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Increased linezolid plasma concentrations are associated with the development of severe toxicity in MDR-TB treatment.

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Summary of article's main point: Our study provides evidence on the association between the detection of even single linezolid trough concentrations >2 mg/L and severe toxicity using comprehensive time-to-toxicity analyses. This paves the way for the design of prospective studies on TDM-based prevention of toxicity.

Abstract

Background: Treatment of multidrug-resistant tuberculosis (MDR-TB) with linezolid is characterized by high rates of adverse events. Evidence on therapeutic drug monitoring to predict drug toxicity is scarce. This study aimed to evaluate the association of linezolid trough concentrations with severe toxicity.

Methods: We retrospectively assessed consecutive patients started on linezolid for MDR-TB between 2011 and 2017. The primary outcome was severe mitochondrial toxicity (SMT) due to linezolid, defined as neurotoxicity or myelotoxicity leading to drug discontinuation. The impact of plasma linezolid trough concentrations >2 mg/L was assessed in multivariate Cox proportional hazards models including time-varying covariates.

Results: SMT occurred in 57 of 146 included patients (39%) at an incidence rate of 0.38 per person-year (95%CI 0.30-0.49). A maximum linezolid trough concentration >2 mg/L was detected in 52 patients (35.6%), while the mean trough concentration was >2 mg/L in 22 (15%). The adjusted hazard ratio for SMT was 2.35 (95%CI 1.26–4.38, p=0.01) in patients with a mean trough concentration >2 mg/L and 2.63 (95%CI 1.55–4.47, p<0.01) for SMT after the first detection of a trough concentration >2 mg/L. In an exploratory analysis, higher maximum trough concentrations were dose-dependently associated with toxicity, while lowering of elevated trough concentrations did not restore baseline risk.

Conclusion: Linezolid trough concentrations >2 mg/L are strongly associated with the development of severe treatment-emergent toxicity in patients treated for MDR-TB. Pending further prospective evidence, an individual risk-benefit assessment on the continuation of linezolid treatment is warranted in any patient with trough concentrations above 2 mg/L.

Introduction

Tuberculosis (TB) is the world's most deadly infectious disease[1]. Multidrug-resistant tuberculosis (MDR-TB) is difficult to treat and burdened by high toxicity rates[2,3]. Following a large meta-analysis of individual patient data, World Health Organization (WHO) guidelines for the treatment of MDR-TB underwent a major revision in 2018: bedaquiline, linezolid and fluoroquinolones were strongly associated with improved outcomes and have been since recommended for all MDR-TB patients[4,5]. However, the safety profile of conventional regimens based on revised WHO guidelines remains sub-optimal[6]. Adverse events during TB treatment reduce adherence and ultimately worsen outcomes[1].

Routine use of therapeutic drug monitoring (TDM) to optimize safety and efficacy of anti-tuberculosis treatment has long been advocated[7–10]. Linezolid is a first-line component of conventional MDR-TB regimens. However, toxicity occurs in more than half of patients during long-term treatment with linezolid[11], and leads to permanent discontinuation in 16% of cases[12]. Linezolid exerts its tissue-dependent toxicity through the inhibition of mitochondrial protein synthesis[13]. Development of toxicity has been linked to daily dose[14], treatment duration, and total number of doses[15]. While the correlation between linezolid AUC/MIC and efficacy is well established[16], the PK/PD-parameter best predicting toxicity risk during long term treatment with linezolid has yet to be determined. Available evidence points towards a role of elevated trough concentrations, rather than the AUC[17–19]. Linezolid trough plasma concentrations above 2 mg/L have been found to be associated with mitochondrial enzyme inhibition and clinical toxicity [20]. However, routine TDM for linezolid is currently not recommended by WHO due to uncertain clinical impact and implementation challenges.

The primary objective of this study was therefore to assess the association of routinely measured linezolid plasma trough concentrations with toxicity in patients treated for MDR-TB.

Methods

Study design and participants

In this retrospective cohort study, we included consecutive MDR-TB patients treated at the referral hospitals of Bligny (Briis-sous-Forges, France), Pitié-Salpêtrière and Bichat (both Paris, France) between January 1st, 2011 and December 31st, 2017 to ensure treatment completion upon final data collection in January 2020. Standard definitions of drug-resistant TB were used[21]. Patients were eligible for inclusion when treated for at least four weeks with linezolid, as linezolid-related adverse events were deemed unlikely at shorter treatment durations. Patients were followed until the end of anti-tuberculosis treatment. Ethical clearance was granted by the Institutional Review Board of Bligny Hospital (January 15th, 2020; CRE 2020 01). The study was registered at Assistance Publique-Hôpitaux de Paris (20201218174223).

