Re-defining Multidrug Resistant Tuberculosis Based on Clinical Response to Combination Therapy

Tawanda Gumbo¹,², Jotam G Pasipanodya³, Peter Wash, André Burger³, Helen McIlleron⁴.

¹Office of Global Health and ²Department of Medicine, UT Southwestern Medical Center, Dallas, Texas, USA, ³Brewelskloof Hospital, Worcester, South Africa, and the ⁴Division of Pharmacology, Department of Medicine, University of Cape Town, Observatory, South Africa

Corresponding author: Helen McIlleron, MBChB, PhD

Division of Clinical Pharmacology
Department of Medicine, University of Cape Town
Observatory, Cape Town 7925, South Africa
E-mail: helen.mcIlleron@uct.ac.za
Phone: +27-21-406-6292
Fax: +27-21-448-1989

Key words: multi-drug resistant tuberculosis, isoniazid, rifampin, critical concentrations, classification and regression tree analysis.

Running title: A new definition of MDR-TB
In tuberculosis treatment, susceptibility is defined by a critical concentration of 1.0 mg/L for rifampin and 0.2 mg/L or 1.0 mg/L for low- and high-level isoniazid resistance, based on epidemiologic cut-off method of the distribution of isolates’ MICs. However, pharmacokinetics/pharmacodynamics based clinical trial simulations suggested that the breakpoints should be 0.0625 mg/L for rifampin and 0.0312 mg/L or 0.125 mg/L for isoniazid. We examined outcomes in 36 patients with drug-susceptible tuberculosis, who had rifampin and isoniazid MICs performed and also had plasma drug concentrations measured, who were part of a prospective cohort study in Western Cape, South Africa. We performed classification and regression tree analysis to identify clinical and laboratory factors which predicted 2-month sputum conversion rates and long-term clinical outcomes. Poor long-term clinical outcomes were defined as microbiological failure, relapse or death, with 2 years of follow up. Peak drug concentrations and area under the concentration-time curve were most predictive of outcomes, and constituted the primary node, similar to our findings with the larger cohort. However, rifampin MIC and isoniazid MIC improved the predictive capacity of the primary decision node by 20% and 17%, respectively in these 36 patients. The rifampin MIC cut-off above which there was therapy failure was 0.125 mg/L, while that for isoniazid was 0.0312 mg/L, which are similar to those derived in clinical trial simulations. The critical concentrations used to define multidrug resistance for clinical decision-making should take into account clinical outcomes.
Patients with drug resistant tuberculosis, especially multi-drug resistant tuberculosis (MDR-TB; defined as tuberculosis resistant to both isoniazid and rifampin) and extremely drug-resistant tuberculosis (XDR-TB; MDR-TB with additional resistance to fluoroquinolones and injectable compounds) need to be accurately categorized from the outset as they need specific management with second-line regimens (1-5). Hence it is crucial that resistance testing identifies infections with a susceptibility threshold beyond which patients receiving the first-line regimen with standard doses of rifampicin and isoniazid will respond poorly. Resistance to isoniazid and rifampin is said to be present when $\geq 1\%$ of *Mycobacterium tuberculosis* culture grows in media supplemented with the critical concentration of each drug. The critical concentrations of rifampin and isoniazid were derived from the MICs of $\geq 95\%$ of wild-type isolates of *M. tuberculosis*, termed epidemiologic cut-off values (6-8). While extensively used, as evidenced by the regular release of MDR-TB worldwide figures by the World Health Organization (3), it is nevertheless unclear if the epidemiologic cut-off values are predictive of clinical responses, and if they are the most accurate. Here, we investigated this possibility based on data from a prospective cohort study.

