


Linezolid toxicity in patients with drug-resistant tuberculosis: a prospective cohort study

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Background: Linezolid is recommended for treating drug-resistant TB. Adverse events are a concern to prescribers but have not been systematically studied at the standard dose, and the relationship between linezolid exposure and clinical toxicity is not completely elucidated.

Patients and methods: We conducted an observational cohort study to describe the incidence and determinants of linezolid toxicity, and to determine a drug exposure threshold for toxicity, among patients with rifampicin-resistant TB in South Africa. Linezolid exposures were estimated from a population pharmacokinetic model. Mixed-effects modelling was used to analyse toxicity outcomes.

Results: One hundred and fifty-one participants, 63% HIV positive, were enrolled and followed for a median of 86 weeks. Linezolid was permanently discontinued for toxicity in 32 (21%) participants. Grade 3 or 4 linezolid-associated adverse events occurred in 21 (14%) participants. Mean haemoglobin concentrations increased with time on treatment (0.03 g/dL per week; 95% CI 0.02–0.03). Linezolid trough concentration, male sex and age (but not HIV positivity) were independently associated with a decrease in haemoglobin >2 g/dL. Trough linezolid concentration of 2.5 mg/L or higher resulted in optimal model performance to describe changing haemoglobin and treatment-emergent anaemia (adjusted OR 2.9; 95% CI 1.3–6.8). SNPs 2706A>G and 3010G>A in mitochondrial DNA were not associated with linezolid toxicity.

Conclusions: Permanent discontinuation of linezolid was common, but linezolid-containing therapy was associated with average improvement in toxicity measures. HIV co-infection was not independently associated with linezolid toxicity. Linezolid trough concentration of 2.5 mg/L should be evaluated as a target for therapeutic drug monitoring.

Introduction

Rifampicin-resistant TB (RR-TB) accounts for an expanding proportion of incident global TB cases and is an ongoing threat to EndTB targets.¹ Linezolid is a repurposed oxazolidinone antimicrobial with bactericidal activity against *Mycobacterium tuberculosis*.² Inclusion of linezolid in treatment regimens for RR-TB is associated with treatment success and mortality reduction,^{3,4} as a result, WHO guidelines now recommend linezolid as a preferred agent for RR-TB.⁵

The major drawback of linezolid is binding to human mitochondrial 16S rRNA, which has a homologous structure to the *M. tuberculosis* target site,⁶ resulting in dose-related

mitochondrial toxicity that manifests most commonly as bone marrow suppression and peripheral neuropathy. These toxic effects may be treatment limiting.^{7,8} The incidence and risk factors for linezolid toxicity have not been systematically studied in TB programmes,⁹ particularly in populations from sub-Saharan Africa with high rates of HIV co-infection, which could increase the risk of toxicity.¹⁰ Factors possibly associated with linezolid toxicity include age, sex and polymorphisms in mitochondrial DNA (mtDNA).^{11–13} Overlapping complications from comorbidities (e.g. peripheral neuropathy secondary to diabetes, HIV, or alcohol abuse) may also contribute. Estimating frequency and identifying risk profiles for serious linezolid toxicity will support deployment of this drug in programmatic settings.

An approach to mitigate toxicity is through optimized linezolid dosing, which requires characterization of the exposure–toxicity relationship.¹⁴ Standard linezolid dosing in RR-TB (600 mg daily) is likely to achieve an *in vitro* efficacy target for *M. tuberculosis* and reduce the emergence of resistance.¹⁵ Trough concentrations are inversely correlated with mitochondrial function,¹⁶ haemoglobin concentration in mouse models^{17,18} and clinical toxicity among patients with Gram-positive infection.^{12,19} Pharmacokinetic (PK)–toxicity targets have been suggested from small clinical studies, but these are not adequately established for patients with TB.^{12,16} Linezolid trough concentrations correlate with AUC (the target PK parameter for efficacy),²⁰ suggesting a potential role for therapeutic drug monitoring (TDM) if a concentration threshold target for clinical toxicity is defined.²¹

We aimed to describe the incidence and determinants of linezolid toxicity, and to determine a drug exposure threshold for toxicity, among patients with RR-TB in a programmatic setting with a high HIV burden.

Patients and methods

Design and population

This analysis was nested in a prospective observational cohort study (PROBeX) conducted at three drug-resistant TB referral hospitals in South Africa. The parent PROBeX study recruited 195 adults with known HIV status and culture-confirmed RR-TB who were initiating treatment with a bedaquiline-containing regimen between April 2016 and March 2018.²² During the study period, local treatment guidelines recommended an 18–24 month regimen. Linezolid was provided at a dose of 600 mg daily, with reduction to 300 mg daily at the discretion of treating clinicians if toxicity developed. Linezolid was recommended for the full treatment course if tolerated, but the duration was determined by treating clinicians. Treatment decisions were informed by clinical assessments and routine toxicity screening, which included monthly full blood counts; linezolid TDM was not performed.