Procedures

Treatment regimens were designed according to then current WHO treatment guidelines and re-evaluated by the French MDR-TB Consilium, a multidisciplinary team coordinated by the French National Reference Center for Mycobacteria (NRC) [4,22,23]. Linezolid was started at 600 mg orally once daily (qd) in all patients; subsequent dose adaptations were subject to clinical judgement. The cumulative linezolid dose was standardized to bodyweight and treatment duration. Sputum smear and cultures were obtained at treatment start, every 14 days until culture conversion and monthly thereafter. Phenotypic drug susceptibility testing (DST) was performed at NRC using the Löwenstein–Jensen method[24]. Genotypic DST was obtained through line-probe assays (GenoType MTBDRplus and

GenoType MTBDRsl; Hain Lifescience, Nehren, Germany) or DNA sequencing. During the study period, French national guidance was to measure linezolid plasma concentrations at least once during treatment and as needed thereafter. There was no specific guidance on how to adjust linezolid dosing[25]. Plasma trough concentrations at the end of a dosing interval (24h, 12h or 8h) were performed by UPLC-MS/MS[26]. Mean trough concentrations were calculated from independent measurements; if only one measurement was available, the mean was replaced by the single available measurement. Maximum and mean trough concentrations were categorized into >2 mg/L and ≤2 mg/L based on previous findings[20]. A TDM-based adaptation of linezolid treatment was defined as any change in linezolid daily dose following plasma concentration measurement. Clinical reasons for dose changes/discontinuation comprised primary prevention of toxicity, prevention of aggravation of early toxicity, and planned treatment completion.

Outcomes

The primary outcome was severe linezolid-related toxicity (severe mitochondrial toxicity, SMT) and defined as follows: (1) myelotoxicity (thrombopenia, leukopenia, and/or anemia), (2) peripheral neuropathy, or (3) optic neuropathy. Severity criteria were either a Grade ≥3 adverse event (CTCAE Severity Scale, v5.0)[27], a serious adverse event according to FDA criteria[28], or an adverse event leading to permanent discontinuation of linezolid. Causality was assessed by WHO-UMC criteria and required to be probable or certain[29]. An event was defined by the timepoint of incipient severe toxicity, i.e. the first report of symptoms or dose adaptation in response to clinical signs of toxicity, whichever came first. Myelotoxicity was diagnosed via weekly routine blood tests. Peripheral neuropathy was diagnosed clinically. If in doubt, electromyography (EMG) and nerve conduction studies (NCS) were

performed. Routine surveillance for optic neuropathy was performed monthly by testing visual acuity, visual field, and color vision. Severity of symptoms was assessed by the main data-extracting investigator (JE) based on medical records. In equivocal cases, a second investigator (LG) was consulted for consensus-based classification.

Data sources and measurement

Routinely collected clinical data were extracted from medical records and anonymized. The data-extracting investigator was not involved in patient care. Comprehensive DST profiles were obtained from NRC. Pharmacological data were obtained from Saint-Joseph and Bichat Hospitals. Data were cleaned, checked for consistency, and reported in accordance with STROBE. Data manipulation and analysis was performed using STATA/IC (v16.1, StataCorp, College Station, TX, USA).

Study sample size

Sample size calculation was based on previously available data showing that 100% (95%CI 82%-100%) and 42% (95%CI 20%-67%) of patients experienced any linezolid-related adverse event at mean linezolid trough concentrations >2 and ≤2 mg/L, respectively[20]. We defined the impact of a mean linezolid trough level ≤2 and > 2mg/L on SMT as the primary endpoint of the study. We assumed a hazard ratio of 2.38, 50% severe adverse events[11], 20% of patients with mean trough concentrations >2 mg/L and a median toxicity-free survival time of 24 months, resulting in a sample size of n=105 to achieve 80% power and 5% type I error rate.

