Pharmacokinetic-pharmacodynamic determinants of clinical outcomes for rifampin-resistant tuberculosis: a multi-site prospective cohort study

Scott K. Heysell$^{1,*}$, Stellah G. Mpagama$^{2,3,*}$, Oleg B. Ogarkov$^{4}$, Mark Conaway$^{5}$, Shahriar Ahmed$^{6}$, Svetlana Zhdanova$^{4}$, Suporn Pholwat$^{1}$, Mohammad H. Alshaer$^{7}$, Anna M. Chongolo$^{2}$, Buliga Mujaga$^{3}$, Margaretha Sariko$^{3}$, Sabrina Saba$^{6}$, S.M. Mazidur Rahman$^{6}$, Mohammad Khaja Mafij Uddin$^{6}$, Alexey Suzdalsnitsky$^{8}$, Elena Moiseeva$^{8}$, Elena Zorkaltseva$^{9}$, Mikhail Koshcheyev$^{8}$, Serhiy Vitko$^{1}$, Blandina T. Mmbaga$^{3}$, Gibson S. Kibiki$^{3}$, Jotam G. Pasipanodya$^{10}$, Charles A. Peloquin$^{7}$, Sayera Banu$^{6,8}$, Eric R. Houpt$^{1,*}$

1. Division of Infectious Diseases and International Health, University of Virginia, VA, USA
2. Kibong’oto Infectious Diseases Hospital, Sanya Juu, Tanzania
3. Kilimanjaro Clinical Research Institute, and Kilimanjaro Christian Medical University College, Moshi, Tanzania
4. Department of Epidemiology and Microbiology, Scientific Centre for Family Health and Human Reproduction Problems, Irkutsk, Russian Federation
5. Department of Public Health Sciences, University of Virginia, VA, USA
6. International Center for Diarrheal Diseases Research, Bangladesh (icddr,b), Dhaka, Bangladesh
7. Infectious Disease Pharmacokinetics Lab, College of Pharmacy, University of Florida, FL, USA
8. Irkutsk Regional Tuberculosis Referral Hospital, Irkutsk, Russian Federation
9. Irkutsk State Medical Academy of Postgraduate Education – Branch of Russian Medical Academy of Continuing Professional Education, Irkutsk, Russian Federation
10. Quantitative Preclinical & Clinical Sciences Department, Praedicare Inc., TX, USA

¶ Authors contributed equally.
& Authors also contributed equally.

*Correspondence to: Scott K. Heysell MD, MPH; 345 Crispell Drive, MR-6; Charlottesville VA, USA, 29908; phone: 1-434-243-9064; email: skh8r@virginia.edu

Running title: PK-PD determinants of RR/MDR-TB outcome

© The Author(s) 2022. Published by Oxford University Press on behalf of Infectious Diseases Society of America. This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs licence (https://creativecommons.org/licenses/by-nc-nd/4.0/), which permits non-commercial reproduction and distribution of the work, in any medium, provided the original work is not altered or transformed in any way, and that the work is properly cited. For commercial re-use, please contact journals.permissions@oup.com
Abstract

Background

Treatment of rifampin-resistant and/or multidrug-resistant tuberculosis (RR/MDR-TB) requires multiple drugs and outcomes remain suboptimal. Some drugs are associated with improved outcome, however whether particular pharmacokinetic-pharmacodynamic relationships predict outcome is unknown.

Methods

Adults with pulmonary RR/MDR-TB in Tanzania, Bangladesh and Russian Federation receiving therapy with local regimens were enrolled from June, 2016 to July, 2018. Serum was collected after two, four, and eight weeks for each drug’s area under the concentration-time curve (AUC₀₋₂₄) and quantitative susceptibility of the Mycobacterium tuberculosis isolate measured by minimum inhibitory concentrations (MIC). Individual drug AUC₀₋₂₄/MIC targets were assessed by adjusted odds ratios (OR) for association with favorable treatment outcome and hazard ratios (HR) for time to sputum culture conversion. K-means clustering algorithm separated the cohort of the most common multidrug regimen into four clusters by AUC₀₋₂₄/MIC exposures.

Results

Among 290 patients, 62 (21%) experienced treatment failure, including 30 deaths. Moxifloxacin AUC₀₋₂₄/MIC target of 58 was associated with favorable treatment outcome [OR 3.75 (1.21, 11.56), p=0.022], while levofloxacin AUC₀₋₂₄/MIC of 118.3, clofazimine AUC₀₋₂₄/MIC of 50.5, and pyrazinamide AUC₀₋₂₄ of 379 mg*h/L were associated with faster culture conversion [HR > 1.0, p<0.05]. Other individual drug exposures were not predictive. Clustering by AUC₀₋₂₄/MIC revealed those with lowest multidrug exposures had slowest culture conversion.

Conclusion

Amidst multidrug regimens for RR/MDR-TB, serum pharmacokinetics and M. tuberculosis MICs were variable, yet defined parameters to certain drugs – fluoroquinolones, pyrazinamide, clofazimine – were predictive and should be optimized to improve clinical outcome.

Key words: multidrug-resistant tuberculosis; pharmacokinetics; pharmacodynamics; minimum inhibitory concentrations
Introduction

Globally, 9.9 million people were estimated to have tuberculosis (TB) disease in 2020, and approximately 500,000 had rifampin-resistant or multidrug-resistant (RR/MDR)-TB [1]. RR/MDR-TB significantly reduces the likelihood of TB treatment success. For decades, antibiotic combinations for RR/MDR-TB utilized drugs of uncertain efficacy with long treatment durations [2]. Subsequently, an individual patient data meta-analysis of RR/MDR-TB treatment outcomes found certain medications of benefit, others of unclear benefit, and still others that may worsen outcomes [3]. This analysis prompted the World Health Organization (WHO) to re-prioritize specific RR/MDR-TB drugs, and numerous trials are now underway to shorten the treatment duration or improve treatment efficacy with combinations of re-prioritized drugs [4]. Nevertheless, the mechanisms have not been fully elucidated to explain why certain drugs proved of benefit in meta-analysis while other drugs, despite in vitro activity, did not.