Procedures

Participants were followed until 6 months after completion of therapy, or up to 24 months after study entry, at the start of bedaquiline therapy. Study visits occurred monthly during the first 6 months of therapy, then 6 monthly until study exit. Phlebotomy was performed at every visit for full blood count and lactate; results of these tests performed in routine care outside of study visits were also obtained. The modified Brief Peripheral Neuropathy Scale (BPNS) was used to screen for peripheral neuropathy.²³ We assessed visual acuity using logMAR charts and colour vision using 14-plate Ishihara charts to screen for optic neuropathy. Neuropathy screening was done at every study visit. Abnormal findings from laboratory and bedside testing were shared with treating clinicians in real time. mtDNA was extracted from stored whole blood and the 16S rRNA gene sequenced to detect two SNPs (2706A>G and 3010G>A) previously associated with linezolid-induced mitochondrial toxicity (Methods S1, available as [Supplementary data](#) at JAC Online).¹¹

PK data

We did intensive PK sampling (pre- and at 1, 2, 3, 4, 5, 6 and 24 h post-dose) on a subgroup of 21 participants at Month 2 and sparse (pre-dose) PK sampling for the full cohort at Months 1, 2 and 6 after initiation of linezolid therapy. Linezolid concentrations were measured in the Division of Clinical Pharmacology at the University of Cape Town using a validated LC–MS/MS assay.²⁰ We developed a population PK model using these

data and derived average linezolid AUC over 24 h (AUC_{0-24}) and trough values for individual participants, based on body weight and time-varying linezolid dose.²⁴

Outcome definitions

The main outcome was linezolid toxicity measured by cytopenia, peripheral and optic neuropathy, and hyperlactataemia. We defined anaemia, thrombocytopenia, leukopenia and hyperlactataemia according to Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events; Version 2.1. Peripheral neuropathy was graded according to the modified BPNS score.²⁵ Optic neuropathy was defined as an increase of 0.3 on the logMAR score in either eye²⁶ or a reduction in colour vision score of >2.²⁷ We performed exploratory data analysis to identify thresholds for toxicity measures by observing distribution and trends of haematological parameters and lactate over time and relationship with baseline values. Early discontinuation of linezolid was defined as a permanent stop prior to 6 months of therapy.

Analysis

Kaplan–Meier survival curves were computed to analyse and plot the timing of event onset; median times were reported for participants who experienced events. The primary outcome of interest was change in haemoglobin concentration from baseline. The key covariates were linezolid exposure, duration on linezolid, age, sex and HIV status. Effect of risk-associated mtDNA SNPs was also explored. To describe changing continuous outcomes, we fitted linear mixed-effects regression models incorporating baseline controlling variables and time-varying covariates (linear time effect and linezolid exposure). We used conditional logistic regression for repeated toxicity events and computed marginal probabilities to represent risk; this approach was selected to incorporate multiple recurring events and account for within-individual correlation through inclusion of participant-specific random effects.²⁸ Internal model validation was performed using a k-fold cross-validation procedure.²⁹ We performed a piecewise (broken stick) regression procedure to identify the optimal threshold value of linezolid exposure that predicted clinical toxicity based on best model fit of linear regression models as measured by Akaike Information Criteria at multiple values of linezolid trough concentrations.^{30,31}

The study was not formally powered as the predictors of linezolid toxicity or PK/pharmacodynamic (PD) relationships are not well characterized. A *post hoc* power calculation showed that a sample size of around 150 participants would have >95% probability of detecting a 95% CI with precision (width) of at least 0.18 for anaemia, given a SD of 0.51 in our sample.

All analyses were performed with Stata/BE 17.0 (Statacorp).

Ethics

This study was approved by the Human Research Ethics Committee at the University of Cape Town (437/2016), Albert Einstein College of Medicine and Emory University. All participants provided written informed consent prior to performance of study procedures. The study was conducted and reported according to STROBE guidelines.