Statistical methods

For descriptive statistics, continuous variables were reported as medians with interquartile range (IQR) or means with standard deviation (SD) and compared using the Wilcoxon rank-sum test or two-sample t-test, as appropriate. Categorical variables were reported as frequencies with percentages and compared using Fisher's exact test or chi-squared test, as appropriate. A two-sided $\alpha < 0.05$ was considered statistically significant. No routine imputation of missing variables was done. For the primary analysis, a counting process survival data set with static and time-varying covariates was used (Supplementary methods). As the mean trough concentration does not take into account timing and level of contributing drug levels, a series of time-to-event analyses were performed with time-varying covariates defining time at risk only after the actual detection of the trough concentration to reduce immortal time bias. Incidence rates were calculated per person-time on linezolid treatment. Toxicity-free survival was analyzed with Kaplan-Meier curves and using multivariate Cox proportional hazards models, reporting adjusted hazard ratios that approximate the incidence rate ratio between categories adjusted for relevant covariates[30]. Where the proportional hazard assumption was not met, survival data was compared using the logrank test. Patients entered the survival analysis upon the first TDM measurement and were censored upon end of treatment, death, or loss to follow-up. Missing end dates of linezolid treatment were replaced by the date of last news. Sensitivity analyses were carried out (restricted analysis at linezolid 600mg qd only, stratification by number of TDM measurements). For regression analysis, a stepwise backwards hierarchical approach was applied for covariate selection and model definition[31]. Variables were included into the model if they predicted the outcome at $p < 0.20$ in univariate analysis and fulfilled the proportional hazards assumption [32] (Supplementary methods).

Results

Population characteristics

During the study period, 155 MDR-TB patients were treated with linezolid (≥4 weeks) in the participating centers. After exclusion of nine patients without TDM measurements, 146 (94.2%) patients were included in the analysis (Table 1). Half of the included patients had pre-XDR- or XDR-TB (50.6%). Prior courses of second-line treatment were reported by 47.9% of patients (Table 1). Additional resistance against at least two more second-line drugs was common (72.6%). The prevalence of HIV co-infection was 6.8%.

Treatment and treatment monitoring

Median treatment duration was 18 months (IQR 15-24). Linezolid was administered at 600 mg qd during 87.6% of person-time (135.1 of 154.1 person-years, Supplementary Figure 4) and 86.6% of TDM measurements were performed at that dose (329/380). Two or more trough concentrations were documented in 96 patients (65.8%). Among all trough concentrations, 18.7% (71/380) were >2 mg/L. A maximum trough level of >2 mg/L was documented in 52 individuals (35.6%), while 22 patients had a mean trough level of >2 mg/L (15.1%, Table 2). Current renal function was reported with 44% of TDM measurements, with eGFR >50 mL/min in all cases. Maximum trough levels >2 mg/L were associated with dose increase, increased dosing frequency or resumption of treatment after a temporary hold, whereas patients with maximum trough concentrations <2 mg/L were more likely to remain on baseline dosing for the entire treatment, and to have linezolid stopped for reasons other than toxicity (Figure 1). The distribution of further baseline covariates, cumulative and

average doses of linezolid were similar between maximum trough concentration groups (Tables 1 and 2).

Treatment-related toxicity and treatment outcomes

Overall, 111 patients (76.0%) experienced a treatment-emergent adverse event (Table 3). Sixty patients (41.1%) were affected by more than one adverse event. Eighty-two patients (56.2%) suffered an adverse event attributed to linezolid. Peripheral neuropathy was the most frequent manifestation (33.6%), followed by myelotoxicity (11.0%,) and optic nerve neuropathy (8.9%). Severe adverse events (SMT) occurred in 57 patients (39%) at an incidence rate of 0.38 events per person-year (95%CI 0.30–0.49) over a total of 161.2 person-years of linezolid treatment. Two patients with comorbidities and extensive disease died one and three months after linezolid discontinuation for peripheral neuropathy, respectively. In neither of these cases, linezolid was identified as the sole culprit but may have contributed to progressive central nervous deficit. Favorable tuberculosis treatment outcome was achieved in 118 patients (84%, Table 3), irrespectively of whether linezolid had to be discontinued prematurely for toxicity or not (83% vs. 86%, p = 0.65).