Pharmacokinetic-pharmacodynamic (PK-PD) relationships are important drivers of Mycobacterium tuberculosis killing and prevention of acquired resistance in vitro [5]. Most anti-TB drugs exert concentration dependent activity as measured by area under the concentration-time curve (AUC), often calculated from serum concentrations, relative to the infecting M. tuberculosis minimum inhibitory concentration (MIC) to that particular drug. While these relationships have been more convincingly demonstrated in humans for drugs used to treat rifampin-susceptible TB whereby higher AUC/MIC exposures improve drug efficacy [6], we and others have found complex relationships for individual drugs commonly used in RR/MDR-TB regimens. Certain drugs such as the fluoroquinolones (levofloxacin and moxifloxacin) exert more consistent exposure dependent activity [7], yet for others such as ethionamide, activity is demonstrated only up to a threshold MIC [8]. Furthermore, drugs such as kanamycin or cycloserine may exert activity only at the highest exposures that risk serious toxicities [9]. Thus, understanding PK-PD relationships within multidrug combination regimens for RR/MDR-TB may better inform drug regimen composition, determine if certain drugs can be considered if dose and serum exposure are optimized, or if other drugs warrant wholesale abandonment.
We present findings from a multi-country prospective cohort of adults with RR/MDR-TB pulmonary TB to determine individual drug and combinatorial PK-PD relationships that predict treatment outcomes.

Methods

Study Design. Patients were enrolled in a prospective cohort at initiation of RR/MDR-TB treatment from regional RR/MDR-TB referral sites: Dhaka, Bangladesh (National Institute of Diseases of the Chest and Hospital); Kilimanjaro region, Tanzania (Kibong’oto Infectious Diseases Hospital); and Irkutsk, Russian Federation (Irkutsk Regional Tuberculosis Referral Hospital). Eligible patients were 18 years or older with pulmonary TB and a respiratory specimen positive by Xpert MTB/RIF with rpoB mutation (Cepheid, Sunnyvale, USA) or cultured for M. tuberculosis complex with phenotypic resistance to rifampin. Patients were excluded if pregnant, unable to undergo sample collection, or treated for RR/MDR-TB within six months of enrollment. The enrollment goal was 125 patients at each site for single site analyses based on a prior PK/PD study that defined individual drug AUC targets for predicting outcome in rifampin-susceptible TB [10], and an estimated proportion with treatment failure in RR/MDR-TB of 25%. The enrollment period covered June, 2016 to July, 2018. All patients provided written informed consent to a protocol following STROBE criteria approved by all affiliated institutional review boards (NCT03559582).

Procedures. RR/MDR-TB treatment regimens were initiated with WHO guidance for weight based dosing by clinicians’ discretion at the enrollment sites. Chest radiographs performed prior to treatment initiation were categorized as cavitary or non-cavitary. Baseline laboratory data from hospital records were hemoglobin (g/dL), creatinine (mg/dL), human immunodeficiency virus (HIV) antibody testing, and CD4 count (cells/mm$^3$), while the study performed point-of-care glycosylated hemoglobin testing (HgbA1c %).
Sputa were collected at baseline and four, eight and 24 weeks after treatment initiation for culture on Lowenstein Jensen slants or Middlebrook 7H11 media, and liquid culture in the Bactec MGIT 960 system (Becton, Dickinson, Franklin Lakes, USA). Positive cultures were confirmed for *M. tuberculosis* complex by Gen-Probe (San Diego, USA). Other sputum mycobacterial cultures for routine care were documented by date of collection and whether positive or negative for *M. tuberculosis*. All positive cultures from baseline specimens and those at eight or 24 weeks had MIC testing with a custom 96-well MYCOTB plate (Trek Diagnostic Systems, OH, USA). Drugs on the custom plate were those most commonly prescribed at the time of study. MIC testing was performed as previously described post-hoc and not available for alteration of patient regimen [11]. In analyses defining susceptibility or resistance, we used the MIC breakpoint closest to the WHO-endorsed critical concentration in liquid media [12]. Pyrazinamide was not available on the plate and therefore binary susceptibility/resistance was determined genotypically for *pncA* mutation by Sanger sequencing.

Serum drug concentrations were measured at two, four and eight weeks after treatment initiation. Blood was collected at one, two, six and twelve hours after directly observed medication administration. Serum was stored at –80°C until shipment to the University of Florida Infectious Diseases Pharmacokinetics Laboratory. Concentrations were measured by validated liquid chromatography–tandem mass spectrometry assays. Estimated total exposures, AUC over 24 hours (AUC$_{0-24}$), were determined by noncompartmental analysis using Phoenix WinNonlin version 8.0 (Certara USA, Princeton, New Jersey), utilizing later hour concentrations in elimination phase.

**Outcomes definitions.** Clinical follow-up was performed at two, four, eight, 24, and 48 weeks after treatment initiation. Time to sputum culture conversion was from treatment initiation to the first negative sputum culture that was not followed by a positive culture. Death was defined as mortality from any cause up to 48 weeks. Favorable treatment outcome was defined as not having death, culture positivity at week 24 or beyond, acquired drug resistance (pre-specified as a four-fold or greater increase in MIC of any
drug in the patient’s regimen), or a lack of improvement of the major TB related symptom at week 24 or beyond (clinical failure).