Another approach that has been used to identify susceptibility breakpoints is pharmacokinetic-pharmacodynamic (PK/PD) based derivation that takes into account pharmacokinetic variability encountered for each drug (9). PK/PD studies in pre-clinical models such as the hollow fiber model as well as in clinical studies of tuberculosis patients have identified peak-to-MIC and area under the concentration-time curve (AUC)-to-MIC ratios below which there is poor outcome (10-14). In addition, pharmacokinetic variability has been shown to be one of the important determinants of therapy failure in patients, and has delineated optimal AUCs and peak concentrations below which patients fail therapy (15,16). Therefore, we have proposed that the critical concentration should be defined as the MIC above which maximum tolerated doses fail to effectively kill *M. tuberculosis* at site of infection (17). If the recommended doses in the
treatment regimen are unable to effectively kill bacilli in patients because of their MIC, then that
MIC defines clinically meaningful drug resistance. Monte Carlo simulations, which utilized
PK/PD exposures associated with optimal outcomes and antibiotic pharmacokinetic variability
encountered in patients, led to the proposal that rifampin critical concentrations should be
lowered from the epidemiologic cut-off derived value of 1.0 mg/L to 0.0625 mg/L, and isoniazid
low- and high-level resistance concentrations from 0.2/1.0 mg/L to 0.0312/0.125 mg/L in
Middlebrook media (17). Here, we used an agnostic machine learning method to identify the
rifampin and isoniazid MICs above which patients fail therapy during treatment with short-
course chemotherapy in the clinic.

METHODS

One hundred and forty two patients with sputum culture positive tuberculosis were enrolled in a
pharmacokinetic and pharmacodynamic study at Brewelskloof Hospital in Worcester, Western
Cape, South Africa, as described in prior studies (15,18). Patients were recruited between August
1999 and February 2002. Thirty six of these 142 patients, randomly chosen, also had isoniazid
and rifampin MICs identified in the isolates at the time of diagnosis. Patients were treated using
the following daily doses during the intensive phase of therapy: 300 mg of isoniazid, 20-35
mg/kg pyrazinamide, 15 mg/kg ethambutol, and 600mg of rifampicin daily if they weighed
>50kg or 450 mg if they weighed less. Patients with prior tuberculosis also received intra-
muscular streptomycin (1 g if they weighed more than 55 kg, 0.75 g if they weighed 38-54kg, or
0.5g if they weighed≤37kg). In the continuation phase all patients received the same isoniazid
and rifampin doses but for 5 in 7 days and patients with prior tuberculosis were continued on
ethambutol. All patients were hospitalized during the first two months of therapy to ensure
directly observed therapy (DOTS), for reasons outlined in the primary pharmacokinetic study
publication (18). Pharmacokinetic studies were performed over 24 hours during the eighth week
of therapy. The compartmental pharmacokinetic analyses have been published elsewhere (15).

Sputum microscopy and culture (using liquid cultures in the BACTEC 460 instrument) were performed after eight weeks of treatment. Patients were then followed prospectively for sputum conversion and long-term outcomes over 2 years.

All patients' isolates had direct sensitivity testing performed using BACTEC. In 36 patients, isoniazid and rifampin MIC assays were performed on the initial isolate at diagnosis. Isoniazid susceptibility was determined in the BACTEC system using the following concentrations: 0.0125, 0.025, 0.05, 0.1 and 0.2 mg/L. For rifampin the following concentrations were examined: 0.06, 0.125, 0.25, 0.5, 1.0 and 2.0 mg/L. MICs of pyrazinamide or ethambutol were not identified.

Classification and regression tree (CART) analysis is a non-parametric method utilizing recursive partitioning to classify data (19-21), which we and others have used for clinical decision making to examine outcomes in several infectious diseases studies (15,22-25). We were interested in classification of the 36 patients into two categories: therapy failure versus success. Two clinical outcome measures were utilized: sputum microbiology status at two months as well as long term outcomes. Poor long-term outcomes were defined as either relapse or microbiologic failure, or death, for up to 2 years of follow up since the start of therapy (15).

Several predictors of outcome were examined, including AUC, peak concentrations, trough concentrations, isoniazid MIC, rifampin MIC, age, gender, weight, HIV status, and treatment with streptomycin. CART analysis examines all these potential predictors of outcome, and examines all possible threshold values of the predictors, to create a tree. The step-by-step description of this analytic method for tuberculosis therapy were described in detail in a prior publication (15). Briefly, the upside tree created starts with a root node, which is the most important potential predictor or decision node. Daughter nodes are added to the tree, and a
score of how much they improve the primary decision node (as a % score of the primary node) is computed. For each outcome, maximal trees were generated by split each daughter node so that each class was homogenous in the examined outcome until further splits were not possible. Maximal trees are important in identifying data structure as well clinically meaningful interactions between covariates, particularly among fewer patients (small-sized daughter nodes). We then pruned the maximal trees based on relative misclassification costs, complexity and maximization of parsimony. Next, we performed a 10-fold validation. In this process, the dataset is randomly split 10 times in order to construct optimal trees, to identify how predictive the primary analysis was with these randomly created “test” data sets. This process obviates the need for a validation data set.