Toxicity risk as a function of linezolid trough concentrations

Both overall linezolid toxicity and SMT were significantly more common in patients with a maximum trough level >2 mg/L (Table 3, Figure 2). The difference in proportions was even more pronounced when analyzed by mean trough level >2 and ≤2 mg/L (overall linezolid toxicity: 81.8% vs. 51.6%, p <0.01; SMT: 68.2% vs. 33.9%, p <0.01). In the prespecified primary time to toxicity analysis, patients with mean trough concentrations >2 mg/L had an incidence rate of 0.91 events per person-year (95%CI 0.55-1.51), while patients with lower

mean trough concentrations only suffered 0.36 events per person-year (95%CI 0.26–0.48) (Figure 3a). In the corresponding Cox proportional hazards model, the adjusted hazard ratio (aHR) for SMT in patients with mean trough concentrations of >2 mg/L was 2.35 (95%CI 1.26–4.38, p=0.01) (Figure 3a, Table 4). The first detection of a linezolid trough concentration >2 mg/L entailed an aHR of 2.63 for SMT (95%CI 1.55–4.47, p<0.01) (Figure 3b). Stratification by number of total measurements as well as restricted analysis of patients who remained at 600 mg qd of linezolid without treatment changes (n = 107/146 (73%)) yielded comparable SMT-free survival (Supplementary Figure 1). A previous trough concentration >4 mg/L was associated with a further increase of SMT compared to trough concentrations between 2 and 4 mg/L (rate ratio 1.72, 95%CI 0.80–3.73, log-rank p = 0.16) (Figure 3c). Conversely, trough level reduction below 2 mg/L after a previously elevated level did not show a strong trend towards reversal of toxicity risk (rate ratio 0.79, 95%CI 0.33–1.85, log-rank p = 0.58). Even though not reaching statistical significance, the detection of several consecutive low trough concentrations <2 mg/L showed a trend towards further risk reduction compared to a single trough level <2 mg/L (rate ratio 0.51, 95%CI 0.23–1.10, log-rank p=0.08) (Figure 3d).. Of note, the cumulative dose of linezolid per kilogram bodyweight corrected for treatment duration was not significantly associated with toxicity (Supplementary Table 3).

Discussion

In this retrospective cohort of patients treated with linezolid as part of individualized MDR-TB regimens, both mean and maximum linezolid trough concentrations >2 mg/L were independently associated with increased risk of linezolid-related severe toxicity. In survival analysis, achievement of a maximum trough concentration >2mg/L more accurately reflected risk modification.

Based on a larger sample, our results confirm findings from a post-hoc analysis of a trial in which 100% of XDR-TB patients treated with linezolid developed attributable toxicity at mean trough concentrations >2 mg/L, compared to 58% at ≤2 mg/L[20]. Corresponding rates in our cohort were 82% and 52%, respectively. A proportion of 68% severe linezolid-related adverse events is also comparable to data from a large meta-analysis reporting 73%[11].

The observed significant association between the detection of even a single trough level >2 mg/L and severe linezolid-related toxicity in our cohort is novel. A subset of the cohort (n=57) has previously been studied, finding an odds ratio (OR) of 2.07 (95%CI 0.6–7.3, p = 0.26) for clinical neurotoxicity at maximum linezolid trough concentrations >2 mg/L[33]. After independent data collection and with increased statistical power, our analysis demonstrated a comparable and highly significant OR of 2.37 (95%CI 1.2– 4.8, p=0.02), strengthening validity of our findings within the study population. Our findings further point towards a lowered toxicity risk with consistently low trough concentrations <2 mg/L and a risk increase upon detection of outliers >4 mg/L. Risk reversal seems to be incomplete when lowering previously elevated trough concentrations, which contrasts findings in earlier studies[34]. However, these findings need to be interpreted with caution given the low TDM

frequency and low number of patients per stratum. In terms of clinical reversibility, established neurotoxicity resolved in 28.6% of patients until end of treatment. Similarly, Jaspard et al. described 22% resolution with a longer follow-up of 12 months after end of treatment[33]. This translates into a number needed to harm for irreversible neurotoxicity of 4.2, highlighting the potential long-term impact on quality of life.

Elevated trough concentrations could represent blips or periods of elevated drug concentrations. In this real-life data, individual sampling timepoints were not systematic and possibly correlated. Stratification by number of total measurements demonstrated a consistent risk difference, making the possibility of an unmeasured risk factor prompting more frequent measurements less likely. The known delay of linezolid neurotoxicity of weeks to months, even at higher doses of linezolid (1200 mg daily), argues against elevated short-term exposure as the cause of toxicity [35]. By contrast, the lack of a risk reversal by lowering previously elevated trough concentrations in our cohort points towards the possibility that even a short period of elevated exposure might be sufficient to promote development of toxicity with latency. This could apply to neurotoxicity in particular with its weak correlation to daily dose as opposed to myelotoxicity[12]. The lack of an association between cumulative dose of linezolid and toxicity in our data further lends support to this notion, contrasting findings from a previous observational study[15].