Results

Characteristics of study population

We included 151 participants out of 195 enrolled in the parent cohort; 44 were excluded because of no documented linezolid prescription ($n=38$) or absent toxicity measure after starting linezolid ($n=6$). Baseline characteristics are shown in Table 1; 63% were HIV positive and 66% had fluoroquinolone-resistant

TB. In addition to linezolid, all participants received bedaquiline; clofazimine, levofloxacin, pyrazinamide, terizidone and para-aminosalicylic acid were provided to over 95%; and ethambutol was prescribed for 74 participants (49%). Prior to starting linezolid, the median haemoglobin was 11.8 g/dL (range 6.4–17.9). Median follow-up from start of linezolid therapy was 86 weeks (range 3–183). A single A>G substitution at position 2706 was detected in 124 (87%) participants; no SNPs were detected at position 3010.

Linezolid therapy and PK

The starting linezolid dose was 600 mg daily in 148 participants and 300 mg in 3 participants. The median duration of linezolid therapy, excluding treatment interruptions, was 336 days (IQR 159–506; range 6–862). Linezolid dose was reduced for 31 (21%) participants at a median time of 69 days (IQR 36–147). Linezolid was permanently discontinued in 32 (21%) participants at a median time of 60 days (IQR 20–99); 10 (31%) patients had either dose reduction or interruption prior to early discontinuation (Table 2 and Figure 1).

The individual PK parameters were derived from a population PK model based on observed concentrations for 95 participants and were predicted (based on weight and dose) for the other 56 participants with no measured linezolid concentrations. Median linezolid AUC_{0–24} was 168.9 mg·h/L (IQR 143–194) and trough concentration was 2.1 mg/L (1.8–2.3) for the 600 mg dose (Figure S1). There was an exponential relationship between AUC_{0–24} and trough concentrations, which were highly correlated (Figure S2).

Linezolid toxicity events

Cumulative incidence of any new grade anaemia or peripheral neuropathy DAIDS event at 6 months was 39% (95% CI 31–47) and 20% (95% CI 14–27), respectively, with similar median time to experiencing the event: 11 weeks (IQR 7–17) for anaemia and 10 weeks (IQR 7–23) for neuropathy (Figure 2). New grade 3 or 4 events occurred in 21 participants: cumulative incidence 14% (95% CI 9–21) at 6 months. Sixteen participants had reductions in visual acuity with a cumulative incidence of 12% (95% CI 8–20) at 24 months; median time to onset was 10 weeks (range 5–79 weeks). Linezolid was dose reduced or permanently discontinued in five participants with reduced visual acuity. Only one participant experienced a reduction in colour vision (Table 3).

Additional toxicity outcomes were defined based on the observed data: anaemia, haemoglobin reduction >2 g/dL; thrombocytopenia, platelet reduction >250 × 10⁹/L; leukopenia, white cell count reduction >4 cells × 10⁹/L; and hyperlactataemia, lactate increase >1.5 mmol/L (Table S1). Using these definitions, cumulative incidence of anaemia at 6 months was 33% (95% CI 26–41), thrombocytopenia 16% (95% CI 11–23), leukopenia 20% (95% CI 14–28) and hyperlactataemia 15% (95% CI 10–22).

Relationship between linezolid exposure and toxicity

A linezolid trough concentration of 2.5 mg/L resulted in optimal model fit to describe association with change in haemoglobin compared with other breakpoint values using piecewise

Table 1. Baseline characteristics

	n (%) or median (IQR)	Denominator
Age (years)	34 (28–42)	151
Female sex	84 (56)	151
Ethnicity		151
Black	127 (84)	
Mixed race	22 (15)	
Other	2 (1)	
Weight (kg)	53 (47–60)	150
BMI (kg/m ²)	20 (18–22)	149
HIV positive	95 (63)	151
CD4 count (cells/mm ³)	212 (111–438)	91
Antiretroviral therapy	81 (85)	95
Previously treated TB	111 (74)	151
Resistance pattern of baseline isolate		141
MDR	12 (9)	
Pre-XDR (injectable)	36 (26)	
Pre-XDR (fluoroquinolone)	32 (23)	
XDR	61 (43)	
Creatinine (μmol/L)	62 (51–71)	150
Creatinine clearance (mL/min)	105 (88–125)	149
Haemoglobin (g/dL)	11.8 (10.4–13.2), range: 6.4–17.9	148
WBC count (×10 ⁹ cells/L)	7.3 (5.3–9.5), range: 1.4–27.1	148
Platelets (×10 ⁹ /L)	345 (271–489), range: 132–1131	148
Venous lactate (mmol/L)	1.7 (1.3–2.3), range: 0.8–6.5	115
BPNS grade		121
0	100 (83)	
1	17 (14)	
2	4 (3)	
logMAR score (right)	0 (range 0–1.0)	113
logMAR score (left)	0 (range 0–0.8)	111
Ishihara score	6 (6–6)	123
mtDNA 16S rRNA polymorphism		
2706A>G	124 (87)	142
3010G>A	0 (0)	142