RESULTS

The full clinical characteristics of the 36 patients in whom isoniazid and rifampin MICs were performed, are shown in Table 1. The characteristics are similar to the entire data set of 142 patients (15), confirming that this subset of patients was randomly chosen from the larger data set. The distribution of isoniazid MICs is shown in Figure 1. The mean isoniazid MIC was 0.07±0.04 mg/L. The epidemiologic cut-off value was 0.2 mg/L. The distribution of rifampin MICs is shown in Figure 2. The mean rifampin MIC was 0.28±0.13 mg/L. The rifampin epidemiologic cut-off value was 0.5 mg/L.

As regards to the two month sputum conversion rates, 18/36 (50%) patients had a positive culture or smear at 8 weeks. The factors most predictive of 2-month sputum conversion included peak pyrazinamide, rifampin and isoniazid concentrations, as identified in the larger 142 patient study (15). However, in the 36 patients, isoniazid and rifampin MICs were also predictive of 2-month sputum conversion, with MIC thresholds shown in Table 2. While 2-
month sputum conversion is an important surrogate, the gold standard of antituberculosis therapy efficacy is still long-term outcomes. As regards to the long term outcomes, pyrazinamide AUC was the primary root node, followed by rifampin AUC, and the isoniazid AUC, consistent with findings from the entire dataset of 142 patients. However, the next root node was of rifampin MIC, followed by isoniazid MIC. Rifampin and isoniazid MICs improved the primary decision node by 20% and 17%, respectively. The rifampin and isoniazid cut-off points associated with outcome are shown in table 2. However, in standard assays, MICs are performed based on two-fold dilution steps, so that the nearest observed MIC that fulfills the non-strict inequality values shown in Table 2 was chosen as the breakpoint MIC (Table 2). The results held in the 10-fold validation process. Since predictive power is defined as performance of the training set–derived tree on the test dataset, our results suggest a good predictive role for therapy failure for these rifampin and isoniazid MICs. As can be seen in the table, the isoniazid MIC breakpoints for patients who failed were 0.0312 mg/L while that for rifampin was 0.0625 mg/L.

DISCUSSION

Isoniazid and rifampin MICs were predictive of clinical outcome, both at the 2-month time point and for long-term outcomes. These data are consistent with classic antimicrobial pharmacokinetic/pharmacodynamics theory, which is that the MIC of a bacterial species is an important determinant of outcome (26-29). Therefore, similar to many other pathogens such as standard gram negative and gram positive bacteria, MICs of anti-tuberculosis drugs affect clinical outcome. The MIC’s effect on microbial kill is considered relative to the drug concentration achieved at site of infection. The drug concentrations achieved in patients have a ceiling at the maximum tolerated dose, and are driven mainly by between-patient pharmacokinetic variability. As the MIC rises in the face of that ceiling concentration, the ratio
of AUC/MIC or peak/MIC fall, leading to less and less kill. Eventually, an MIC is reached above which microbial killing is not effective in most patients and which defines clinical resistance. This MIC above which therapeutic failure occurs is not necessarily linked to the 95% epidemiologic cut-off value derived from the MIC distribution. Indeed, there should be no mathematical or physiological reason to expect patient response to be linked to Gaussian parameters of *M. tuberculosis* isolates’ drug MICs. On the other hand, a shift in the 95% epidemiologic cut-off value at different time periods indicates that the population of isolates from the locale is moving more and more towards drug resistance, making it an excellent index for epidemiologic and public health work tracking of MDR-TB and XDR-TB. Our current study suggests that for clinical decision making during combination therapy, however, susceptibility breakpoint values should be lowered to 0.0312 mg/L for isoniazid, and 0.125 mg/L for rifampin, and do not coincide with the 95% epidemiologic cut-off value. The implication is that MDR-TB rates in the world are likely multiple-fold higher than currently assumed.