The study has limitations. The prespecified primary analysis of trough concentrations by an approximated mean was based on a limited number of measurements per patient. As a retrospective marker, aggregate mean trough concentrations cannot be used for predictive purposes and introduce immortal time bias in survival analysis[36]. Our time-varying analyses of risk after exceeding the specified trough level threshold attenuate the latter. Moreover, the retrospective study design restricts information on toxicity to clinical

documentation favoring classification errors. Diagnosis and grading of peripheral neuropathy were not standardized, nor was the management of SMT by clinicians. The dilemma of subjectivity is highlighted by the two covariates included in the multivariate Cox model: Awareness of the crucial efficacy of linezolid and limited remaining treatment options could explain that patients with a challenging treatment profile developed linezolid toxicity later (Supplementary figure 2). Similarly, other severe adverse events may have lowered the threshold to declare subsequent linezolid toxicity. However, neither of these covariates confounded the independent effect of elevated trough concentrations on toxicity.

Certain strengths of the study also deserve mentioning. The design as a high-resolution survival dataset allowed for detailed assessment of the temporal relationship between treatment monitoring, dose changes and toxicity. Despite the limited number of TDM measurements per patient in our study, the prevalence of linezolid trough concentrations ≤2mg/L in patients receiving 600 mg qd in our data is in line with findings from a large PK/PD meta-analysis[37]. This validates our results from a real-life setting and suggests broader applicability. Our study further represents a comprehensive evaluation of linezolid TDM in the absence of clearly-defined indications and management strategies: therefore, our results may have relevant implications from a cost-benefit perspective and inform the design of future studies. Limited sampling strategies and centralised TDM could provide a feasible framwork[38].

In conclusion, TDM shows promise for the optimization of the risk-benefit-ratio in linezolid-based MDR-TB regimens. Our findings corroborate and extend existing evidence on the association between increased linezolid trough concentrations and toxicity. Yet, they also highlight the need for more longitudinal pharmacometric data such as the PanDR

substudy within the EndTB trials (ClinicalTrials.gov: NCT03827811) to better understand how to maintain linezolid in target range[39]. In any case, the topic will remain highly relevant as linezolid maintains a pivotal role in the new shortened regimens[40]. Pending additional evidence, we suggest a thorough individual risk-benefit assessment for the continuation of linezolid treatment, including assessment of disease severity and remaining treatment options, in any patient with linezolid trough concentrations above 2 mg/L.

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Conflict of interest

None of the authors reports any conflicts of interest.

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Tables

Table 1: Baseline disease characteristics and demographics

Table 2: Treatment

Table 3: Toxicity and outcomes

Table 4: Multivariate Cox proportional hazards model

Table 1: Baseline disease characteristics and demographics, by maximum linezolid trough concentrations (≤2 vs. >2 mg/L). Continuous variables are presented as medians with interquartile ranges unless otherwise stated. Categorical variables are presented in absolute numbers (percentage %).

		Max. trough level		
	Total	*≤2 mg/L*	*>2 mg/L*	*p-value*
	N=146	N=94	N=52	
Age	34 (11)	34 (11)	34 (12)	0.95
Male gender	108 (74.0%)	75 (79.8%)	33 (63.5%)	0.03
Referred from MDR-TB reference center[a]	73 (50.0%)	41 (43.6%)	32 (61.5%)	0.04
Origin: Eastern WHO European Region	82 (56.2%)	50 (53.2%)	32 (61.5%)	0.33
Imprisonment	18 (12.3%)	11 (11.7%)	7 (13.5%)	0.76
HIV	10 (6.8%)	8 (8.5%)	2 (3.8%)	0.29
BMI (kg/m2)	20.2 (2.9)	20.2 (2.9)	20.1 (2.8)	0.91
Intravenous drug use	37 (25.3%)	24 (25.5%)	13 (25.0%)	0.94
Drug resistance level				0.76
RR-TB/MDR-TB	72 (49.3%)	48 (51.1%)	24 (46.2%)	
Pre XDR-TB	37 (25.3%)	24 (25.5%)	13 (25.0%)	
XDR-TB	37 (25.3%)	22 (23.4%)	15 (28.8%)	
Previous second-line treatment	70 (47.9%)	42 (44.7%)	28 (53.8%)	0.29
LZD resistance	1 (0.7%)	1 (1.1%)	0 (0.0%)	0.47
Additional second-line drug resistance[b]	106 (72.6%)	67 (71.3%)	39 (75.0%)	0.63
Bilateral pulmonary involvement	92 (63.0%)	57 (60.6%)	35 (67.3%)	0.42
Cavitary lung disease	101 (69.2%)	64 (68.1%)	37 (71.2%)	0.70
Sputum smear positive[c]	105 (71.9%)	69 (73.4%)	36 (69.2%)	0.59
Sputum culture positive[c]	119 (81.5%)	79 (84.0%)	40 (76.9%)	0.29
Extrapulmonary TB	41 (28.1%)	29 (30.9%)	12 (23.1%)	0.32