Statistical analysis. For initial analyses that did not include PK-PD determinants, patients that had received a given drug and had M. tuberculosis isolates with MIC values known to be susceptible to that drug were compared to patients that had either received the drug and were found to be resistant or that did not receive the drug. Multivariable logistic regression models using Firth’s penalized likelihood method defined the odds of favorable treatment outcome, and Weibull regression models estimated the hazard ratios for time to sputum culture conversion. Given that some drugs were prescribed to low numbers of patients, regression models were adjusted for site of enrollment only.

To first assess the impact of PK exposure, we sought the individual drug AUC$_{0-24}$/MIC that yielded at least N = 20 patients with values above and below that target that generated the maximum odds ratios and 95% confidence intervals for treatment failure and hazard ratios and 95% confidence intervals for time to sputum culture conversion. Mean AUC$_{0-24}$ across dosing intervals was utilized with the robustness of the PK sampling strategy determined by D-optimality design. AUC$_{0-24}$ values that could not be calculated from measured concentrations from that dosing interval (two, four or eight weeks) were imputed by random multiple imputation. Missing values for MIC were imputed using site-specific regression of median MIC values. These models were adjusted for site of enrollment, age, sex, diabetes, HIV, known cavitary disease and body mass index.

Then, among patients with the most commonly prescribed medication regimens across sites, a K-means clustering algorithm with four clusters was applied to the AUC$_{0-24}$/MIC values at 2 weeks, the time point of earliest drug exposure measurement, whereby patients in each cluster had a similar pattern of values for each drug [13]. The difference in time to sputum conversion among clusters was estimated using the interval censored Weibull model, and a likelihood ratio test assessed differences.
Results

A total of 340 patients were enrolled with 50 patients (15%) unable to complete the first sampling procedures due to early death or loss to follow-up (N=26 from Bangladesh, N=17 from Tanzania, and N=7 from Russian Federation). The enrollment target was not met for the Russian Federation following temporary closure of the enrollment hospital. Thus analyses included all sites, with adjustment for site at a minimum, for 290 patients. The median age was 35 years (interquartile range 29-46 years) and 208 (72%) were male. There were expected differences in clinical characteristics among the sites (Table 1).

RR/MDR-TB drug regimen composition. Supplementary Figure 1 displays the individual drug frequencies. There were 53 distinct multi-drug regimens (11 from Tanzania, 14 from Bangladesh and 28 from the Russian Federation). There were site-specific preferred regimens such as 116 (93.5%) of patients from Tanzania received one of four regimens including 4-5 drugs, and 124 (100%) received a regimen that contained pyrazinamide and a fluoroquinolone (levofloxacin or moxifloxacin). In Bangladesh, 101 (87.1%) received one of three regimens, and 115 (99.1%) received a regimen containing a fluoroquinolone (levofloxacin or moxifloxacin) and 114 (98.2%) received pyrazinamide. Regimens were more varied in the Russian Federation.

Individual drug exposure variability over the 2-8 week treatment interval. Figure 1 displays the individual drug mean AUC$_{0-24}$ at two, four and eight weeks following treatment initiation, revealing drug specific variability, and further variability among sites. Complete site-to-site variability of AUC$_{0-24}$/ MIC relative to mg/kg dosing is represented in Supplementary Table 1.

Treatment outcomes and impact of individual drugs in the multidrug regimen. Of the 290 participants, 228 (79%) had a favorable treatment outcome. Of 62 (21%) failures, 30 died. There was no significant difference in the time to death among sites (Supplementary Figure 2). Of the remaining
participants categorized as treatment failure, 13 (4.4%) were culture positive at week 24 or beyond, 14
(4.8%) had acquired resistance, and 10 (3.4%) had clinical failure. Interval-censored time to culture
conversion was significantly faster for patients from the Russian Federation compared to those in
Tanzania (p=0.018) and Bangladesh (p=0.038) (Supplementary Figure 3).

To quantify the impact of individual drugs without PK-PD influence, the odds of favorable treatment
were compared in those that had received the drug and were known susceptible to those that had received
the drug and were found to be resistant or did not receive the drug. Sequencing for pncA mutations was
able to be performed in 122 patients’ isolates with 88 (72%) determined susceptible to pyrazinamide and
the proportion susceptible did not significantly vary across sites (p=0.164). In adjusted regression models,
patients who received pyrazinamide were significantly more likely to have favorable treatment
(Supplementary Figure 4a). Pyrazinamide, moxifloxacin, and clofazimine were associated with a shorter
time to sputum culture conversion (Supplementary Figure 4b).

PK-PD relationship to treatment failure and time to sputum culture conversion for individual
drugs. We then examined whether individual drug exposures and AUC\textsubscript{0-24}/MIC refined or strengthened
these associations with better treatment response. Moxifloxacin AUC\textsubscript{0-24}/MIC target of 58 was associated
with favorable treatment outcome (OR 3.75, 95% CI 1.21-11.56, p=0.022, Table 2). AUC\textsubscript{0-24}/MIC for
other drugs did not demonstrate significant associations. Table 3 shows the same analysis for time to
culture conversion, and revealed that AUC\textsubscript{0-24}/MIC targets were significantly associated with faster time
to culture conversion for levofloxacin, and clofazimine, and AUC\textsubscript{0-24} for pyrazinamide.