MDR, multidrug resistant (resistance to rifampicin plus isoniazid); XDR, extensively drug resistant (additional resistance to fluoroquinolones and injectable agents); pre-XDR (additional resistance to either fluoroquinolones or injectable agents).

regression (Table S2 and Figure S3). There was a clear time trend for the onset of anaemia, defined as a drop in haemoglobin >2 g/dL, during the first 6 months of linezolid therapy: of the 47 participants who experienced anaemia, 43 (91%) events occurred within 120 days. Eight out of 9 (89%) participants with a linezolid trough concentration above 2.5 mg/L in this period had anaemia; 38% (21/55) with trough concentrations below this threshold had no anaemia (Figure 3).

Table 2. Details of linezolid interruption

Parameter	n=151
Linezolid changes	
Dose reduction	31 (21%)
Interruption then dose reduction	6 (4%)
Interruption then same dose	10 (7%)
Early discontinuation	32 (21%)
Linezolid duration	
Until first interruption/change	69 days (IQR 36–147; range 4–530)
Duration of interruption	42 days (IQR 28–85; range 5–315)
Until early discontinuation	60 days (IQR 20–99; range 12–179)
Total duration	336 days (IQR 159–506; range 6–862)

Data are number (percentage) or median (IQR).

Early discontinuation defined as permanent discontinuation before 6 months.

Ten (31%) patients had either dose reduction or interruption prior to early discontinuation.

Total duration excludes time off linezolid during treatment interruptions.

Factors associated with linezolid toxicity measures

Mean haemoglobin was predicted to increase with time on treatment (0.03 g/dL per week; 95% CI 0.02–0.03) and with a higher pre-treatment haemoglobin (0.6 g/dL; 95% CI 0.5–0.7); and to decrease with increasing linezolid trough concentrations (–0.2 g/dL per 1 mg/L; 95% CI –0.3 to –0.1), HIV positivity (–0.5 g/dL; 95% CI –1.0 to –0.1) and age (–0.3 g/dL per 10 years; 95% CI –0.5 to –0.1) (Figure 4 and Figure S4A). Thirty-one percent of total variability was due to inter-individual variability. Model-predicted haemoglobin at 4 weeks was 8.2 g/dL (95% CI 7.8–8.8) for an HIV-positive participant with the lowest pre-

treatment haemoglobin of 6.4 g/dL (at observed values of other parameters).

Average platelet count, WBC count and lactate decreased over time when adjusted for baseline values, HIV status, age, gender and linezolid trough concentrations (Figure S4B–D). Lactate increase was associated with linezolid trough concentrations (0.08 mmol/L increase per 1 mg/L linezolid trough, 95% CI 0.01–0.2). There was an inverse association between linezolid trough concentrations and both platelet count (–11.4 × 10⁹/L, 95% CI –19.7 to –3.1) and white cell count (–0.2 cells × 10⁹/L, 95% CI –0.3 to –0.02). Sensitivity analysis was done for all outcomes including only PK estimates from measured concentrations, without substantial change in parameter estimates.

Factors independently associated with anaemia, defined as a reduction in haemoglobin >2 g/dL, were linezolid trough concentration [adjusted OR (aOR) 1.4 per 1 mg/L increase, 95% CI 1.1–1.8], male sex (aOR 3.4, 95% CI 1.5–8.1) and age (aOR 1.7 per 10 year increase, 95% CI 1.2–2.3). HIV positivity was not a significant predictor (aOR 1.2, 95% CI 0.5–2.9). There was large inter-individual variability (rho=0.47). Marginal predictions for probability of anaemia are shown in Figure 5. A linezolid trough concentration ≥2.5 mg/L was associated with 2.9-fold increased odds (95% CI 1.3–6.8) of anaemia in the adjusted model. There was also a significant association between linezolid trough concentration and thrombocytopenia and hyperlactataemia (Table S3 and Table S4), but not with neuropathy (Table S5). There was no effect modification with inclusion of the mtDNA A2706G mutation in any model (data not shown). Model performance and parameter estimates were similar for all toxicity outcomes when AUC_{0–24} was tested instead of trough concentration (data not shown).

Discussion

In this cohort of South African RR-TB patients with an HIV prevalence of 63%, mild anaemia and peripheral neuropathy occurred frequently, and linezolid was prematurely discontinued in a fifth of patients. However, severe adverse events were infrequent and, on average, linezolid use in a multidrug regimen was associated with a positive treatment effect on haemoglobin over time. We identified a trough concentration threshold that predicted higher risk of anaemia, the most specific measure of linezolid toxicity, which, if validated, could be used for TDM.