a = Pitié Salpêtrière and Bichat University Hospitals, Paris. b = Two or more second line drugs other than linezolid, fluoroquinolones and aminoglycosides. c = at treatment start.

Abbreviations: TB = tuberculosis; WHO = World Health Organization; BMI = body-mass index; MDR-TB = multidrug-resistant tuberculosis; RR-TB = rifampicin-resistant tuberculosis; XDR-TB = extensively drug resistant tuberculosis

Table 2: Treatment, by maximum linezolid trough concentrations inferior to and greater than 2 mg/L. Continuous variables are presented as medians with interquartile ranges unless otherwise stated. Categorical variables are presented in absolute numbers (percentage %).

		Max. trough level		
	Total	*≤ 2 mg/L*	*> 2 mg/L*	*p-value*
	N=146	N=94	N=52	
Anti-tuberculosis treatment				
Total treatment duration (months)	18 (15-24)	18 (15-24)	18 (15-23)	0.80
Adjunctive surgical treatment	32 (21.9%)	19 (20.2%)	13 (25.0%)	0.50
Drugs with potential neuro- or myelotoxicity[a]				
Fluoroquinolone	105 (71.9%)	67 (71.3%)	38 (73.1%)	0.82
Ethionamide	42 (28.8%)	29 (30.9%)	13 (25.0%)	0.45
Cycloserine	118 (80.8%)	79 (84.0%)	39 (75.0%)	0.18
Linezolid treatment				
Total duration (days)	359 (168-553)	374 (137-584)	346 (201-535)	0.92
Average dose (mg/d, mean)	594 (59)	593 (39)	596 (83)	0.78
Cumulative dose (mg/kg*d, mean)	10.19 (1.95)	10.04 (1.76)	10.46 (2.26)	0.21
Therapeutic drug monitoring				
N° of measurements (n per 90 days, mean)	0.9 (0.7)	0.8 (0.6)	1.0 (0.7)	0.04
Time to first TDM (days)	22 (12-40)	22 (12-39)	21 (9.5-43)	0.70
Dose adaptation following TDM (n)	30 (20.5%)	14 (14.9%)	16 (30.8%)	0.02
Mean LZD trough level (unit)	1.20 (1.10)	0.62 (0.17)	2.24 (1.54)	<0.01
Mean LZD trough level > 2 mg/L (n)	22 (15.1%)	0 (0.0%)	22 (42.3%)	<0.01

a = used for at least four weeks during treatment. Abbreviations: LZD linezolid; TDM therapeutic drug monitoring.

Table 3: Toxicity and outcomes, by maximum linezolid trough concentrations inferior to and greater than 2 mg/L. Continuous variables are presented as medians with interquartile ranges unless otherwise stated. Categorical variables are presented in absolute numbers (percentage %).