PK-PD relationship and time to sputum culture conversion for combinatorial patterns. Since
multiple drugs are used for treating TB, we examined the most commonly prescribed drug combination
across sites, a 5-drug regimen prescribed in N=95 patients. Patterns of pyrazinamide AUC\textsubscript{0-24} and
levofloxacin AUC\textsubscript{0-24}/MIC most clearly separated clusters (Figure 2a). Figure 2b demonstrates the
differences in time to culture conversion when separated by AUC$_{0-24}$/MIC pattern cluster, such that the
cluster with the slowest time to culture conversion (HR= 0.59 +/- SE 0.29, nominal p= 0.065) had both the
lowest levofloxacin and pyrazinamide exposures. Proportions reaching PK-PD targets identified in Table
3 for drugs within each cluster are described in Supplementary Table 2.

Discussion

In this multi-country prospective cohort of adults with pulmonary RR/MDR-TB, individual drug and
combinatorial PK-PD relationships significantly influenced clinical outcomes. It was already evident
from prior meta-analyses that bedaquiline, moxifloxacin or levofloxacin, linezolid and clofazimine were
associated with better treatment outcomes [3]. Drugs such as pyrazinamide, ethambutol, ethionamide or
prothionamide, para-aminosalicylic acid, delaminid, imipenem-cilastin, and amikacin have been
recommended only when a prioritized medication is unavailable or if the \textit{M. tuberculosis} isolate is
resistant; and kanamycin and capreomycin were no longer recommend due to association with poor
outcome [4]. Our PK-PD findings support this prioritization but with notable refinements.

First, we found pyrazinamide to be important. Inclusion of pyrazinamide in a regimen when the \textit{M.
tuberculosis} isolate was susceptible was significantly associated with favorable treatment outcome and a
faster time to culture conversion. Among all patients that received pyrazinamide, an AUC$_{0-24}$ target (379
mg*h/L) was found that significantly associated with faster time to culture conversion. Furthermore, in
adjusted cluster analyses of the most common prescribed drug regimens, a pattern of below target
pyrazinamide AUC$_{0-24}$ (along with low levofloxacin AUC$_{0-24}$/MIC) identified patients with the slowest
time to culture conversion. The AUC$_{0-24}$ target identified in this study was similar to that in serum for
predicting outcomes in rifampin-susceptible TB patients (363 mg*h/L) [10], and in a hollow fiber model
that translated to exposure within lung tissue [14]. Pyrazinamide has enhanced the \textit{in vitro} and animal
model activity of new TB drugs and remains one of the few anti-TB agents with sterilizing activity
against \textit{M. tuberculosis} in differing phases of growth [15]. Yet most PK-PD studies in humans with
pyrazinamide have been performed in rifampin-susceptible TB where modeling studies have found only very high doses and exposures could further shorten treatment duration [16]. Our findings rekindle the importance of pyrazinamide as an effective drug in treating RR/MDR-TB, particularly when susceptibility can be determined, and when a minimal target AUC$_{0-24}$ is achieved.

We found benefits to fluoroquinolones as well. Use of moxifloxacin was associated with shorter time to conversion (versus not using moxifloxacin), however when restricting the analysis to just those participants that received the drug (N=112) a specific AUC$_{0-24}$/MIC target could not be found that significantly associated with a faster time to culture conversion (Table 3). These findings may reflect the different background regimens as moxifloxacin was commonly prescribed in the 7-drug regimen studied in the STREAM trials and included clofazimine [17]. Clofazimine, a drug whose inclusion was significantly associated with improved sputum culture conversion (Supplementary Figure 3b) and for which a target AUC$_{0-24}$/MIC could be derived that improved time to culture conversion (Table 3), may have masked individual moxifloxacin impact. Levofloxacin, which was less commonly prescribed with clofazimine, did yield an AUC$_{0-24}$/MIC target associated with a faster time to sputum culture conversion (Table 3) and provided important discrimination in cluster analyses. The levofloxacin AUC$_{0-24}$/MIC target identified was also similar to the AUC$_{0-24}$/MIC of 146 predictive of maximal *M. tuberculosis* kill in the hollow fiber model [7].

We did not find PK-PD targets predictive of outcomes for other drugs. Statistical associations were expectedly limited by the frequency of the drugs utilized in the countries, or more narrow ranges of observed PK exposures [9]. Nevertheless, not only were no PK/PD targets found for ethionamide and cycloserine, inclusion of these drugs in the regimen even when susceptible significantly associated with slower culture conversion. We did not find PK/PD justification for inclusion of kanamycin in the RR/MDR-TB regimen, but did find serum exposures that may have placed patients at high risk of ototoxicity [18].
Along with site-specific trends in MIC distribution for a certain drugs (Supplemental Table 1), we noted regional and temporal variation in AUC\textsubscript{0-24} (Figure 1). Prior to this study, most anti-TB drug serum exposure was assumed as stable after the first few weeks of therapy following induction of genes related to absorption and metabolism [19]. Globally, TB medicines are dosed using weight bands and not personalized to an individual’s serum exposure or a PK-PD parameter such as AUC\textsubscript{0-24}/MIC. Our findings conceptually support expanded effort toward PK-PD interventions to facilitate individual exposure based dosing, or highly regionalized dosing informed by local PK-PD studies [20]. For RR/MDR-TB, drugs of priority should include fluoroquinolones and pyrazinamide where in vitro and clinically identified targets now appear to align. Alternative sample matrices such as saliva [21] and urine [22] that employ spectrophotometric methods may bring such personalized dosing closer to the point of care.

