Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

_____ _____

Daniel Sans Graciaa Date

Approval Sheet

Linezolid Use and Cytopenias in Patients Treated for Multidrug-resistant Tuberculosis

By

Daniel Sans Graciaa, M.D., M.P.H.
Master of Science

Clinical Research

_____
Russell R. Kempker, M.D., M.Sc.
Advisor

_____
Sara Auld, M.D., M.Sc.
Committee Member

_____
Matthew Magee, Ph.D., M.P.H.
Committee Member

Accepted:

_____
Lisa A. Tedesco, Ph.D.
Dean of the James T. Laney School of Graduate Studies

_____
Date

Linezolid Use and Cytopenias in Patients Treated for Multidrug-resistant Tuberculosis

By

Daniel Sans Graciaa
M.D., Emory University, 2014
M.P.H., Emory University, 2014

Advisor: Russell R. Kempker, M.D., M.Sc.

An abstract of
A thesis submitted to the Faculty of the
James T. Laney School of Graduate Studies of Emory University
in partial fulfillment of the requirements for the degree of
Master of Science
in Clinical Research
2021

Abstract

Linezolid Use and Cytopenias in Patients Treated for Multidrug-resistant Tuberculosis
By Daniel Sans Graciaa

Background: Treatment of multidrug-resistant (MDR) TB requires long courses of therapy of 6-18 months. Linezolid is an oxazolidinone antibiotic used for MDR-TB treatment; while effective, its use is associated with adverse events including low blood counts (cytopenias) with treatment over 4 weeks. Data on toxicities with long-term use of linezolid and drug pharmacodynamics in TB treatment are limited and are barriers to implementation.

Methods: This was a secondary analysis of a National Institutes of Health (NIH)-funded retrospective cohort study of patients treated for MDR-TB in the country of Georgia from 2015-2017 that collected clinical and laboratory data via medical chart review and patient interview. The standard daily linezolid dose was 600 mg daily. Intensive blood sampling 4-6 weeks after treatment initiation generated serum linezolid trough concentration (C_{min}) and area under the curve. Linezolid PK exposure was defined using literature-reported thresholds and receiver operating characteristic curves. Cytopenias were defined using an NIH adverse event (AE) scale with grades of increasing severity from 1-4. Logistic regression was used to estimate odds ratios (OR) and evaluate the relationship between linezolid PK exposure with development and severity of cytopenias.

Results: Among 100 patients enrolled, 80 had linezolid in their baseline regimen; 76 had PK data available. Cytopenia adverse events occurred in 31 (40.8%) for an incidence rate of 53 per 100 person-years. No patients required dose reduction or interruption due to cytopenias. Cytopenias were associated with linezolid PK parameters (OR 5.78 [95% CI 1.08-30.3] for thrombocytopenia with $C_{min} > 2$mg/L) and higher-grade cytopenia AE were associated with higher PK parameters (OR 5.70 [95% CI 1.11-29.2] for thrombocytopenia with $C_{min} > 2$mg/L).

Conclusions: Cytopenias occur frequently with long-term use of linezolid 600 mg/day and are associated with pharmacokinetic parameters early in therapy. However, this dose appears to be well-tolerated as there were no treatment-limiting cytopenia adverse events.

Linezolid Use and Cytopenias in Patients Treated for Multidrug-resistant Tuberculosis

By

Daniel Sans Graciaa
M.D., Emory University, 2014
M.P.H., Emory University, 2014

Advisor: Russell R. Kempker, M.D., M.Sc.

A thesis submitted to the Faculty of the
James T. Laney School of Graduate Studies of Emory University
in partial fulfillment of the requirements for the degree of
Master of Science
in Clinical Research
2021

Acknowledgements

I would like to thank the Thesis Committee for their flexibility and guidance in conducting the analysis and presenting these findings. Thanks to my thesis advisor and mentor, Dr. Russell Kempker, for his vision, specific advice, and general encouragement during my clinical and research training and this project. Thanks to Dr. Henry Blumberg for connecting me to the Emory-Georgia TB research community and the MSCR program. Thanks to Dr. Wendy Armstrong and the Division of Infectious Diseases for the opportunity to continue my clinical and research career at Emory.

Most importantly, thanks to my family for their daily support throughout my many years of training. I could not have done it without them.

TABLE OF CONTENTS

INTRODUCTION

Prior to the emergence of coronavirus disease 2019 (COVID-19), tuberculosis (TB) was the leading infectious cause of mortality globally, with an estimated 10 million incident cases of TB and 1.4 million deaths due to TB in 2019 (1). Multidrug-resistant (MDR) TB, defined as a *Mycobacterium tuberculosis* isolate with resistance to the first-line drugs isoniazid and rifampin, presents a major barrier to ending the TB epidemic. There were an estimated 465,000 incident cases of MDR-TB in 2019. These cases are difficult to treat, with worse outcomes compared to drug-susceptible TB. Historically, MDR-TB treatment resulted in approximately 50-60% favorable outcomes, defined as a cure or treatment completion, compared to 90% or better for drug-susceptible TB (1, 2). Previous standard treatment regimens for MDR-TB required up to 24 months of therapy and were associated with significant toxicities for patients, including nephrotoxicity (kidney damage) and ototoxicity (hearing loss), which can be irreversible. However, in recent years, new anti-TB drugs and existing drugs repurposed to treat TB have improved outcomes and shortened treatment regimens to 6-18 months (3-6). Understanding how to best use these new and repurposed drugs is essential for safe and effective treatment of MDR-TB and for the preservation of these drugs for ongoing use.

Evaluating MDR-TB treatment is best done in settings with a high burden of disease. The country of Georgia, similar to other former Soviet republics, has one of the highest rates of MDR-TB in the world (7). MDR-TB accounted for 12% of new cases and 31% of previously treated cases in recent years (1). The national TB program (NTP) implemented new (bedaquiline and delamanid) and repurposed (linezolid, clofazimine, and imipenem/cilastatin) drugs into routine programmatic use in 2015. Linezolid is included in the most recent WHO guidelines on the treatment of drug-resistant TB (DR-TB) released in 2019, but caution is still advised with use over 6 months due to potential toxicities including cytopenias, or low blood counts (8). Data on long-term use of linezolid and development of cytopenias remain limited, but recent evidence suggests pharmacokinetic measures may inform safe use. The goal of the present study was to utilize pharmacokinetic data to assess the development of cytopenias with long-term use of linezolid to support safe and effective treatment of MDR-TB.

BACKGROUND

Linezolid is an oxazolidinone antibiotic which was approved by the United States Food and Drug Administration (FDA) in 2000 for Gram-positive bacterial infections (9). It was also found to have activity against *M. tuberculosis*. However, long term use of linezolid has been limited due to toxicities associated with therapy lasting > 4 weeks (9). The primary toxicities of concern include cytopenias and neuropathies of the peripheral or optic nerves. Development of cytopenias has been found to be related to total linezolid exposure in both a time- and dose-dependent fashion, though the mechanism is not well-defined (10). Hypothesized mechanisms for anemia include impaired mitochondrial protein synthesis, and for thrombocytopenia a combination of myelosuppression and reticuloendothelial system clearance of platelets bound with linezolid via an immune-mediated process (11, 12). The duration of linezolid therapy preceding the development of hematologic toxicities is variable, typically 10-14 days but as short as 5 days (9, 13). This is substantially shorter than the current 9 to 18-month durations of MDR-TB treatment regimens and hence raises serious concerns about its prolonged use for TB treatment.