Our results redefine MDR-TB, and by extension XDR-TB, given that these definitions are dependent on critical concentrations of isoniazid and rifampin. In the case of rifampin, four sets of case reports from New Zealand, Australia and the Netherlands, in which a total of 11 clinical isolates from these 3 centers had mutations in the β subunit of RNA polymerase (*rpoB*) flagged as rifampin-resistant by Gene-Xpert® technology, were found to have rifampin MICs of 0.125-1.0 mg/L and failed rifampin containing multiple drug therapy (30-32). The authors termed this “phenotypically occult” resistance. We propose, instead, that the rifampin breakpoint should in fact be lower than current standards, at ≤0.125 mg/L. Moreover, our proposed breakpoint is based on failure of patients to respond to therapy, and is therefore not defined by chromosomal mutations as is the case with Gene-Xpert. That is because not all drug-resistance is due to mutations; some *M. tuberculosis* isolates have naturally high MICs, while other mechanisms of drug resistance such as efflux pump induction could also lead to drug-resistance (33-36). There
have not been any similar case reports for isoniazid as the 11 cases for rifampin since isoniazid
does not as of yet have a rapid molecular test to identify resistant mutants. Nevertheless,
mechanisms such as efflux pumps are also known to play a role in isoniazid resistance, which
means the final definition of isoniazid resistance will ultimately have to be based on phenotypic
tests such as MICs (11,13,37,38). Our current findings suggest the critical concentration for
isoniazid should also be lowered for clinical decision making. In essence, there is now clinical
support to change these critical concentrations that define MDR-TB. These new concentrations
should be considered for clinical decision making by the several bodies important in the clinical
care of tuberculosis around the world such as the WHO and STOP TB, as well as by groups
designing assays for diagnosis of MDR-TB.

The approach using hollow fiber PK/PD and pharmacokinetic variability in Monte Carlo
simulations to identify provisional and definitive susceptibility breakpoints of antituberculosis
agents (17) identified virtually the same breakpoints as those identified in our current clinical
study. Similarly, the breakpoint of pyrazinamide was correctly identified using this method, and
was also recently identified using CART to be identical (25). This is noteworthy because CART
analyses did not pre-specify the susceptibility breakpoints that should be selected but were
“agnostic” in both identifying MIC as a predictor of clinical outcome from among several clinical
factors, but also calculated threshold MICs that classifies patients into those who fail or succeed
therapy without relying on a prior choice. Therefore, the pharmacometric pathway consisting of
(a) pre-clinical PK/PD studies of monotherapy to identify optimal drug exposures in the hollow
fiber or other pre-clinical models, followed by (b) use of population pharmacokinetics in Monte
Carlo experiments, and (c) the choice of a susceptibility breakpoint based on >10% of patients
failing to achieve optimal exposures, is validated for setting susceptibility breakpoints of anti-TB
drugs. We propose its use for the new and experimental antituberculosis therapies currently
being studied.
Our study has several limitations. First is the size of the clinical study, which could limit generalizability of the findings. However, CART has been able to correctly identify thresholds with similarly small populations in the past (24,25,39). Second, several other clinical factors also determine clinical outcome, including drug concentrations, cavitary disease, and bacterial burden. However, these factors need not exclude a role for MICs in outcome. Indeed, our CART analysis also examined some of these as possible predictors, and with the exception of drug concentrations, they were outranked by MICs. Third, one potential limitation of CART is overfitting and biasing toward covariates with many possible splits. Thus our findings should be taken with these three factors in context. Nevertheless, cross-validation identified the same MIC threshold values, which were virtually identical to Monte Carlo simulations results published 5 years earlier (17). This means that the same breakpoints have now been identified using two completely independent methods. In the case of rifampin, retrospective case reports lead to the same conclusion, adding a third independent method. Thus, critical concentrations of 0.125 mg/L for rifampicin, and 0.0312 mg/L for isoniazid should be used to define MDR-TB. Such PK/PD evaluation should form the basis for accurate susceptibility breakpoints. The continued use of breakpoints which disregard the drug exposures in patients administered recommended doses, will lead to incorrect categorization of patients and treatment with inappropriate regimens (40).
FUNDING

This work was supported by the National Institutes of Health via the NIH Director New Innovator Award (National Institutes of General Medical Sciences DP2 OD001886 for JGP and TG, and the National Institute of Allergy and Infectious Diseases R01AI079497 for JGP and TG). Funding for recruitment of patients and for Helen McIlleron was provided by the Division of Pharmacology of the University of Cape Town, and the Medical Research Council of South Africa.