Limitations of this study include fewer patients treated with the currently prioritized drugs of bedaquiline and linezolid. Furthermore, for analyses that included pyrazinamide susceptibility, we used Sanger sequencing of the pncA gene and classified as resistant if a mutation was previously associated with phenotypic resistance or was likely to be associated with resistance, but excluded mutations with insufficient evidence, documented neutrality or likely neutrality [23]. Pyrazinamide resistance in rifampin-resistant TB has been documented higher proportions, yet likely represents an overestimation when using any pncA mutation. While the current study enrolled a majority of men, not atypical for TB studies, serum exposures for drugs such as a pyrazinamide may differ by sex [22]. Additionally, the overall rate of favorable treatment outcomes in this study may be higher than experienced in other RR/MDR-B settings, but is similar to contemporary trials with similar regimens [17]. We included the patient centered outcome of clinical failure in the composite of favorable treatment outcome [24]. While an uncommon cause of unfavorable treatment outcome alone, this may have modestly underestimated conventional treatment success, but did not factor into the analyses of time to sputum culture conversion. Lastly, early death or loss to follow-up prior to the first PK procedures may have been overrepresented.
among patients with more severely altered PK-PD, although baseline characteristics did not significantly
differ among those with completed procedures.

In summary, this multi-country “real-world” cohort of PK-PD and RR/MDR-TB treatment outcomes
found that not only the use of certain anti-TB drugs, but PK-PD targets of those drugs could be defined
that were predictive of clinically important outcomes. Therefore, RR/MDR-TB treatment centers that use
pyrazinamide, fluoroquinolones, and clofazimine should consider implementation studies of individual
exposure based dosing. Lastly, new RR/MDR-TB regimens containing bedaquiline and pretomanid
appear highly efficacious [25], yet as those drugs are utilized for RR/MDR-TB removed from their
original clinical trials, our findings would predict that similar PK-PD principles will drive outcomes over
time and result in another hierarchy of importance of certain drugs over others.

NOTES
Acknowledgements
We are indebted to the participants for their generosity.

Funding
This work was supported by the National Institute of Allergy and Infectious Diseases of the National
Institutes of Health (grant number U01AI115594 to SKH and ERH). Metadata and standard operating
procedures are available upon request to the corresponding author.

Potential conflicts
All authors declare no competing interests. SKH, SGM, OO, GK, SB and ERH conceptualized and
designed the study. SGM, OO, SB, SA, SZ, BTM, AC, and EZ managed patient recruitment and oversaw
the study sites. MHA and CAP performed pharmacokinetic analyses. AS, MK, EM, BM, MS, SS,
SMMR, MKMU, and SV contributed to data collection. SKH, SGM, SA, and SZ verified the source data.
MC, JP and SKH performed statistical analysis. SKH, SGM, SP, SB and ERH interpreted the data. SKH
wrote the first draft of the manuscript and MC produced the figures. All authors revised and edited the
final version of the manuscript. SKH, CAP, and ERH report grants or contracts from the National
Institutes of Health outside of the submitted work.
References


diagnostics for drug-resistant tuberculosis cases in South Africa. Int J Tuberc Lung Dis, 2017; 21:
1106-111.

3. Collaborative Group for the Meta-Analysis of Individual Patient Data in MDR-TB treatment–
2017, Ahmad N, Ahuja SD, Akkerman OW, et al. Treatment correlates of successful outcomes in


Intermediate Susceptibility Dose-Dependent Breakpoints For High-Dose Rifampin, Isoniazid, and
Pyrazinamide Treatment in Multidrug-Resistant Tuberculosis Programs. Clin Infect Dis, 2018;
67:1743-1749.

Pharmacokinetics/Pharmacodynamics, Dosing, Susceptibility Breakpoints, and Artificial
Intelligence in the Treatment of Multidrug-resistant Tuberculosis. Clin Infect Dis, 2018; 67:
S293-S302.

Pharmacokinetics/Pharmacodynamics-derived Dose, the Role of MICs in Clinical Outcome, and
the Resistance Arrow of Time in Multidrug-resistant Tuberculosis. Clin Infect Dis, 2018; 67:
S317-S326.