Despite its associated toxicities, due to limited options for drug-resistant TB treatment, the WHO added linezolid to treatment recommendations for certain cases of DR-TB in 2006 (14). As evidence of its effectiveness accumulated, WHO included linezolid in the most recent DR-TB treatment guidelines published in 2019 as a "Group A" drug for MDR-TB treatment (8). Drugs in group A, which is composed of the fluoroquinolones (levofloxacin or moxifloxacin), bedaquiline, and linezolid, should be included in all MDR-TB regimens unless contraindicated. However, these guidelines still advise caution with use of linezolid lasting longer than 6 months, noting that treatment may need to be shortened due to toxicity. Data on long-term use of linezolid and development of toxicities remain limited, particularly in the treatment of TB (15).

Recent clinical trials and observational studies have provided some evidence for linezolid-associated toxicities in patients treated for TB. In the Nix-TB trial with 109 patients with either MDR or XDR-TB treated with linezolid-containing regimens, 52 (48%) developed cytopenias, with the majority occurring in the first 2 months of therapy, though the proportion was not quantified in study results (5).

As concerning, 88 (81%) patients developed neuropathy, and 93 (85%) required linezolid interruption or dose reduction due to an adverse event (AE). Notably, linezolid was dosed at 1200 mg/day in this trial, while the dose used in programmatic settings via National TB programs is variable but most commonly 600 mg/day. Observational data from the country of Georgia suggest that a dose of 600 mg/day is associated with good tolerability. Among 100 patients treated for DR-TB with a linezolid-containing regimen, 12 (12%) patients experienced a linezolid AE, with only 4 due to cytopenias, though all 4 required either linezolid interruption or dose reduction (16). Pharmacokinetic/pharmacodynamic data may supplement these clinical data to optimize linezolid use for TB (15).

Pharmacokinetic (PK) and pharmacodynamic (PD) data provide an additional source of information on linezolid therapy for TB. Pharmacokinetic parameters that have been proposed as informing linezolid safety include the trough or minimum serum concentration (C_{min}) and area under the curve (AUC). While PK parameters for efficacy in TB treatment are not yet defined, a C_{min} threshold > 2 mg/L for linezolid efficacy in Gram-positive infections has been proposed based on available data (17, 18). However, values above 2 mg/L have also been associated with mitochondrial toxicity, which is a potential mechanism for cytopenias (19). A higher threshold of 7 mg/L has been associated with thrombocytopenia (low platelets), prompting a proposed target range of 2-8 mg/dL for efficacy while minimizing toxicity. The proposed thresholds for AUC are > 160 mg x h/L for efficacy and > 280 mg x h/L for thrombocytopenia (17, 20). Pharmacologic data for linezolid in the treatment of TB remain limited. Mouse models have demonstrated an association between hemoglobin and linezolid dose and C_{min}, but not AUC or C_{max} (21). PK modeling utilizing patient data from several different settings including Georgia suggests that the 600 mg/day dose may maximize efficacy while minimizing toxicity compared to 900 mg or 1200 mg daily dosing, assuming a low minimum inhibitory concentration (22). PK/PD studies tend to have small sample sizes, particularly those evaluating linezolid in TB. An existing cohort study of patients treated for MDR-TB in Georgia provides one of the largest such populations in the literature. The project goal was to incorporate PK/PD data with clinical outcome data to assess the

development of cytopenias with long-term use of linezolid to support safe and effective treatment of MDR-TB.

METHODS

To accomplish the study goal, we investigated the following specific aims and associated hypotheses:

1. To describe the incidence of cytopenias among patients with MDR-TB receiving linezolid 600mg daily.
2. To evaluate the relationship between linezolid drug exposure, as measured by PK parameters, and development of cytopenias among patients with MDR-TB receiving linezolid.
   Hypothesis: Incident cytopenias are associated with a linezolid C_{min} > 2 mg/L and AUC > 160 mg x h/L.
3. To describe the dose-response relationship between linezolid drug exposure and severity of cytopenias among patients with MDR-TB receiving linezolid.
   Hypothesis: Higher grades of cytopenia are associated with higher linezolid C_{min} and AUC.

Design/population

This was a secondary analysis of a National Institutes of Health / National Institute of Allergy and Infectious Diseases (NIH/NIAID) funded prospective observational cohort of patients with MDR-TB initiating treatment at the National Center for Tuberculosis and Lung Diseases (NCTLD) in Tbilisi, Georgia (23). Eligible patients were those 16 years of age or older with sputum culture-confirmed TB and multidrug resistance who initiated therapy with bedaquiline, linezolid, clofazimine, and/or delamanid from December 2015 to May 2017. Treatment regimens were individualized based on drug susceptibility testing (DST) results following National TB Program (NTP) and WHO guidelines. Treatment regimens were determined by the NTP Drug Resistance Committee, which meets twice weekly. The standard of care for treatment duration during the study period was 20–24 months. All treatment was administered through directly observed therapy (DOT). Medical records and patient interview were used to collect demographics, medical history, and clinical and laboratory data including adverse events. Written

informed consent was required and ethics approvals were obtained from the institutional review boards of Emory University and the NCTLD.

Among a total of 156 patients who initiated therapy for MDR-TB during the study period and were approached for enrollment, 100 enrolled (Figure 1). Of the 56 excluded, 36 were due to distance from the study site, 13 to patient declining participation, 3 to late screening, 3 to cognitive impairment, and 1 due to critical illness. Linezolid was included in the initial regimen for 80 patients. Of these, 76 had blood drawn for PK evaluation.

Measurements

Laboratory evaluation included monthly sputum cultures until conversion, defined as 2 consecutive negative sputum cultures ≥ 28 days apart, and at least through 12 months of treatment. Patients were asked to return approximately 6 months posttreatment for follow-up and to provide a sputum sample. Sputum samples underwent acid-fast bacilli (AFB) smear microscopy, and AFB sputum cultures were performed at the National Reference Laboratory using Löwenstein-Jensen–based solid medium and the BACTEC mycobacterial growth indicator tube 960 broth culture system. Positive cultures were confirmed to be *M. tuberculosis* complex using the *M. tuberculosis* related antigen test. Phenotypic first- and second-line drug susceptibility testing (DST) were conducted as previously described, and the MTBDR*plus* assay was performed on positive culture isolates (24).

Complete blood counts (CBC) were collected monthly for 12 months and up to 3 later time points at 13, 18 and 20-24 months. For pharmacokinetic analyses, intensive blood sampling was done 4-6 weeks after treatment initiation. Patients were hospitalized and receiving drugs via DOT for at least 7 days prior to PK sampling. Drug concentrations were measured at at the Infectious Diseases Pharmacokinetics Laboratory at the University of Florida. Minimum serum concentration (C_{min}), maximum serum concentration (C_{max}), and area under the curve (AUC) were calculated using a non-compartment model.

Primary Outcome

Incident cytopenias were evaluated using two potential definitions. First were the AIDS Clinical Trial Group (ACTG) criteria, which were used in the initial linezolid clinical trials supporting FDA approval (17). These criteria use a percentage decrease from baseline in the CBC parameters white blood cells (WBC), hemoglobin, and platelets to define categories of cytopenias. This may not be directly clinically relevant, as most clinical decisions are made on the basis of CBC parameter threshold. Therefore, we also examined the NIH Division of AIDS (DAIDS) adverse event (AE) grading scale, which assigns increasing severity of AE from 1 through 4 with decreasing CBC parameter (25). For platelets, a value < 125 $x10^3/\mu L$ is grade 1, < 100 $x10^3/\mu L$ is grade 2, < 50 $x10^3/\mu L$ is grade 3, and < 25 $x10^3/\mu L$ is grade 4. For hemoglobin, a value < 11.0 g/dL is grade 1, < 10.0 g/dL is grade 2, < 9.0 g/dL is grade 3, and < 7.0 g/dL is grade 4. To ensure only incident cytopenias were captured, if baseline CBC values were below the threshold for AE, the minimum value in follow-up labs was set as the next lowest value after CBC count recovery above the AE threshold.

