.31 (4.94, 12.9)</td>
</tr>
<tr>
<td>Rate of decrease of maximum growth rate in MGIT culture of rapidly killed bacteria (wk (^{-1}))</td>
<td>0.863 (0.582, 1.83)</td>
</tr>
<tr>
<td>Rate of decrease of maximum growth rate in MGIT culture of slowly killed bacteria (wk (^{-1}))</td>
<td>0.283 (0.223, 0.370)</td>
</tr>
<tr>
<td>Maximum carrying capacity of MGIT</td>
<td>0.158 (0.133, 0.212)</td>
</tr>
<tr>
<td>Population variability in rate of decrease in maximum growth rate of bacteria in MGIT (%)</td>
<td>56.6 (34.1, 71.6)</td>
</tr>
</tbody>
</table>

**FIG 3** Typical relative amount of *Mycobacterium tuberculosis* bacteria processed from patients’ weekly sputum samples and inoculated into the MGIT.
grow in the MGIT tube during treatment, (iii) saturable growth in the MGIT system, and (iv) the numerical nature of the MGIT data, i.e., the time-to-event-type data. Although the time to positivity in MGIT data was highly variable, a general upward trend with time on treatment can be seen in Fig. 1, with the boxes plateauing late on treatment because of the censoring of the data at 42 days. The visual predictive check using Kaplan-Meier plots shows that our model can predict the observed data quite well.

To test the potential use of our model, we investigated the effect of 2 covariates on some model parameters. Our finding that patients with lung cavitation have larger amounts of bacteria that are killed slowly is in accord with previous studies that showed lower 2-month sputum conversion rates (15, 25) and a shorter time to detection (or days to positivity) (26, 27) for patients with lung cavitation. The small number of patients with HIV infection may explain our failure to identify HIV infection as a significant covariate. However, other studies have also shown that HIV infection does not appear to have an effect on sputum conversion rates (28–30).

Depending on the type of bacteria in culture, the model best describing their growth can differ (24), with the Gompertz model performing better than the logistic model in some cases (31), or vice versa (23). In our case, the logistic model described the data better. It is probable that a positive MGIT culture result will have been recorded by the time the carrying capacity of the MGIT system is reached. Indeed, our model predicts that the carrying capacity could be reached only with the baseline sputum sample (data not shown); none of the subsequent samples had enough bacteria to reach the carrying capacity, even when cultured for up to 42 days. Nonetheless, the correct growth model must be used. The lag time that was estimated by the model is probably due to the bacteria having to recover from harsh treatment with sodium hydroxide and other antibiotics used during the decontamination process for the sputum before the inoculum can start growing in the MGIT culture system. Another possible reason for a lag time would be postantibiotic effects of the drugs administered to the patients during treatment. This presents a particularly important issue in that different drug classes may have differing postantibiotic effects, which could confound the association between time to positivity and the number of viable bacteria in the inoculum and negatively impact the ability of time to positivity to serve as a surrogate for the bacterial load when comparing the efficacies of different drug regimens. Care should be taken to address this possibility by testing the drug regimens as covariates on the estimated lag times. A drug regimen with greater postantibiotic effects would therefore have a longer lag time.

Although we report a biexponential model, a model with 3 exponents had an objective function value (OFV) that was 11 points lower than that of the biexponential model. However, due to the small difference in the OFV upon the addition of 2 parameters (intercept and slope) as well as wide confidence intervals from a bootstrap, the biexponential model was chosen, since it was more stable. The marginally lower OFV for a triexponential model for bacillary decline in patients suggests the existence of at least a third bacterial population. Richer data than our once-weekly MGIT results may be able to properly define the third population. The difference between the OFV for the biexponential model and that for the monoexponential model was 29 points; hence, the
biexponential model was significantly better and was deemed to describe data adequately.

A strength of our model is that it can differentiate an increase in days to positivity due to killing of the bacteria in the patient from an increase due to changes in the growth kinetics of the bacteria in MGIT culture. We report a decrease in the proliferative ability of the bacteria in the MGIT culture as time on treatment progresses, suggesting a change in the fitness of the bacteria. A possible explanation for the decrease in proliferative ability with time is that as treatment progresses, only persistor mycobacteria remain in the sputum, and these may take a longer time to grow in the MGIT culture. Another possibility is drug pressure resulting in the selection for or acquisition of some mutations at a fitness cost (32–35), although this does not occur indefinitely, since an evolution of compensatory mutations (36, 37) is likely to occur. One other possible explanation is the development of an adaptive mechanism of change in expression of different pathways by the mycobacteria in response to the altered environment caused by drug pressure and other stresses. We report 57% population variability in the rate of change of the bacterial growth rate in the MGIT culture. A previous report found genetically similar mycobacteria obtained from different patients having different fitness (38), showing that host factors also play a role. The higher rate of declining fitness of the rapidly killed bacteria might be because of their higher rate of multiplication in the human host, such that they acquire mutations that impede fitness much faster than the bacteria that are already growing slowly, since mutations can occur during DNA replication (39).

Although our semimechanistic model predicts the data well and is based on biologically plausible mechanisms, in vitro data to support our findings are lacking. Further work to determine the in vitro trends in growth rates and changes in the fitness of different subpopulations of M. tuberculosis during treatment is needed. To develop an even more mechanistic model, experiments to identify a calibration curve to relate days to positivity from 0 to 42 with initial bacterial amounts, followed by identifying the exact (possibly nonlinear) relationship between oxygen consumption and the hazard of a positive test result, would be ideal. It is also essential in this case to know the metabolic activity of the bacteria upon introduction to the culture. Identification of the exact threshold in oxygen tension and other factors that trigger a positive result would also enable more mechanistic models to be developed. We made an attempt to separate the bacterial growth compartment from the oxygen consumption compartment in the model. This resulted in a worse fit than the model that we present. This, however, means that what is in the bacterial growth compartment with the logistic function is actually a combination of bacterial growth and oxygen consumption and that $K_{\text{growth}}$ is a combination of the rate of bacterial growth and oxygen consumption. We also tried to estimate between-subject variability in the amount of bacteria, but this could not be supported by the data and created model instability. Only one random effect could be estimated (as is typical for time-to-event-type data), and this was on the change in viability of bacteria with time on treatment.

In summary, we present a novel method for analyzing the response of tuberculosis patients to treatment using data comprised of days to positivity in MGIT cultures of serially collected sputum samples. This model can be used to investigate covariates such as various drug regimens, drug exposure, strains of mycobacteria, patient/disease characteristics, or any other covariates on any of the model parameters in a manner similar to what we have shown with lung cavitation.

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