Table 1. Demographic and clinical characteristics by site of enrollment

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total N = 290</th>
<th>Tanzania N=124</th>
<th>Bangladesh N=116</th>
<th>Russian Federation N=50</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median in years (IQR)</td>
<td>35.5 (29-46)</td>
<td>42 (32-50)</td>
<td>32 (25-42)</td>
<td>35 (30-39)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Duration of symptoms, median # of days (IQR)</td>
<td>90 (52-180)</td>
<td>91 (58-165)</td>
<td>90 (45-180)</td>
<td>126 (60-249)</td>
<td>0.051</td>
</tr>
<tr>
<td>Prior history of TB, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>75 (26%)</td>
<td>32 (26%)</td>
<td>19 (16%)</td>
<td>24 (49%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Yes</td>
<td>211 (73%)</td>
<td>89 (72%)</td>
<td>97 (84%)</td>
<td>25 (50%)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>4 (1%)</td>
<td>3 (2%)</td>
<td>0</td>
<td>1 (1%)</td>
<td></td>
</tr>
<tr>
<td>HIV infection, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>214 (74%)</td>
<td>72 (58%)</td>
<td>116 (100%)</td>
<td>26 (52%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Positive</td>
<td>72 (25%)</td>
<td>48 (39%)</td>
<td>0 (0%)</td>
<td>24 (48%)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>4 (1%)</td>
<td>4 (3%)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>CD4 count in HIV Positive, median</td>
<td>227</td>
<td>224</td>
<td>--</td>
<td>237</td>
<td>0.444</td>
</tr>
<tr>
<td>On antiretroviral therapy, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>44 (62%)</td>
<td>41 (85%)</td>
<td>--</td>
<td>3 (13%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No</td>
<td>28 (38%)</td>
<td>7 (15%)</td>
<td>--</td>
<td>21 (87%)</td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>251 (87%)</td>
<td>119 (96%)</td>
<td>83 (72%)</td>
<td>49 (98%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Yes</td>
<td>39 (13%)</td>
<td>5 (4%)</td>
<td>33 (28%)</td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>HgbA1c in diabetes mellitus, median</td>
<td>9.2</td>
<td>9.1</td>
<td>9.5</td>
<td>6.6</td>
<td></td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>140 (48%)</td>
<td>80 (65%)</td>
<td>52 (45%)</td>
<td>8 (16%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Yes</td>
<td>145 (50%)</td>
<td>39 (31%)</td>
<td>64 (55%)</td>
<td>42 (84%)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>5 (2%)</td>
<td>5 (4%)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Alcohol, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>205 (71%)</td>
<td>84 (68%)</td>
<td>105 (91%)</td>
<td>16 (32%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Yes</td>
<td>80 (27%)</td>
<td>36 (29%)</td>
<td>11 (9%)</td>
<td>33 (66%)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>5 (2%)</td>
<td>4 (3%)</td>
<td>0</td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Recreational injection drugs, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>262 (90%)</td>
<td>116 (94%)</td>
<td>107 (92%)</td>
<td>39 (78%)</td>
<td>0.002</td>
</tr>
<tr>
<td>Yes</td>
<td>26 (9%)</td>
<td>6 (5%)</td>
<td>9 (8%)</td>
<td>11 (22%)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>2 (1%)</td>
<td>2 (1%)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>BMI, median (IQR)</td>
<td>19.0 (16.9-21.2)</td>
<td>17.8 (16.3-20.2)</td>
<td>19.0 (17.3-21.1)</td>
<td>20.6 (18.3-21.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MUAC, median (IQR)</td>
<td>23.0 (20.5-25.1)</td>
<td>22.0 (19.5-25.0)</td>
<td>23.1 (21.0-25.0)</td>
<td>24.5 (22.0-27.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>11.5 (10.1-12.7)</td>
<td>10.5 (9.1-11.8)</td>
<td>12.0 (11.3-12.8)</td>
<td>12.4 (9.9-14.0)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>------------------</td>
<td>-----------------</td>
<td>-----------------</td>
<td>-----------------</td>
<td>-----------------</td>
<td>--------</td>
</tr>
<tr>
<td>Creatinine Median (IQR)</td>
<td>0.90 (0.80-1.00)</td>
<td>0.90 (0.79-1.01)</td>
<td>0.88 (0.70-1.00)</td>
<td>0.90 (0.90-1.00)</td>
<td>0.019</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cavitary disease, baseline chest x-ray, n (%)</th>
<th>No</th>
<th>Yes</th>
<th>Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>82 (28%)</td>
<td>61 (49%)</td>
<td>13 (11%)</td>
</tr>
<tr>
<td>Yes</td>
<td>118 (41%)</td>
<td>49 (40%)</td>
<td>31 (27%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>90 (31%)</td>
<td>14 (11%)</td>
<td>72 (62%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Extrapulmonary involvement of TB, n (%)</th>
<th>No</th>
<th>Yes</th>
<th>Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>278 (96%)</td>
<td>121 (98%)</td>
<td>116 (100%)</td>
</tr>
<tr>
<td>Yes</td>
<td>12 (4%)</td>
<td>3 (2%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

IQR = intraquartile range. TB = tuberculosis. HIV = human immunodeficiency virus. HgbA1c = glycosylated hemoglobin. BMI = body mass index. MUAC = mean upper arm circumference.
<table>
<thead>
<tr>
<th>Drug (N= number of people prescribed)</th>
<th>Number below cut point (%)</th>
<th>Cut point AUC_{0-24}/MIC</th>
<th>Odds Ratio (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pyrazinamide (N=283)</td>
<td>46 (16%)</td>
<td>443*</td>
<td>1.92 (0.89, 4.13)</td>
<td>0.098</td>
</tr>
<tr>
<td>kanamycin (N=199)</td>
<td>64 (32%)</td>
<td>63</td>
<td>0.92 (0.43, 1.98)</td>
<td>0.834</td>
</tr>
<tr>
<td>cycloserine  (N=184)</td>
<td>148 (80%)</td>
<td>83</td>
<td>2.14 (0.75, 6.06)</td>
<td>0.153</td>
</tr>
<tr>
<td>levofloxacin (N=178)</td>
<td>25 (14%)</td>
<td>129</td>
<td>1.49 (0.52, 4.16)</td>
<td>0.448</td>
</tr>
<tr>
<td>prothionamide (N=141)</td>
<td>60 (43%)</td>
<td>1.78</td>
<td>1.62 (0.58, 4.37)</td>
<td>0.436</td>
</tr>
<tr>
<td>ethionamide (N=140)</td>
<td>28 (20%)</td>
<td>1.76</td>
<td>1.66 (0.62, 4.47)</td>
<td>0.315</td>
</tr>
<tr>
<td>moxifloxacin (N=112)</td>
<td>21 (19%)</td>
<td>58</td>
<td>3.75 (1.21, 11.56)</td>
<td>0.022</td>
</tr>
<tr>
<td>clofazimine (N=105)</td>
<td>50 (48%)</td>
<td>62</td>
<td>2.01 (0.74, 5.43)</td>
<td>0.168</td>
</tr>
<tr>
<td>ethambutol (N=105)</td>
<td>48 (46%)</td>
<td>2.58</td>
<td>0.76 (0.28, 2.07)</td>
<td>0.594</td>
</tr>
<tr>
<td>isoniazid (N=98)</td>
<td>37 (38%)</td>
<td>11.1</td>
<td>1.22 (0.41, 3.54)</td>
<td>0.719</td>
</tr>
</tbody>
</table>