Primary Exposure

Linezolid exposure as measured by pharmacokinetic parameters was evaluated using several methods. First, values reported in the literature were considered. As noted above, previous studies have proposed a toxicity threshold of C_{min} > 7 mg/L or AUC > 280 mg x h/L, and an efficacy threshold of C_{min} > 2 mg/L or AUC > 160 mg x h/L. C_{min} > 2 mg/L has also been associated with mitochondrial toxicity. Among the study population, the median C_{min} was 0.235 mg/L (interquartile range, IQR 0.069-0.529) and median AUC was 89.6 mg x h/L (IQR 69.2-116.2). As the majority of the study population had PK measures below the literature-reported thresholds for C_{min} and AUC, a receiver operating characteristic (ROC) curve was created for these two parameters modeling any cytopenia as the outcome (Figure 2). The AUC for these curves was 0.595 for C_{min} and 0.614 for linezolid AUC. Cutoffs were selected to maximize sensitivity and specificity, resulting in a C_{min} cutoff of 0.27 mg/L and an AUC cutoff of 90.4 mg x h/L. Among the 76 participants with PK data, 33 (43.4%) had C_{min} above the ROC threshold of 0.27 mg/L, 7 (9.2%) were above the 2 mg/L efficacy/mitochondrial toxicity threshold, and 0 were above the 7

mg/L thrombocytopenia threshold. For linezolid AUC, 36 (48.6%) had values above the ROC threshold of 90.4 mg x h/L, 10 (13.2%) had values above the 160 mg x h/L efficacy threshold, and 1 (1.3%) had a value above the 280 mg x h/L toxicity threshold.

Covariates included the baseline characteristics of body mass index, diabetes status, tobacco use, hepatitis B virus infection, cavitary TB disease, and extensive drug resistance, as well as treatment characteristics such as duration of linezolid therapy, duration of second-line drug therapy, and duration of initial hospitalization.

Analytic Plan

Analytic methods for each Aim were as follows:

1. To describe the incidence of cytopenias with cumulative proportion and incidence rate. Person-time was calculated as time to incident cytopenia or assumed to be the 12 month study duration.
2. To evaluate the relationship between linezolid pharmacokinetic exposure and development of cytopenias using logistic regression with the following model:

$$\ln\left(\frac{P\,(cytopenia = 1)}{1 - P(cytopenia = 1)}\right) = \beta_0 + \beta_1 PK\_parameter$$

   The outcomes were any cytopenia, anemia, or thrombocytopenia anemia as defined by the NIH/DAIDS AE grading table. Exposures were C_{min} and AUC cutoffs from the literature and generated from the ROC curve.

3. To describe the dose-response relationship between linezolid pharmacokinetic exposure and severity of cytopenia using ordinal logistic regression with the following model:

$$ln\left[\frac{P(AEordinal \geq g|PK)}{P(AEordinal < g|PK)}\right] = \beta_{g=1} + \beta_{g=2} + \beta_3 PK1 + \beta_4 PK2$$

   The outcomes were grade of anemia or thrombocytopenia as defined by the NIH/DAIDS AE grading table. The three levels of the outcome were none, Grade 1, and Grade 2-3 combined.

There were no grade 4 AEs. Exposures were C_{min} and AUC cutoffs from the literature and generated from the ROC curve, as well as tertiles of exposure. The PK2 term was only included in the model when using tertiles of exposure. The proportional odds assumption was assessed using the score test. If this assumption was not met, polytomous logistic regression was used instead of ordinal logistic regression.

Age and gender were included in adjusted models because of the variability in CBC parameters with these characteristics. In univariate analysis, alcohol use was associated with cytopenia outcomes (any cytopenia AE OR 5.6, 95% CI 1.4-22.7) but other potential covariates were not. A second adjusted model including alcohol use and hepatitis C virus infection was evaluated because of the potential clinical effect of these factors on CBC parameters via myelosuppression or liver disease. There was no evidence of collinearity between covariates. Models were compared by the likelihood ratio test for nested models.

RESULTS

Among the 100 enrolled participants, the median age was 37.9 years (IQR 28.4-50.9) and the majority (80%) were male (Table 1). Tobacco use (51%) and alcohol use (31%) were common. 13 participants had diabetes mellitus, 21 had antibodies against hepatitis C virus, and 2 had human immunodeficiency virus (HIV) infection. 60 participants had cavitary TB disease, and 21 had isolates with additional resistance to fluoroquinolones and injectable agents, known as extensively drug-resistant TB. Overall, treatment outcomes were favorable in 72 (72%), with 91 (91%) achieving sputum culture conversion (Table 2). The median time to culture conversion was 40 days (IQR 28-63). There were 7 treatment failures and 3 deaths. Linezolid was included in the initial regimen for 80 patients and was administered at some point during therapy to 94 patients. Of the 80 receiving linezolid at baseline, 76 had blood drawn for PK evaluation and were included in the primary analysis. The median baseline CBC values for these patients were within normal limits, with WBC count 9.4 x 10^3/μL (IQR 7.5-11.0), hemoglobin 12.5 g/dL (IQR 11.3-14.0), and platelets 373 x 10^3/μL (IQR 306-489) (Table 1). There were 21 individuals with baseline hemoglobin and 2 with baseline platelets below the AE threshold. The median number of follow up CBC collections was 8 (IQR 5-12). The difference between baseline and lowest values in follow up for hemoglobin was a median of 0.9 g/dL and for platelets a median of 163 x 10^3/μL (Table 3).

Aim 1

When defining cytopenias using the ACTG criteria, 64 participants (84.2%) developed any cytopenia (Table 3). Cytopenia of WBC (leukopenia) occurred in 26 (34.2%), of hemoglobin (anemia) in 4 (5.3%), and of platelets (thrombocytopenia) in 61 (80.3%). As noted, due to its superior clinical utility, the NIH/DAIDS cytopenia definition was used for primary analysis. Applying the NIH/DAIDS AE grading scale, 31 participants (40.8%) had any cytopenia AE. An AE for individual CBC parameters occurred in occurred in 1 (1.3%) for WBC, in 24 (31.6%) for hemoglobin, and in 11 (14.5%) for platelets.

Most of these were grade 1 (23, 74.2%) or grade 2 (11, 35.5%) AE. There were 1 each grade 3 AE for hemoglobin and platelets, and no grade 4 AE.

The incidence rate of any cytopenia AE among those with linezolid in their baseline regimen was 53 per 100 person-years. The incidence rate for anemia was 39 per 100 person-years, and for thrombocytopenia it was 32 per 100 person-years. However, only 4 participants stopped linezolid due to any AE, and these were for neuropathy (n=2) and hepatotoxicity (n=2), not for cytopenias. No participants required dose reduction. Incident cytopenias occurred at a median of 4 months (IQR 3-7) for any cytopenia, 4 months (IQR 3-6) for anemia, and 11 months (IQR 5-13) for thrombocytopenia.