Linezolid-associated haematological and neurological toxicity is a major concern for prescribers.³² The most recent systematic review, published in 2015, summarized data from 14 retrospective studies and 1 randomized controlled trial; all but 1 study included fewer than 50 patients, and there was large heterogeneity in outcome definitions and treatment. The pooled proportion of adverse events leading to linezolid discontinuation was 29%, with anaemia and peripheral neuropathy reported in 31% and 27%, respectively.⁷ Importantly, none of the included studies was conducted in Africa where high rates of HIV co-infection and limited monitoring capability may exacerbate the risk of linezolid toxicity.¹⁰ A recent small prospective study (n=63) among South African patients with RR-TB and a high HIV prevalence described similar proportions with anaemia and neuropathy at the 600 mg dose, with linezolid interruption or

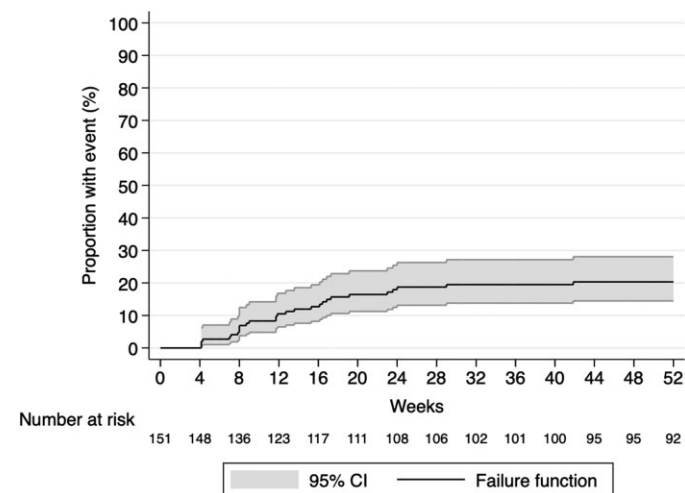


Figure 1. Time to early discontinuation of linezolid at 52 weeks. Kaplan-Meier plot with survival estimates for early discontinuation of linezolid, defined as permanent stop prior to completing 6 months of therapy.

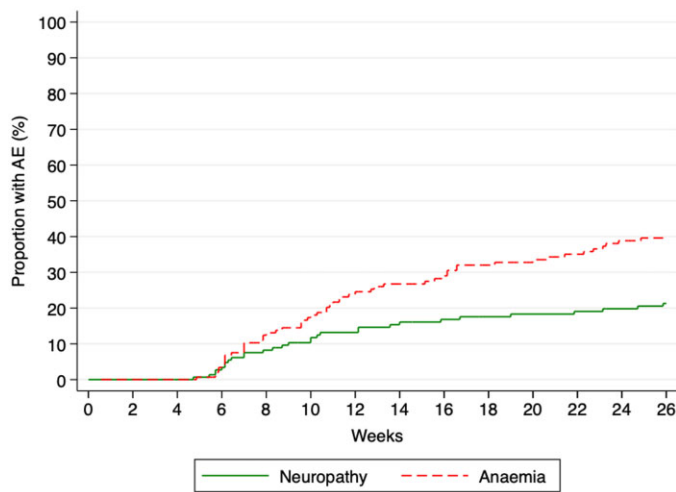


Figure 2. Time to development of any grade adverse event for anaemia or peripheral neuropathy. Kaplan–Meier plot with superimposed individual survival estimates for anaemia and neuropathy. Anaemia defined as any new grade on DAIDS grading and neuropathy defined as any new grade on BPNS score. AE, adverse event. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

discontinuation in 35%, but severity was not reported, and it is unclear how outcomes were ascertained.⁹

To obtain more reliable estimates of toxicity, we defined haematological events using the established DAIDS grading system and used the validated BPNS scale to screen for peripheral neuropathy. We identified incident severe (grade 3 or 4) adverse events in 14% of our participants. Anaemia followed by mild

peripheral neuropathy were the most common adverse events, which is in line with other TB studies.^{3,8} Most adverse events occurred within the first 4 months of therapy, with similar timing of onset for anaemia and neuropathy at a median of around 10 weeks. Neuropathy has occurred relatively later than myelosuppression in some studies, leading to suggestions of a duration-dependent effect for neurotoxicity.^{3,33,34} However, these studies were limited by small size and lack of consistent outcome definitions, and there is no clear biological explanation for this hypothesis. The onset of peripheral neuropathy in the Nix-TB trial, which used a higher dose of linezolid, occurred mainly in the initial 3 months of treatment, consistent with our findings.⁸ Cumulative incidence of reduced visual acuity was 12% at 24 months in our cohort, the earliest detected at 5 weeks after starting linezolid. This is within the range reported from other studies,⁷ but is likely an overestimate of true linezolid-induced optic neuropathy because visual acuity testing lacks specificity,³⁵ and many participants were on concomitant ethambutol, which can also cause ocular toxicity. In the Nix-TB trial, optic nerve disorders were suspected in 11.9% by bedside testing, but confirmed optic neuropathy only occurred in two (<2%) participants.^{8,36}