		Max. trough level		
	Total	*≤ 2 mg/L*	*> 2 mg/L*	*p-value*
	N=146	N=94	N=52	
Treatment-emergent adverse event (all)	111 (76.0%)	65 (69.1%)	46 (88.5%)	<0.01
LZD-related toxicity	82 (56.2%)	45 (47.9%)	37 (71.2%)	<0.01
Severe mitochondrial toxicity (SMT)	57 (39.0%)	27 (28.7%)	30 (57.7%)	<0.01
Time to SMT (days)	214 (131-420)	200 (84-461)	235 (155-412)	0.45
Other drugs stopped for toxicity				
Any time	79 (54.1%)	46 (48.9%)	33 (63.5%)	0.09
Before linezolid	73 (50.0%)	43 (45.7%)	30 (57.7%)	0.17
Myelotoxicity	16 (11.0%)	11 (11.7%)	5 (9.6%)	0.70
Myelotoxicity (severe[a])	6 (37.5%)	4 (36.4%)	2 (40.0%)	0.89
Time to severe toxicity (days)	292.5 (154-491)	448.5 (292.5-509.5)	92 (30-154)	0.06
Resolution of symptoms	11 (73.3%)	6 (60.0%)	5 (100.0%)	0.10
Peripheral neuropathy	49 (33.6%)	25 (26.6%)	24 (46.2%)	0.02
Peripheral neuropathy (severe[a])	41 (83.7%)	18 (72.0%)	23 (95.8%)	0.02
Time to severe toxicity (days)	214 (103-376)	158.5 (68-358)	234 (155-412)	0.11
Resolution of symptoms	14 (28.6%)	8 (32.0%)	6 (25.0%)	0.59
Optic neuropathy	13 (8.9%)	7 (7.4%)	6 (11.5%)	0.41
Optic neuropathy (severe[a])	10 (76.9%)	5 (71.4%)	5 (83.3%)	0.61
Time to severe toxicity (days)	309.5 (181-467)	200 (181-505)	367 (252-466)	0.92
Resolution of symptoms	9 (69.2%)	6 (85.7%)	3 (50.0%)	0.16
Outcome (WHO)				0.81
Cure/Completion	118 (80.8%)	78 (83.0%)	40 (76.9%)	
Failure	4 (2.7%)	2 (2.1%)	2 (3.8%)	
Died	2 (1.4%)	1 (1.1%)	1 (1.9%)	
Loss to follow up	22 (15.1%)	13 (13.8%)	9 (17.3%)	
Time to culture conversion (days)	70 (40-103)	70 (38-102)	76 (41-106)	0.86
Treatment adherence	111 (79.3%)	71 (79.8%)	40 (78.4%)	0.85

a = Fulfilling definition of severe mitochondrial toxicity. Abbreviations: LZD linezolid; SMT severe mitochondrial toxicity.

Table 4: Multivariate Cox proportional hazards model for severe mitochondrial toxicity.

	aHR	p-value	95%CI	
Linezolid mean trough level (> 2mg/L)	2,35	0,01	1,26	4,38
Challenging treatment profile[a]	0,42	<0,01	0,24	0,74
Other severe treatment-emergent adverse event[b]	1,61	0,09	0,92	2,79

Adjustment for age after linear transformation. Abbreviations: aHR adjusted hazard ratio, CI confidence interval. a = Composite variable defined by one or more of the following: Increased drug resistance level (MDR vs. Pre-XDR/XDR-TB), intravenous drug use, alcohol abuse, history of imprisonment, previous second line anti-tuberculosis treatment. b = Adverse event preceding toxicity due to linezolid (time-varying).

Figure legends

Figure 1: Proportion of patients on a specific linezolid treatment status by maximum trough level category over 24 months of follow-up. The stacked bar plots represent relative proportions of treatment status in the study population still on MDR-TB treatment at a given month (upper graph) and the entire study population including patients after end of treatment (lower graph). The baseline dose was 600 mg per day. A dose reduction as a response to therapeutic drug monitoring results is distinguished from a dose reduction or temporary hold due to incipient toxicity. The resumption of a previously held treatment as well as any increase in dose or frequency are summed up in the category "Increased/Restart" as a potential risk action. Linezolid discontinuation for other reason refers to permanent preventive or clinically-guided discontinuation (planned treatment duration and/or efficacy achieved). Regarding favorable outcome, any patient declared as having a favorable outcome by completion of treatment before 18 months either had very limited disease or had been partly treated abroad prior to re-initiation of treatment in France.

Figure 2: Swimmer plot comparing the individual linezolid treatment course of the entire cohort stratified by a maximum trough level ≤2 and >2 mg/L. Bar color transitions from grey (= no TDM measurement performed, i.e. not at risk yet), via blue (= maximum trough ≤2 mg/L) to red (= maximum trough level >2 mg/L) correspond to at risk and exposure status in survival analysis. The upper cluster of each side shows patients affected by severe mitochondrial toxicity, the lower cluster patients who discontinued linezolid treatment for

other reasons (i.e. preventively or for clinical reasons (planned, efficacy achieved)). Each bar represents an individual patient (Y-axis). The X-axis represents days elapsed since initiation of treatment with linezolid.