Aim 2

Logistic regression modeling for Aim 2 demonstrated that linezolid pharmacokinetic exposure as defined by C_{min} and AUC thresholds is associated with development of cytopenias when evaluating the three outcomes of any cytopenia, anemia, and thrombocytopenia (Table 4). First, for any cytopenia AE, the odds of cytopenia among those with $C_{min} > 2$ mg/L were 4.13 times the odds among those with $C_{min} \leq 2$ mg/L (95% confidence interval 0.75-22.9). This association is somewhat attenuated in the model adjusted for age and gender (adjusted OR, aOR 3.70, 95% CI 0.65-21.2) and in the model further adjusted for alcohol use and hepatitis C virus infection (aOR 2.78, 95% CI 0.43-18.2). A similar, though smaller magnitude association is shown with the ROC-generated C_{min} threshold of 0.27 mg/L. With linezolid AUC as the exposure, the odds of cytopenia among those with AUC > 160 mg x h/L were 2.46 times the odds among those with AUC < 160 mg x h/L (95% CI 0.63-9.58). Similar to C_{min}, this association is attenuated in the partially-adjusted (aOR 1.92, 95% CI 0.46-7.93) and fully-adjusted (aOR 1.20, 95% CI 0.24-5.96) models, and a similar association is shown with the ROC-generated AUC threshold of 90.4 mg x h/L.

For hemoglobin cytopenia AE, the odds of anemia among those with $C_{min} > 2$ mg/L were 3.27 times the odds among those with $C_{min} \leq 2$ mg/L (95% confidence interval 0.67-15.9). This association is attenuated in the partially-adjusted (aOR 2.89, 95% CI 0.56-14.0) and fully-adjusted (aOR 2.00, 95% CI

0.34-11.9) models. A similar, though smaller magnitude association is demonstrated with the ROC-generated C_{min} threshold of 0.27 mg/L. With linezolid AUC as the exposure, the odds of anemia among those with AUC > 160 mg x h/L were 2.47 times the odds among those with AUC < 160 mg x h/L (95% CI 0.64-9.53). This association is attenuated in the partially-adjusted (aOR 1.74, 95% CI 0.41-7.32) and fully-adjusted (aOR 1.29, 95% CI 0.28-6.00) models. A similar, though smaller magnitude association is shown with the ROC-generated AUC threshold of 90.4 mg x h/L.

For platelet cytopenia AE, the odds of thrombocytopenia among those with C_{min} > 2 mg/L were 5.78 times the odds among those with $C_{min} \leq 2$ mg/L (95% confidence interval 1.08-30.3). The direction of this association remains in the partially-adjusted (aOR 5.64, 95% CI 1.01-31.4) and fully-adjusted (aOR 6.43, 95% CI 0.90-45.9) models. A similar, though smaller magnitude association is demonstrated with the ROC-generated C_{min} threshold of 0.27 mg/L (cOR 2.62, 95% CI 0.70-9.89). With linezolid AUC as the exposure, the odds of thrombo cytopenia among those with AUC > 160 mg x h/L were 3.11 times the odds among those with AUC < 160 mg x h/L (95% CI 0.67-14.5). This association is slightly more pronounced in partially-adjusted (aOR 3.24, 95% CI 0.63-16.7) and fully-adjusted (aOR 3.51, 95% CI 0.56-22.2) models. A similar, though higher magnitude association is shown with the ROC-generated AUC threshold of 90.4 mg x h/L; the crude model produces cOR 6.33 (95% CI 1.27-31.7), the partially-adjusted model aOR 6.81 (95% CI 1.32-35.2), and the fully-adjusted model aOR 8.12 (95% CI 1.39-47.3).

Aim 3

Ordinal logistic regression modeling for Aim 3 demonstrates that linezolid pharmacokinetic exposure as defined by C_{min} and AUC thresholds is associated in a dose-response fashion with grade of cytopenia AE for anemia and thrombocytopenia. The proportional odds assumption was not met for the models evaluating anemia AE using linezolid PK thresholds gathered from the literature (C_{min} of 2 mg/L and AUC of 160 mg x h/L), likely because there were no grade 1 AE in these categories; polytomous logistic regression for Grade 2-3 anemia versus no anemia was used for these models. The odds of higher-

grade anemia among patients with C_{min} > 2 mg/L were 10.9 times the odds among those with C_{min} ≤ 2 mg/L (95% CI 1.9-60.8) (Table 5). A slightly higher magnitude association is shown in the adjusted model, though the confidence interval is very wide (aOR 16.7, 95% CI 2.2-127). Using tertiles of C_{min} as exposure categories, there appears to be a dose-response relationship as the OR for higher-grade anemia among patients with C_{min} > 0.35 mg/L compared to those with C_{min} ≤ 0.12 mg/L was 2.02 (95% CI 0.66-6.23), while among those with C_{min} > 0.12-0.35 mg/L the OR was 0.60 (0.17-2.19). A similar association is seen using the ROC-generated cutoff of C_{min} > 0.27 mg/L and after adjusting for age and gender. Using AUC categories of exposure, the odds of higher-grade anemia among patients with AUC > 160 mg x h/L were 9.4 times the odds among those with AUC ≤ 160 mg x h/L (95% CI 2.01-44.1). This association is attenuated by adjustment for age and gender (aOR 7.15, 95% CI 1.35-37.8). As for C_{min}, there appears to be a dose-response relationship between higher AUC and higher-grade anemia, with cOR 2.81 (95% CI 0.88-8.93) for AUC > 105 mg x h/L compared to ≤ 75.4 mg x h/L, and cOR 0.78 (95% CI 0.21-2.90) for AUC > 75.4-105 mg x h/L. A similar association holds using the ROC-generated cutoff of AUC > 90.4 mg x h/L and after adjusting for age and gender.

For thrombocytopenia, the odds of higher-grade AE among patients with C_{min} > 2 mg/L were 5.7 times the odds among those with C_{min} ≤ 2 mg/L (95% CI 1.11-29.2), with a similar association after adjusting for age and gender (aOR 5.95, 95% CI 1.11-32.1) (Table 6). Using tertiles of C_{min} as exposure categories, there appears to be a dose-response relationship as the cOR for higher-grade thrombocytopenia among patients with C_{min} > 0.35 mg/L compared to those with C_{min} ≤ 0.12 mg/L was 3.76 (95% CI 0.68-20.8), while among those with C_{min} > 0.12-0.35 mg/L the cOR was 1.55 (0.23-10.4). A similar association is seen using the ROC-generated cutoff of C_{min} > 0.27 mg/L and after adjusting for age and gender. Examining AUC categories of exposure, the odds of higher-grade thrombocytopenia among patients with AUC > 160 mg x h/L were 3.15 times the odds among those with AUC ≤ 160 mg x h/L (95% CI 0.69-14.5), with a similar association after adjusting for age and gender (aOR 3.31, 95% CI 0.65-16.9). As for C_{min}, there appears to be a dose-response relationship between higher AUC and higher-grade thrombocytopenia, with cOR 3.58 (95% CI 0.64-19.9) for AUC > 105 mg x h/L compared to ≤ 75.4

mg x h/L, and cOR 1.62 (95% CI 0.25-10.7) for AUC > 75.4-105 mg x h/L. A similar association holds using the ROC-generated cutoff of AUC > 90.4 mg x h/L and after adjusting for age and gender.

DISCUSSION

Our study findings demonstrate that cytopenia adverse events are common among patients receiving linezolid 600 mg/day for treatment of MDR-TB, occurring in 41% of patients at an incidence rate of 53 per 100 person-years. However, these AE were not treatment-limiting, as no patients required linezolid interruption or dose reduction due to cytopenias. These findings suggest that while cytopenias occur frequently with long-term use of linezolid 600 mg/day, this dose appears well-tolerated and safe for long term use. This contrasts with other TB treatment cohorts using 1200 mg/day in which nearly all patients required linezolid interruption or dose reduction due to cytopenias, as well as a clinical trial using 600 mg/day in which 18% experienced a grade 3 or higher cytopenia AE (5, 26). This contrast is likely because most cytopenia AEs in the current study were low-grade and unlikely to be clinically significant. However, the apparent tolerability of the 600 mg/day dose is consistent with PK modeling data that suggests this dose may minimize toxicity while maintaining efficacy (22). Our study provides needed safety data to support use of this essential drug for treatment of MDR-TB.