There were no grade 3 or 4 thrombocytopenia events in our cohort. Platelets are acute-phase reactants, increasing in response to systemic inflammation, including from TB,^{37,38} while haemoglobin changes in the opposite direction.³⁹ The negative correlation between platelet counts and haemoglobin over time in our data suggests that reductions in platelets represent reduction in systemic inflammation due to treatment rather than linezolid toxicity. Therefore, platelets are not a good PD marker for linezolid toxicity in TB.

Average haemoglobin increased over time after adjustment for other factors. HIV positivity was independently associated

Table 3. New adverse events after starting linezolid

	Number of participants with any event (n=151)	Grade 1	Grade 2	Grade 3	Grade 4	Cumulative incidence of any grade at 6 months (95% CI)	Event rate (per 100 person-weeks) (95% CI)
Anaemia	58	24	14	13	6	39% (31–47)	0.8 (0.6–1.0)
Thrombocytopenia	10	8	2	0	0	6% (3–11)	0.1 (0.0–0.2)
Leukopenia	6	5	0	1	0	4% (2–9)	0.1 (0.0–0.2)
Peripheral neuropathy	37 ^a	32	4	0	1	20% (14–27)	0.4 (0.3–0.5)
Reduced visual acuity	16 ^b	–	–	–	–	9% (5–15)	0.1 (0.1–0.2)
Worsening colour vision	1 ^c	–	–	–	–	–	–
Hyperlactataemia	51 ^d	43	8	–	–	31% (24–40)	0.6 (0.5–0.8)

These data are for the highest-grade adverse event experienced by individual participants.

Anaemia, thrombocytopenia, leukopenia and hyperlactataemia were defined according to DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Events; Version 2.1.

Data on grade 3 or 4 hyperlactataemia were not collected as associated symptoms were not ascertained and pH was not measured.

Peripheral neuropathy was graded according to the modified BPNS score.

Optic neuropathy was defined as an increase of 0.3 on the logMAR score in either eye or a reduction in colour vision score of >2 on a 14-plate Ishihara chart.

^an=121.

^bn=119.

^cn=123.

^dn=115.

with reduced haemoglobin, but not with anaemia (reductions >2 g/dL). This effect was likely related to underlying HIV-related myelosuppression, as only two participants were on zidovudine. In the Nix-TB trial, there was also no increase in linezolid-associated adverse events among HIV-positive participants.⁸ Independent predictors of anaemia in our cohort were age, male sex and linezolid trough concentrations, which have been associated with linezolid toxicity in patients with Gram-positive infections.^{12,13} In our cohort, the predicted probability of substantial haemoglobin reduction was $\sim 10\%$ for male participants at the median values of age and linezolid trough concentrations (Figure 5), indicating relative safety of the 600 mg daily dose in our population. Despite this, linezolid was interrupted, dose reduced, or discontinued early in over half of participants, suggesting either the presence of unmeasured adverse events or a low threshold by clinicians to alter or stop therapy due to concerns about toxicity potential.³²

Hyperlactataemia is a complication of linezolid therapy due to mitochondrial injury,⁴⁰ and there have been case reports of lactic acidosis.^{41,42} Data are scarce on the incidence of hyperlactataemia in cohort studies. In the Nix-TB trial, there were only eight cases (three had lactic acidosis), much lower than the 30% incidence in our study.^{8,36} Possible reasons for this discrepancy include technical issues relating to sample processing, a sicker population in our study and different definitions of hyperlactataemia. Nonetheless, there were relatively few severe events, with only 12 grade 2 episodes and 15% with increases >1.5 mmol/L at 6 months; on average, lactate decreased over time on linezolid therapy.