CONFLICTS OF INTEREST

TG founded Jacaranda Biomed. JGP and HM have no conflicts of interest.

ACKNOWLEDGEMENTS.

We would like to acknowledge the patients who participated in the study, the nursing staff of Brewelskloof Hospital, Western Cape, for taking care of patients, and Dr Andrew Whitelaw for MIC determination. We would also like to acknowledge the University of Cape Town’s Department of Medicine and UT Southwestern Medical Center’s Office of Global Health for making this collaboration possible.
REFERENCES


mutations in phenotypically occult multidrug-resistant Mycobacterium tuberculosis.

Int. J. Tuberc. Lung Dis. 16:216-20


Figure 1. Isoniazid MIC distribution in isolates from 36 patients.

The Gaussian distribution has a right skew, and a slightly higher epidemiologic cut-off value than in the literature (6).
Gaussian distribution of rifampin MICs in patients, means that there is no one “MIC” to a drug in clinical isolates. Thus the standard notion of stating that rifampin clinical isolates have an MIC of say 0.125 mg/L is meaningless.
**Table 1. Clinical and demographic factors in 35 patients treated for tuberculosis in Cape Town, South Africa.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Estimate or median</th>
<th>Range or percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex-female (%)</td>
<td>23</td>
<td>65.71%</td>
</tr>
<tr>
<td>Age in years</td>
<td>35</td>
<td>20-71</td>
</tr>
<tr>
<td>Self-identified “race” or ethnic group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed Race South African</td>
<td>31</td>
<td>88.57%</td>
</tr>
<tr>
<td>Black South African</td>
<td>4</td>
<td>11.43%</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td>47.3</td>
<td>30-68</td>
</tr>
<tr>
<td>HIV infection (%)</td>
<td>3</td>
<td>8.57%</td>
</tr>
<tr>
<td>Dose and range in mg/kg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rifampin</td>
<td>10.71</td>
<td>8.38-15.00</td>
</tr>
<tr>
<td>Isoniazid</td>
<td>6.39</td>
<td>4.41-10.00</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td>35.14</td>
<td>19.69-50.00</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>23.87</td>
<td>12.88-30.77</td>
</tr>
<tr>
<td>Prior tuberculosis (%)</td>
<td>23</td>
<td>65.71%</td>
</tr>
<tr>
<td>Hemoglobin g/dL (range)</td>
<td>12.00</td>
<td>7.40-14.00</td>
</tr>
<tr>
<td>White cell count x10⁶ cells/mL</td>
<td>8.60</td>
<td>3.80-25.70</td>
</tr>
<tr>
<td>Platelet count x10⁹ cells/mL</td>
<td>410.0</td>
<td>66.0-813.0</td>
</tr>
<tr>
<td>ESR in mm/hr</td>
<td>58</td>
<td>12-136</td>
</tr>
<tr>
<td>Total protein in g/L</td>
<td>77</td>
<td>62-92</td>
</tr>
<tr>
<td>Albumin in g/L</td>
<td>34.00</td>
<td>21-43</td>
</tr>
</tbody>
</table>
Table 2. Classification and regression tree analysis derived MIC breakpoints in 36 patients.

<table>
<thead>
<tr>
<th></th>
<th>Two month sputum conversion</th>
<th>Long term outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cut-off point</td>
<td>2 log scale</td>
</tr>
<tr>
<td>Isoniazid MIC (mg/L)</td>
<td>( \leq 0.150 )</td>
<td>0.125</td>
</tr>
<tr>
<td>Rifampin MIC (mg/L)</td>
<td>( \leq 0.188 )</td>
<td>0.125</td>
</tr>
</tbody>
</table>