AUC_{0-24}/MIC = area under the concentration time curve over the 24 hours/ minimum inhibitory concentration. *Pyrazinamide MIC was not measured, cut point is AUC_{0-24} mg*h/L only. Other drugs (capreomycin, para-aminosalicylic acid, linezolid, bedaquiline, delaminid) were not prescribed in enough patients to generate cut points with at least 20 patients with AUC_{0-24}/MIC values above and below a maximum cut point. Adjusted for site of enrollment, age, sex, diabetes, HIV, cavitary disease and body mass index.
Table 3. Impact on time to sputum culture conversion of maximum individual drug AUC$_{0-24}$/MIC cut points yielding at least N = 20 above and below the cut point.

<table>
<thead>
<tr>
<th>Drug (N= number of people prescribed)</th>
<th>Number below cut point (%)</th>
<th>Cut point AUC$_{0-24}$/MIC</th>
<th>Hazard Ratio (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pyrazinamide (N=283)</td>
<td>20</td>
<td>379*</td>
<td>1.83 (1.02, 3.27)</td>
<td>0.042</td>
</tr>
<tr>
<td>kanamycin (N=199)</td>
<td>20</td>
<td>30.1</td>
<td>1.53 (0.89, 2.62)</td>
<td>0.121</td>
</tr>
<tr>
<td>cycloserine (N=184)</td>
<td>115</td>
<td>57.5</td>
<td>1.19 (0.82, 1.74)</td>
<td>0.362</td>
</tr>
<tr>
<td>levofloxacin (N=178)</td>
<td>23</td>
<td>118.3</td>
<td>2.0 (1.15, 3.48)</td>
<td>0.015</td>
</tr>
<tr>
<td>prothionamide (N=141)</td>
<td>37</td>
<td>0.86</td>
<td>1.27 (0.77, 2.07)</td>
<td>0.347</td>
</tr>
<tr>
<td>ethionamide (N=140)</td>
<td>26</td>
<td>1.52</td>
<td>1.36 (0.83, 2.24)</td>
<td>0.224</td>
</tr>
<tr>
<td>moxifloxacin (N=112)</td>
<td>20</td>
<td>54.1</td>
<td>1.35 (0.74, 2.49)</td>
<td>0.323</td>
</tr>
<tr>
<td>clofazimine (N=105)</td>
<td>46</td>
<td>50.5</td>
<td>1.60 (1.01, 2.55)</td>
<td>0.046</td>
</tr>
<tr>
<td>ethambutol (N=105)</td>
<td>22</td>
<td>1.63</td>
<td>1.65 (0.95, 2.86)</td>
<td>0.073</td>
</tr>
<tr>
<td>isoniazid (N=98)</td>
<td>59</td>
<td>18.7</td>
<td>0.95 (0.58, 1.53)</td>
<td>0.825</td>
</tr>
</tbody>
</table>

AUC$_{0-24}$/MIC= area under the concentration time curve over the 24 hours/ minimum inhibitory concentration. *Pyrazinamide MIC was not measured, cut point is AUC$_{0-24}$ mg*h/L only. Other drugs (capreomycin, para-aminosalicylic acid, linezolid, bedaquiline, delaminid) were not prescribed in enough patients to generate cut points with at least 20 patients with AUC$_{0-24}$/MIC values above and below a maximum cut point. Adjusted for site of enrollment, age, sex, diabetes, HIV, cavitary disease and body mass index.
FIGURE LEGENDS:

Fig 1. Change in total serum exposure. Change expressed as population mean and standard errors for the area under the concentration time curve during a 24 hour dosing interval (AUC_{0-24} \text{mg}*h/L) measured at 2, 4 and 8 weeks after treatment initiation as visualized for each drug at each site: Tanzania (blue), Bangladesh (green), Russian Federation (red), all sites averaged (black). Total patients for each drug at 2, 4 and 8 week time points: pyrazinamide (266, 171, 127); kanamycin (193, 141, 88); cycloserine (163, 91, 79); levofloxacin (164, 101, 68); prothionamide (118, 69, 45); ethionamide (132, 105, 86); clofazimine (56, 13, 13); ethambutol (98, 28, 22); isoniazid (96, 64, 41); capreomycin (33, 25, 21); para-amino salicylic acid (20, 14, 8); bedaquiline (6, 4, 3). Delamanid and linezolid not displayed given infrequency of use.

Fig 2a. Pharmacokinetic-pharmacodynamic pattern for the most common multidrug drug regimen. Clusters identified by AUC_{0-24}/ minimum inhibitory concentration (MIC) pattern at 2 weeks after treatment initiation for the regimen of pyrazinamide, levofloxacin, kanamycin, cycloserine, and ethionamide, N=95. Pyrazinamide values represent AUC_{0-24} only (pyrazinamide MIC not performed).