The presence of SNPs at positions 2706 and 3010 in mitochondrial 16S rRNA have been reported in association with hyperlactataemia during linezolid therapy and are hypothesized to confer genetic susceptibility to linezolid toxicity through enhanced binding to mitochondrial structures.¹¹ The G3010A SNP

was not detected in any of our participants and the presence of A2706G was not associated with any toxicity measure, corroborating findings from a trial among Korean drug-resistant TB patients.¹⁶

Linezolid is a good candidate for TDM in RR-TB because of its narrow therapeutic margin and large inter-individual variability.^{20,24} Linezolid trough concentrations are consistently associated with haematological toxicity measures,^{12,16–18,43} including in our cohort. A trough threshold of 2 mg/L has been suggested based on the high proportion of clinical events observed above that value among Korean XDR-TB patients in a small trial ($n=38$).¹⁶ Although this target is now widely applied in PK/PD analyses, the specificity is poor, and it has not been validated in other cohorts. Using a model-based approach, we found that a trough concentration of ≥ 2.5 mg/L described change in haemoglobin better than other tested values and had a large effect on risk of significant haemoglobin drop after adjustment for other factors—this finding has potential for use in TDM to reduce risk of adverse events. Where TDM is unavailable, close clinical and haematological (especially haemoglobin) monitoring could trigger linezolid dose changes once toxicity develops.⁴⁴

There are limitations to consider when interpreting our findings. There was no planned phlebotomy or neuropathy screening in the first month of our study, which may have contributed to the low event rate observed within the first few weeks of linezolid. Although we obtained all full blood count results from routine care, bedside haemoglobin testing was not captured, neither were blood transfusions, potentially masking more severe anaemia. However, a strength of our study is that it reflects real-world practice and outcomes. The observational nature of the study resulted in unbalanced visits and missing observations, but random effects models are valid under flexible missing data assumptions, including missingness at random,⁴⁵

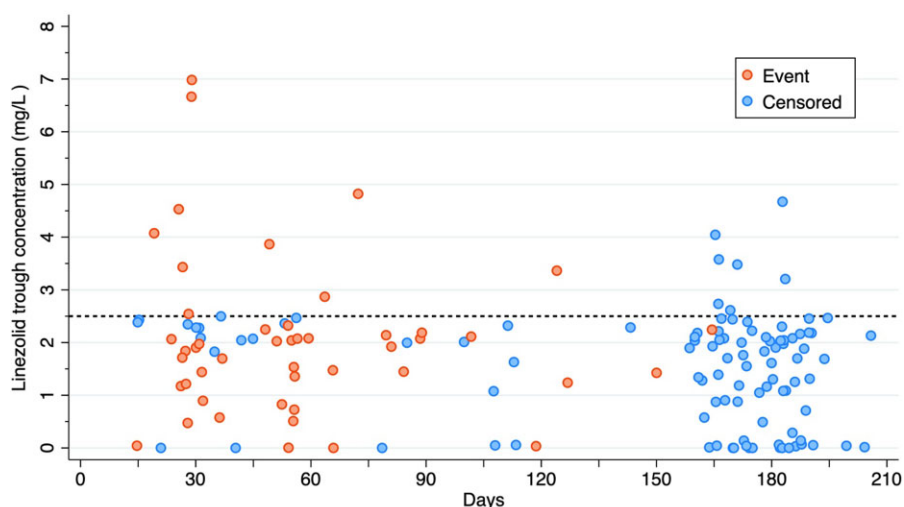


Figure 3. Observed relationship between anaemia events and linezolid trough concentrations during the first 6 months of linezolid therapy. Events defined as reduction in haemoglobin >2 g/dL in red circles; censoring at 6 months without anaemia, and for lost to follow-up, and death in blue circles. Dashed line indicates trough concentration of 2.5 mg/L. This figure appears in colour in the online version of JAC and in black and white in the print version of JAC.

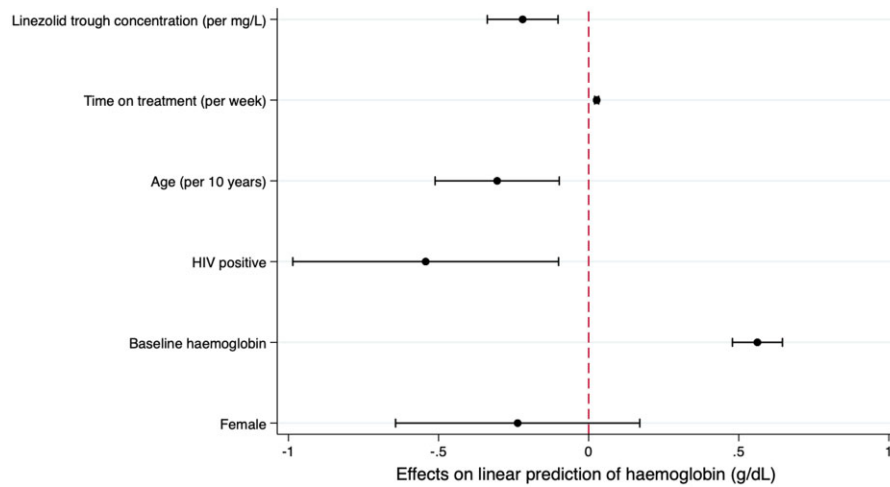


Figure 4. Predictors of longitudinal haemoglobin measures over the study period. Estimates of mean effects on haemoglobin from the mixed-effects linear regression model. Dots indicate point estimate; black lines indicate 95% CI; dashed red line indicates no effect. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