Regression modeling suggests that cytopenia AE, particularly thrombocytopenia, are associated with linezolid C_{min} and AUC using several different cutoffs, and higher grade cytopenias are associated with higher PK parameter values. Relatively few (7/76, 9.2%) patients in this cohort had C_{min} values above the literature-defined 2 mg/L mitochondrial toxicity threshold, compared to 58% of 30 patients receiving linezolid 600mg or 300mg daily in a study of drug-resistant TB in South Africa (18). This could reflect known heterogeneity between linezolid PK studies (27). However, the proportion above the toxicity threshold in the South African cohort was higher than in other studies, which may be related to HIV co-infection or drug-drug interactions with HIV therapy, though the authors did not find evidence of the latter in their analysis (18). Contrasting findings in different populations may also reflect the distribution of mitochondrial ribosomal RNA polymorphisms that have shown to have possible association with linezolid mitochondrial toxicity (19). Populations in the Caucasus region have low mitochondrial genetic diversity, so including patients from other settings could strengthen the findings of studies based in Georgia (28).

While pharmacokinetic parameters could potentially identify patients at risk of linezolid toxicity, PK analysis for therapeutic drug monitoring (TDM) is costly, time-intensive, and primarily available in research laboratories (29). Although strategies have been proposed to address these issues, such barriers limit the generalizability of TDM to settings where most patients with MDR-TB are treated (30). Alternative linezolid dosing strategies, such as an intensive phase of 1200 mg daily followed by dosing modification or discontinuation, have been proposed (18, 31). However, given the apparent safety and efficacy of long-term use of linezolid 600 mg/day, alternative dosing or PK monitoring may not be necessary. Indeed, the frequent CBC monitoring of these patients currently in use for the duration of therapy may not be needed. This would be particularly impactful in low-resource settings, where regular laboratory monitoring is challenging. Studies suggest that cytopenias develop early in therapy (13). The evidence from general use of linezolid indicates this is within a few weeks, while data from patients with TB suggest the majority occur within 2 months. However, median time to development of cytopenias in this cohort was 4 months for anemia and 11 months for thrombocytopenia; further analysis will inform whether CBC monitoring can be less frequent or even stopped after 3-4 months.

A strength of our study is its size; while not a large cohort study, the 76 participants with linezolid pharmacokinetic data constitute a large population for a PK/PD study, particularly for linezolid, and is the largest in the literature focused on the PK of linezolid use in TB. Limitations include the low frequency of certain AE outcomes, which may affect model fit. Few patients had thrombocytopenia at baseline, so our data do not provide evidence on safety of linezolid in this group. Missing CBC data remained despite supplemental data collection, which could result in missed cytopenia AE or over-representation of cytopenias in the study population. The PK data are from a single time point, which may limit the association of PK parameters with incident cytopenias late in therapy, but most cytopenias occurred early in the treatment course. Additionally, PK data for other drugs have not yet been analyzed, though the drugs included in treatment regimens for these patients are unlikely to influence the development of cytopenias either directly or via drug-drug interactions. Future directions include a time

to event analysis for linezolid-associated cytopenias utilizing Kaplan-Meier curves and survival analysis to evaluate whether cytopenias truly occurred early in therapy in this cohort.

In conclusion, while cytopenias occur frequently with long-term use of linezolid 600 mg/day in treatment of MDR-TB and may be associated with pharmacokinetic parameters, the lack of treatment-limiting adverse events on this dose suggest this essential anti-TB drug is safe for use in programmatic settings.

REFERENCES

1. World Health Organization. Global Tuberculosis Report 2020. Geneva, 2020.
2. Kim DH, Kim HJ, Park SK, et al. Treatment outcomes and long-term survival in patients with extensively drug-resistant tuberculosis. *Am J Respir Crit Care Med* 2008;178(10):1075-82.
3. Borisov SE, Dheda K, Enwerem M, et al. Effectiveness and safety of bedaquiline-containing regimens in the treatment of MDR- and XDR-TB: a multicentre study. *Eur Respir J* 2017;49(5).
4. World Health Organization. Multidrug and extensively drug-resistant TB (M/XDR-TB): 2010 global report on surveillance and response. Geneva, 2010.
5. Conradie F, Diacon AH, Ngubane N, et al. Treatment of Highly Drug-Resistant Pulmonary Tuberculosis. *N Engl J Med* 2020;382(10):893-902.
6. Schnippel K, Ndjeka N, Maartens G, et al. Effect of bedaquiline on mortality in South African patients with drug-resistant tuberculosis: a retrospective cohort study. *Lancet Respir Med* 2018;6(9):699-706.
7. Frank M, Adamashvili N, Lomtadze N, et al. Long-term Follow-up Reveals High Posttreatment Mortality Rate Among Patients With Extensively Drug-Resistant Tuberculosis in the Country of Georgia. *Open Forum Infect Dis* 2019;6(4):ofz152.
8. World Health Organization. WHO consolidated guidelines on drug-resistant tuberculosis treatment. Geneva, 2019.
9. Vinh DC, Rubinstein E. Linezolid: a review of safety and tolerability. *J Infect* 2009;59 Suppl 1:S59-74.
10. Takahashi Y, Takesue Y, Nakajima K, et al. Risk factors associated with the development of thrombocytopenia in patients who received linezolid therapy. *J Infect Chemother* 2011;17(3):382-7.
11. De Vriese AS, Coster RV, Smet J, et al. Linezolid-induced inhibition of mitochondrial protein synthesis. *Clin Infect Dis* 2006;42(8):1111-7.

12. Bernstein WB, Trotta RF, Rector JT, et al. Mechanisms for linezolid-induced anemia and thrombocytopenia. *Ann Pharmacother* 2003;37(4):517-20.

13. Cattaneo D, Orlando G, Cozzi V, et al. Linezolid plasma concentrations and occurrence of drug-related haematological toxicity in patients with gram-positive infections. *Int J Antimicrob Agents* 2013;41(6):586-9.

14. World Health Organization. WHO treatment guidelines for multidrug- and rifampicin-resistant tuberculosis, 2018 update. Geneva, 2018.

15. Lan Z, Ahmad N, Baghaei P, et al. Drug-associated adverse events in the treatment of multidrug-resistant tuberculosis: an individual patient data meta-analysis. *Lancet Respir Med* 2020;8(4):383-94.

16. Mikiashvili L, Kipiani M, Schechter MC, et al. Linezolid use for drug-resistant tuberculosis in Georgia: a retrospective cohort study. *Int J Tuberc Lung Dis* 2020;24(4):436-43.

17. Pea F, Viale P, Cojutti P, et al. Therapeutic drug monitoring may improve safety outcomes of long-term treatment with linezolid in adult patients. *J Antimicrob Chemother* 2012;67(8):2034-42.

18. Wasserman S, Denti P, Brust JCM, et al. Linezolid Pharmacokinetics in South African Patients with Drug-Resistant Tuberculosis and a High Prevalence of HIV Coinfection. *Antimicrob Agents Chemother* 2019;63(3).

19. Song T, Lee M, Jeon HS, et al. Linezolid Trough Concentrations Correlate with Mitochondrial Toxicity-Related Adverse Events in the Treatment of Chronic Extensively Drug-Resistant Tuberculosis. *EBioMedicine* 2015;2(11):1627-33.

20. Roger C, Roberts JA, Muller L. Clinical Pharmacokinetics and Pharmacodynamics of Oxazolidinones. *Clin Pharmacokinet* 2018;57(5):559-75.