Fig 2b. Time to sputum culture conversion to negative for patients receiving the most common regimen as clustered by pharmacokinetic-pharmacodynamic pattern. Hazard ratio=0.59 +/-SE 0.29, nominal p= 0.065, for difference between cluster 4 and other clusters.
Figure 1
216x279 mm (x DPI)
Figure 2

216x279 mm (x DPI)
Please excuse the presence of this and the following test pages, which have been added to a small number of article PDFs for a limited time as part of our process of continual development and improvement.
Lorem ipsum dolor sit amet, consectetur adipiscing elit, sed do eiusmod tempor incididunt ut labore et dolore magna aliqua. Ut enim ad minim veniam, quis nostrud exercitation ullamco laboris nisi ut aliquip ex ea commodo consequat. Duis aute irure dolor in reprehenderit in voluptate velit esse cillum dolore eu fugiat nulla pariatur. Excepteur sint occaecat cupidatat non proident, sunt in culpa qui officia deserunt mollit anim id est laborum. Lorem ipsum dolor sit amet, consectetur adipiscing elit, sed do eiusmod tempor incididunt ut labore et dolore magna aliqua. Ut enim ad minim veniam, quis nostrud exercitation ullamco laboris nisi ut aliquip ex ea commodo consequat. Duis aute irure dolor in reprehenderit in voluptate velit esse cillum dolore eu fugiat nulla pariatur. Excepteur sint occaecat cupidatat non proident, sunt in culpa qui officia deserunt mollit anim id est laborum. Lorem ipsum dolor sit amet, consectetur adipiscing elit, sed do eiusmod tempor incididunt ut labore et dolore magna aliqua. Ut enim ad minim veniam, quis nostrud exercitation ullamco laboris nisi ut aliquip ex ea commodo consequat. Duis aute irure dolor in reprehenderit in voluptate velit esse cillum dolore eu fugiat nulla pariatur. Excepteur sint occaecat cupidatat non proident, sunt in culpa qui officia deserunt mollit anim id est laborum. Lorem ipsum dolor sit amet, consectetur adipiscing elit, sed do eiusmod tempor incididunt ut labore et dolore magna aliqua. Ut enim ad minim veniam, quis nostrud exercitation ullamco laboris nisi ut aliquip ex ea commodo consequat. Duis aute irure dolor in reprehenderit in voluptate velit esse cillum dolore eu fugiat nulla pariatur. Excepteur sint occaecat cupidatat non proident, sunt in culpa qui officia deserunt mollit anim id est laborum. Lorem ipsum dolor sit amet, consectetur adipiscing elit, sed do
eiusmod tempor incididunt ut labore et dolore magna aliqua. Ut enim ad 
minim veniam, quis nostrud exercitation ullamco laboris nisi ut aliquip ex 
ea commodo consequat. Duis aute irure dolor in reprehenderit in 
voluptate velit esse cillum dolore eu fugiat nulla pariatur. Excepteur sint 
occacaeat cupidatat non proident, sunt in culpa qui officia deserunt mollit 
anim id est laborum. Lorem ipsum dolor sit amet, consectetur adipiscing 
elit, sed do eiusmod tempor incididunt ut labore et dolore magna aliqua. 
Ut enim ad minim veniam, quis nostrud exercitation ullamco laboris nisi 
ut aliquip ex ea commodo consequat. Duis aute irure dolor in reprehenderit 
in voluptate velit esse cillum dolore eu fugiat nulla pariatur. Excepteur sint 
occacaeat cupidatat non proident, sunt in culpa qui officia deserunt mollit 
anim id est laborum. Lorem ipsum dolor sit amet, consectetur adipiscing 
elit, sed do eiusmod tempor incididunt ut labore et dolore magna aliqua. 
Ut enim ad minim veniam, quis nostrud exercitation ullamco laboris nisi 
ut aliquip ex ea commodo consequat. Duis aute irure dolor in reprehenderit 
in voluptate velit esse cillum dolore eu fugiat nulla pariatur. Excepteur sint 
occacaeat cupidatat non proident, sunt in culpa qui officia deserunt mollit 
anim id est laborum. Lorem ipsum dolor sit amet, consectetur adipiscing 
elit, sed do eiusmod tempor incididunt ut labore et dolore magna aliqua.
Ut enim ad minim veniam, quis nostrud exercitation ullamco laboris nisi ut aliquip ex ea commodo consequat. Duis aute irure dolor in reprehenderit in voluptate velit esse cillum dolore eu fugiat nulla pariatur. Excepteur sint occaecat cupidatat non proident, sunt in culpa qui officia deserunt mollit anim id est laborum. Lorem ipsum dolor sit amet, consectetur adipiscing elit, sed do eiusmod tempor incididunt ut labore et dolore magna aliqua. Ut enim ad minim veniam, quis nostrud exercitation ullamco laboris nisi ut aliquip ex ea commodo consequat. Duis aute irure dolor in reprehenderit in voluptate velit esse cillum dolore eu fugiat nulla pariatur. Excepteur sint occaecat cupidatat non proident, sunt in culpa qui officia deserunt mollit anim id est laborum. Lorem ipsum dolor sit amet, consectetur adipiscing elit, sed do eiusmod tempor incididunt ut labore et dolore magna aliqua. Ut enim ad minim veniam, quis nostrud exercitation ullamco laboris nisi ut aliquip ex ea commodo consequat. Duis aute irure dolor in reprehenderit in voluptate velit esse cillum dolore eu fugiat nulla pariatur. Excepteur sint occaecat cupidatat non proident, sunt in culpa qui officia deserunt mollit anim id est laborum. Lorem ipsum dolor sit amet, consectetur adipiscing elit, sed do eiusmod tempor incididunt ut labore et dolore magna aliqua. Ut enim ad minim veniam, quis nostrud exercitation ullamco laboris nisi ut aliquip ex ea commodo consequat. Duis aute irure dolor in reprehenderit in voluptate velit esse cillum dolore eu fugiat nulla pariatur. Excepteur sint occaecat cupidatat non proident, sunt in culpa qui officia deserunt mollit anim id est laborum.