supporting our conclusions. Linezolid concentrations were missing for a third of participants and individual drug exposures were predicted from a population PK model based on measured body weight and dose. The model was developed using rich data from a subgroup of participants in our cohort and, on sensitivity analysis, inclusion of sparse concentrations did not alter model performance. A limitation of using trough values is that they are

strongly influenced by other model parameters, plus uncertainty in dosing timing. We addressed this by including separate additive error and additive lag variability relative to reported time of the dose to account for uncertainty in unobserved dosing (affecting sparse samples).²⁴ Additionally, there was no effect modification on parameter estimates when only values with observed concentrations were included in toxicity outcome models.

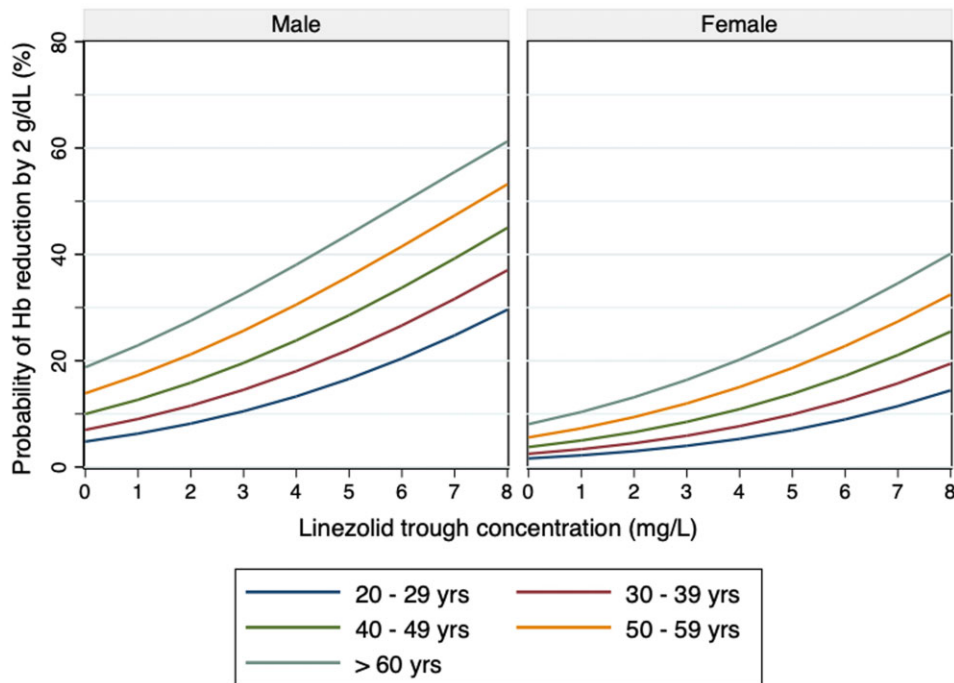


Figure 5. Predicted probability of anaemia by sex. Marginal predictions from mixed-effects logistic regression model for probability of anaemia, defined as reduction in haemoglobin ≥ 2 g/dL. Coloured lines indicate age ranges, defined in the key. Hb, haemoglobin. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

Finally, our study was not formally powered, influencing the precision of our estimates and ability to detect relationships with smaller effects. There were relatively few observations above our identified toxicity concentration threshold of 2.5 mg/L, emphasizing the need to validate this finding. However, our sample size is larger than that used in previous studies, which successfully identified PK/PD relationships for first-line TB treatment,^{46,47} and for other studies evaluating linezolid toxicity.^{9,16}

In summary, we characterized linezolid toxicity in a drug-resistant TB treatment programme among patients with high HIV prevalence. Severe events were uncommon at the standard dose of 600 mg daily in this setting and, overall, linezolid use was associated with improvement haemoglobin and other toxicity measures. A trough concentration threshold of 2.5 mg/L should be further evaluated as a potential target for TDM of this important TB drug.

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Transparency declarations

None to declare.

Supplementary data

Tables S1 to S5, Figures S1 to S4 and Methods S1 are available as Supplementary data at JAC Online.

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