21. Bigelow KM, Deitchman AN, Li SY, et al. Pharmacodynamic Correlates of Linezolid Activity and Toxicity in Murine Models of Tuberculosis. *J Infect Dis* 2020.

22. Alghamdi WA, Al-Shaer MH, An G, et al. Population Pharmacokinetics of Linezolid in Tuberculosis Patients: Dosing Regimen Simulation and Target Attainment Analysis. *Antimicrob Agents Chemother* 2020;64(10).

23. Kempker RR, Mikiashvili L, Zhao Y, et al. Clinical Outcomes Among Patients With Drug-resistant Tuberculosis Receiving Bedaquiline- or Delamanid-Containing Regimens. *Clin Infect Dis* 2020;71(9):2336-44.

24. Tukvadze N, Kempker RR, Kalandadze I, et al. Use of a molecular diagnostic test in AFB smear positive tuberculosis suspects greatly reduces time to detection of multidrug resistant tuberculosis. *PLoS One* 2012;7(2):e31563.

25. U.S. Department of Health and Human Services National Insititues of Health, National Institute of Allergy and Infectious Diseases, Division of AIDS. Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2.0. (https://rsc.niaid.nih.gov/sites/default/files/daids-ae-grading-table-v2-nov2014.pdf).

26. Lee M, Lee J, Carroll MW, et al. Linezolid for treatment of chronic extensively drug-resistant tuberculosis. *N Engl J Med* 2012;367(16):1508-18.

27. Millard J, Pertinez H, Bonnett L, et al. Linezolid pharmacokinetics in MDR-TB: a systematic review, meta-analysis and Monte Carlo simulation. *J Antimicrob Chemother* 2018;73(7):1755-62.

28. Nasidze I, Ling EY, Quinque D, et al. Mitochondrial DNA and Y-chromosome variation in the caucasus. *Ann Hum Genet* 2004;68(Pt 3):205-21.

29. Alsultan A, Peloquin CA. Therapeutic drug monitoring in the treatment of tuberculosis: an update. *Drugs* 2014;74(8):839-54.

30. Vu DH, Bolhuis MS, Koster RA, et al. Dried blood spot analysis for therapeutic drug monitoring of linezolid in patients with multidrug-resistant tuberculosis. *Antimicrob Agents Chemother* 2012;56(11):5758-63.

31. Chang KC, Yew WW, Cheung SW, et al. Can intermittent dosing optimize prolonged linezolid treatment of difficult multidrug-resistant tuberculosis? *Antimicrob Agents Chemother* 2013;57(7):3445-9.

TABLES/FIGURES

Table 1. Baseline characteristics of patients with MDR-TB initiating treatment at the NCTLD from December 2015 – May 2017.

	Overall n=100, n	Patients with available linezolid PK data n=76, n (%)
Age, years (median IQR)	37.9 (28.4, 50.9)	36.8 (27.7, 49.9)
Body mass index, kg/m^2 (median IQR)	19.9 (18.4, 22.5)	19.9 (18.4, 22.4)
Male	80	59 (77.6)
Any tobacco use[a]	51	37 (48.7)
More than 1 pack per day	27	19 (25.0)
Any alcohol use[a]	31	23 (30.3)
Heavy[b]	12	8 (10.5)
Diabetes mellitus	13	10 (13.2)
HIV infection	2	2 (2.6)
Hepatitis C antibody positive	21	19 (25.0)
Hepatitis B	2	-
Case definition		
New	47	34 (44.7)
Relapse	20	15 (19.7)
After loss to follow up	14	10 (13.2)
After failure	11	10 (13.2)
Other	8	7 (9.2)
Case definition		
New	46	33 (43.4)
Prior treatment with first-line drugs	15	10 (13.2)
Prior treatment with second-line drugs	39	33 (43.4)
Disease location		
Pulmonary only	97	75 (98.7)
Pulmonary and extrapulmonary	3	1 (1.3)
Cavitary disease	60	47 (61.8)
AFB sputum smear positive[c]	75	54 (71.1)
Extensive drug resistance	21	16 (21.1)
Baseline laboratory values (median IQR)		
White blood cells x10^3/µL	8.95 (7.4-10.8)	9.4 (7.5-11.0)
Hemoglobin g/dL	12.6 (11.3-14.1)	12.5 (11.3-14.0)
Platelets x10^3/µL	370 (291-459)	373 (306-488.5)
Creatinine µmol/L	72 (61-84)	71 (61-84)
Bilirubin µmol/L	10 (8-14)	10 (7-14)
Albumin g/dL	3.6 (3.2-4.0)	3.6 (3.2-4.0)

Abbreviations: NCTLD, National Center for Tuberculosis and Lung Diseases; MDR-TB, multidrug-resistant TB; TB, tuberculosis; PK, pharmacokinetic; IQR, interquartile range; HIV, human immunodeficiency virus

[a]Reported within 12 weeks of diagnosis

[b]>15 drinks per week for men, >8 drinks per week for women

[c]At time of new drug initiation

Table 2. Treatment characteristics and outcomes of patients with MDR-TB initiating treatment at the NCTLD from December 2015 – May 2017

	Overall n=100	Patients with available linezolid PK data n=76, n (%)
Treatment characteristics, median (IQR)		
Initial hospitalization duration, days	105 (62-189)	99 (64-144)
SLD treatment duration, days	608 (455-624)	609 (461-622)
New drug treatment duration, days	539 (360-609)	532 (348-608)
Linezolid duration, days	499 (283-599)	526 (305-607)
Treatment time not receiving linezolid, days	61 (4-136)	27.5 (0-112)
Initial companion drugs, n (%)		
Bedaquiline	64	50 (65.8)
Delamanid	30	22 (28.9)
Clofazimine	73	53 (69.7)
Imipenem	20	18 (23.7)
Pyrazinamide	11	8 (10.5)
Ethambutol	6	4 (5.3)
Levofloxacin or moxifloxacin	41	30 (39.5)
Capreomycin or kanamycin	64	45 (59.2)
Para-aminosalicylic acid	21	15 (19.7)
Cycloserine	83	62 (81.6)
Prothionamide	34	21 (27.6)
Effective drugs received, median (IQR)	4.0 (3.5–5)	4.0 (3-5)
Linezolid dose reduced	6	-
Linezolid stop reason		
Adverse event	5	4 (5.3)
Poor outcome	14	13 (17.1)
End of treatment	29	19 (25.0)
Clinical outcomes, n (%)		
Sputum culture conversion	91	69 (90.8)
Days to culture conversion (median IQR)	40 (28-63)	50 (31-63)
Culture conversion within 180 days	89	68 (98.6)
Treatment outcome		
Cured	62	45 (59.2)
Completed	10	8 (10.5)
LFU	18	16 (21.1)
Failure	7	5 (6.6)
Death	3	2 (2.6)
Favorable outcome	72	53 (69.7)
Any acquired drug resistance	17	12 (16.2)

Abbreviations: NCTLD, National Center for Tuberculosis and Lung Diseases; MDR-TB, multidrug-resistant TB; TB, tuberculosis; IQR, interquartile range; LFU, lost to follow up

Table 3. Laboratory follow up of patients with MDR-TB initiating treatment at the NCTLD from December 2015 – May 2017.

	Overall n=100	Linezolid PK data n=76, n (%)
Laboratory values, median (IQR)		
Last value		
White blood cells x10^3/mm^3 (n=57)	6.9 (5.4-8.0)	7.9 (4.9-7.9)
Hemoglobin g/dL (n=59)	14.0 (12.8-14.9)	13.9 (12.8-14.6)
Platelets x10^3/µL (n=46)	273 (192-331)	275 (189-331)
Maximum decrease from baseline		
White blood cells	3.5 (1.8-6.0)	3.7 (1.8-6.3)
Hemoglobin	0.9 (0-1.5)	0.9 (0.2-1.5)
Platelets	150.5 (77.5-216)	163.0 (89.5-262.0)
Laboratory outcomes, n (%)		
ACTG criteria, decrease from baseline	79 (79)	64 (84.2)
White blood cells by 50%	31 (31)	26 (34.2)
Hemoglobin by 25%	5 (5)	4 (5.3)
Platelets by 25%	75 (75)	61 (80.3)
Adverse event, any grade	39 (39)	31 (40.8)
White blood cells	1 (1)	1 (1.3)
Hemoglobin	31 (31)	24 (31.6)
Platelets	13 (13)	11 (14.5)
Adverse event grade 1		
White blood cells <2.5 x10^3/mm^3	1 (1)	1 (1.3)
Hemoglobin <11.0 g/dL	17 (17)	14 (18.4)
Platelets <125 x10^3/µL	10 (10)	8 (10.5)
Adverse event grade 2		
White blood cells <2.0 x10^3/mm^3	-	-
Hemoglobin <10.0 g/dL	12 (12)	9 (11.8)
Platelets <100 x10^3/µL	2 (2)	2 (2.6)
Adverse event grade 3		
White blood cells <1.5 x10^3/mm^3	-	-
Hemoglobin <9.0 g/dL	2 (2)	1 (1.3)
Platelets <50 x10^3/µL	1 (1)	1 (1.3)
Adverse event grade 4		
White blood cells <1.0 x10^3/mm^3	-	-
Hemoglobin <7.0 g/dL	-	-
Platelets <25 x10^3/µL	-	-
Follow up CBC, median (IQR)	8 (5-12)	8 (5-12)
Months to cytopenia, median (IQR)		
Any cytopenia		4 (3-7)
Hemoglobin		4 (3-6)
Platelets		11 (5-13)

Table 4. Logistic regression analysis of cytopenia adverse events among patients with MDR-TB initiating treatment at the NCTLD from December 2015 – May 2017.

Outcome	N=76, n (%)	cOR (95% CI)	aOR* (95% CI)	aOR^ (95% CI)
Any cytopenia AE	31 (40.8)			
C_{min} > 2 mg/L	5/7 (71.4)	4.13 (0.75-22.9)	3.70 (0.65-21.2)	2.78 (0.43-18.2)
C_{min} ≤ 2 mg/L	26/69 (37.8)	Ref	Ref	Ref
C_{min} > 0.27 mg/L	16/33 (48.5)	1.76 (0.70-4.44)	1.50 (0.57-3.92)	1.48 (0.52-4.19)
C_{min} ≤ 0.27 mg/L	15/43 (34.9)	Ref	Ref	Ref
AUC > 160 mg/L x h	6/10 (60.0)	2.46 (0.63-9.58)	1.92 (0.46, 7.93)	1.20 (0.24-5.96)
AUC ≤ 160 mg/L x h	25/66 (37.9)	Ref	Ref	Ref
AUC > 90.4 mg/L x h	19/36 (52.8)	2.61 (1.02-6.68)	2.34 (0.89-6.20)	1.98 (0.68-5.79)
AUC ≤ 90.4 mg/L x h	12/40 (30.0)	Ref	Ref	Ref
Platelet AE	11 (14.5)			
C_{min} > 2 mg/L	3/7 (42.9)	5.78 (1.08-30.3)	5.64 (1.01-31.4)	6.43 (0.90-45.9)
C_{min} ≤ 2 mg/L	8/69 (11.6)	Ref	Ref	Ref
C_{min} > 0.27 mg/L	7/33 (21.2)	2.62 (0.70-9.89)	2.62 (0.67-10.3)	2.89 (0.67-12.4)
C_{min} ≤ 0.27 mg/L	4/43 (9.3)	Ref	Ref	Ref
AUC > 160 mg/L x h	3/10 (30.0)	3.11 (0.67-14.5)	3.24 (0.63-16.7)	3.51 (0.56-22.2)
AUC ≤ 160 mg/L x h	8/66 (12.1)	Ref	Ref	Ref
AUC > 90.4 mg/L x h	9/36 (25.0)	6.33 (1.27-31.7)	6.81 (1.32-35.2)	8.12 (1.39-47.3)
AUC ≤ 90.4 mg/L x h	2/40 (5.0)	Ref	Ref	Ref
Hemoglobin AE	24 (31.6)			
C_{min} > 2 mg/L	4/7 (57.1)	3.27 (0.67-15.9)	2.89 (0.56-14.0)	2.00 (0.34-11.9)
C_{min} ≤ 2 mg/L	20/69 (29.0)	Ref	Ref	Ref
C_{min} > 0.27 mg/L	12/33 (36.4)	1.48 (0.56-3.91)	1.16 (0.41-3.25)	1.08 (0.35-3.36)
C_{min} ≤ 0.27 mg/L	12/43 (27.9)	Ref	Ref	Ref
AUC > 160 mg/L x h	5/10 (50.0)	2.47 (0.64-9.53)	1.74 (0.41-7.32)	1.29 (0.28-6.00)
AUC ≤ 160 mg/L x h	19/66 (28.8)	Ref	Ref	Ref
AUC > 90.4 mg/L x h	14/36 (38.9)	1.91 (0.72-5.09)	1.57 (0.56-4.41)	1.14 (0.36-3.63)
AUC ≤ 90.4 mg/L x h	10/40 (25.0)	Ref	Ref	Ref

Abbreviations: NCTLD, National Center for Tuberculosis and Lung Diseases; MDR-TB, multidrug-resistant TB; TB, tuberculosis; AE = adverse event; cOR = crude odds ratio; aOR = adjusted odds ratio cRR = crude risk ratio; aRR = adjusted risk ratio; CI = confidence interval

*Adjusted for age & gender

^Adjusted for age, gender, alcohol use & hepatitis C virus infection

Table 5. Ordinal logistic regression analysis of linezolid exposure and degree of anemia among patients with MDR-TB initiating treatment at the NCTLD from December 2015 – May 2017.

Measure	Grade AE N=76, n (%)			Higher grade anemia	
	None	1	2-3	cOR (95% CI)	aOR* (95% CI)
Linezolid C_{min}	52 (68.4)	14 (18.4)	10 (13.2)		
> 2 mg/L	3/7 (42.9)	-	4/7 (57.1)	10.9 (1.9-60.8)^	16.7 (2.20-127)^
≤ 2 mg/L	49/69 (71.0)	14/69 (20.3)	6/69 (8.7)	Ref	Ref
> 0.35	15/26 (57.7)	4/26 (15.4)	7/26 (26.9)	2.02 (0.66-6.23)	1.55 (0.47-5.05)
> 0.12 – 0.35	20/25 (80.0)	3/25 (12.0)	2/25 (8.0)	0.60 (0.17-2.19)	0.64 (0.17-2.38)
≤ 0.12	17/25 (68.0)	7/25 (28.0)	1/25 (4.0)	Ref	Ref
> 0.27 mg/L	21/33 (63.6)	5/33 (15.2)	7/33 (21.2)	1.70 (0.66-4.42)	1.38 (0.51-3.75)
≤ 0.27 mg/L	31/43 (72.1)	9/43 (20.9)	3/43 (7.0)	Ref	Ref
Linezolid AUC					
> 160 mg/L x h	5/10 (50.0)	-	5/10 (50.0)	9.40 (2.01-44.1)^	7.15 (1.35-37.8)^
≤ 160 mg/L x h	47/66 (71.2)	14/66 (21.2)	5/66 (7.6)	Ref	Ref
> 105	14/26 (53.9)	5/26 (19.2)	7/26 (26.9)	2.81 (0.88-8.93)	2.33 (0.70-7.77)
> 75.4 – 105	20/25 (80.0)	2/25 (8.0)	3/25 (12.0)	0.78 (0.21-2.90)	0.86 (0.23-3.22)
≤ 75.4	18/25 (72.0)	7/25 (28.0)	-	Ref	Ref
> 90.4 mg/L x h	22/36 (61.1)	6/36 (16.7)	8/36 (22.2)	2.21 (0.84-5.80)	1.85 (0.67-5.06)
≤ 90.4 mg/L x h	30/40 (75.0)	8/40 (20.0)	2/40 (5.0)	Ref	Ref

Abbreviations: NCTLD, National Center for Tuberculosis and Lung Diseases; MDR-TB, multidrug-resistant TB; TB, tuberculosis; AE = adverse event; cOR = crude odds ratio; aRR = adjusted odds ratio; CI = confidence interval; Cmin, trough serum concentration; AUC, area under curve

*Adjusted for age & gender

^ Polytomous logistic regression for Grade 2-3 vs none

Table 6. Ordinal logistic regression analysis of linezolid exposure and degree of thrombocytopenia among patients with MDR-TB initiating treatment at the NCTLD from December 2015 – May 2017.

Measure	Grade AE N=76, n (%)			Higher grade thrombocytopenia	
	None	1	2-3	cOR (95% CI)	aOR* (95% CI)
Linezolid C_{min}	65 (85.5)	8 (10.5)	3 (3.9)		
> 2 mg/L	4/7 (57.1)	2/7 (28.6)	1/7 (14.3)	5.70 (1.11-29.2)	5.95 (1.11-32.1)
≤ 2 mg/L	61/69 (88.4)	6/69 (8.7)	2/69 (2.9)	Ref	Ref
> 0.35	20/26 (76.9)	3/26 (11.5)	3/26 (11.5)	3.76 (0.68-20.8)	4.11 (0.71-23.9)
> 0.12 – 0.35	22/25 (88.0)	3/25 (12.0)	-	1.55 (0.23-10.4)	1.50 (0.22-10.1)
≤ 0.12	23/25 (92.0)	2/25 (8.0)	-	Ref	Ref
> 0.27 mg/L	26/33 (78.8)	4/33 (12.1)	3/33 (9.1)	2.80 (0.75-10.5)	2.85 (0.73-11.1)
≤ 0.27 mg/L	39/43 (90.7)	4/43 (9.3)	-	Ref	Ref
Linezolid AUC					
> 160 mg/L x h	7/10 (70.0)	2/10 (20.0)	1/10 (10.0)	3.15 (0.69-14.5)	3.31 (0.65-16.9)
≤ 160 mg/L x h	58/66 (87.9)	6/66 (9.1)	2/66 (3.0)	Ref	Ref
> 105	20/26 (76.9)	4/26 (15.4)	2/26 (7.7)	3.58 (0.64-19.9)	3.78 (0.65-22.0)
> 75.4 – 105	22/25 (88.0)	2/25 (8.0)	1/25 (4.0)	1.62 (0.25-10.7)	1.61 (0.24-10.7)
≤ 75.4	23/25 (92.0)	2/25 (8.0)	-	Ref	Ref
> 90.4 mg/L x h	27/36 (75.0)	6/36 (16.7)	3/36 (8.3)	6.53 (1.30-32.8)	7.07 (1.36-36.7)
≤ 90.4 mg/L x h	38/40 (95.0)	2/40 (25.0)	-	Ref	Ref

Abbreviations: NCTLD, National Center for Tuberculosis and Lung Diseases; MDR-TB, multidrug-resistant TB; TB, tuberculosis; AE = adverse event; cOR = crude odds ratio; aRR = adjusted odds ratio; CI = confidence interval; Cmin, trough serum concentration; AUC, area under curve

*Adjusted for age & gender

Figure 1. Study population of patients with MDR-TB initiating treatment at the NCTLD from December 2015 – May 2017.

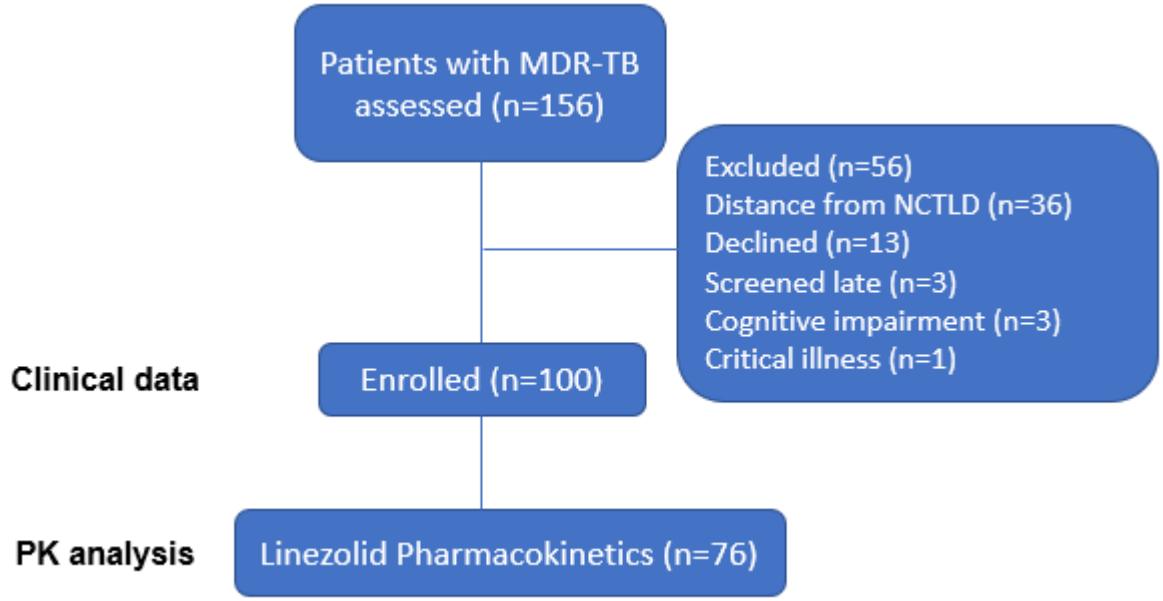
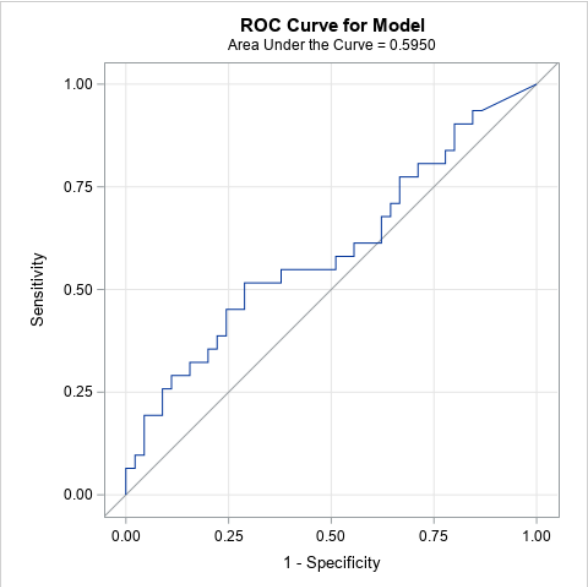


Figure 2. Receiver operating characteristic curves modeling any cytopenia as outcome with linezolid pharmacokinetic parameters (C_{min} and AUC) as exposure.

C_{min}



AUC