Figure 3: Kaplan-Meier Survival curves for toxicity free survival from severe mitochondrial toxicity. (A) By mean trough level >2 mg/L or ≤2 mg/L. Panels B, C and D represent time varying analyses by acquired status over time avoiding immortal time bias: (B) After achievement of a maximum trough level >2 mg/L or ≤2 mg/L. (C) Semi-quantitative assessment after a maximum trough level <2 mg/L, between 2 and 4 mg/L and above 4 mg/L. (D) Risk modification by trough concentrations sequences examining consistency and reversibility.

All graphs: Y-axis = probability of freedom from severe mitochondrial toxicity. X-axis = days since treatment start. The table below the x-axis shows individuals at risk.

Figures

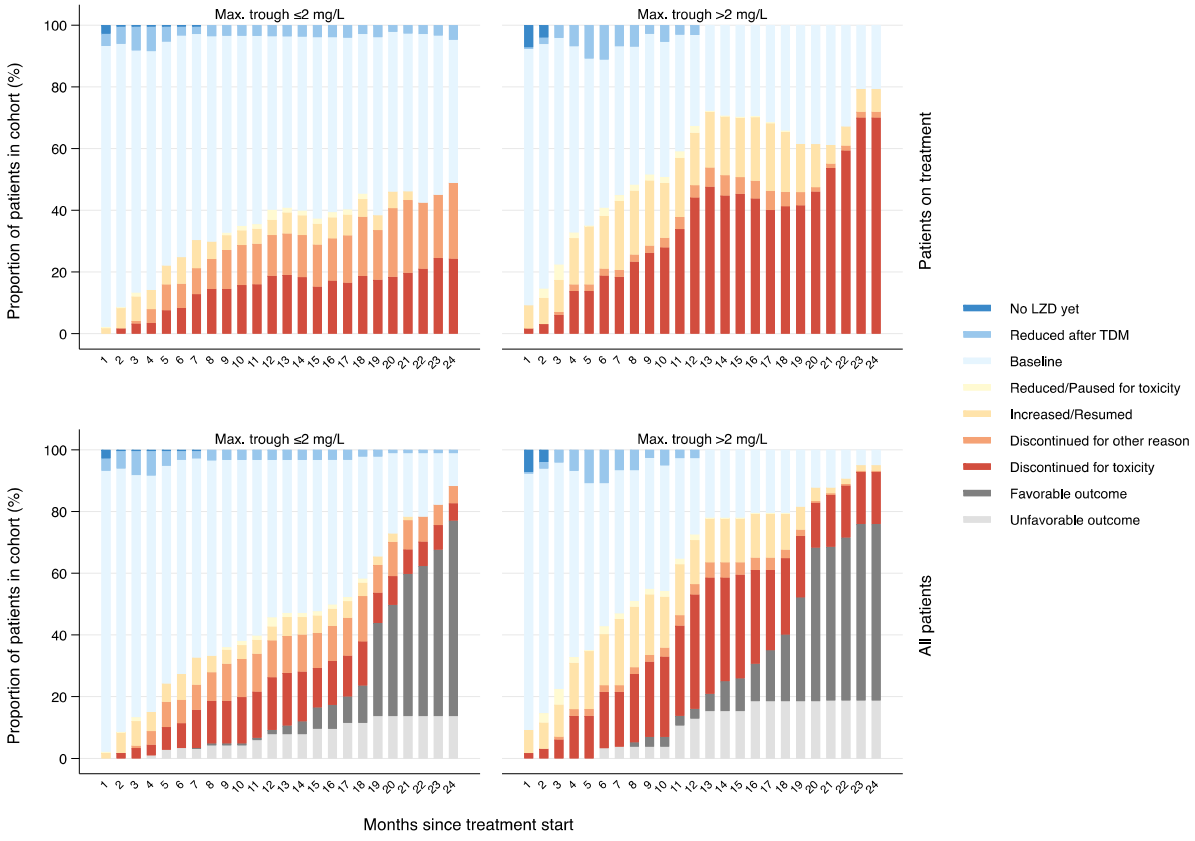


Figure 1

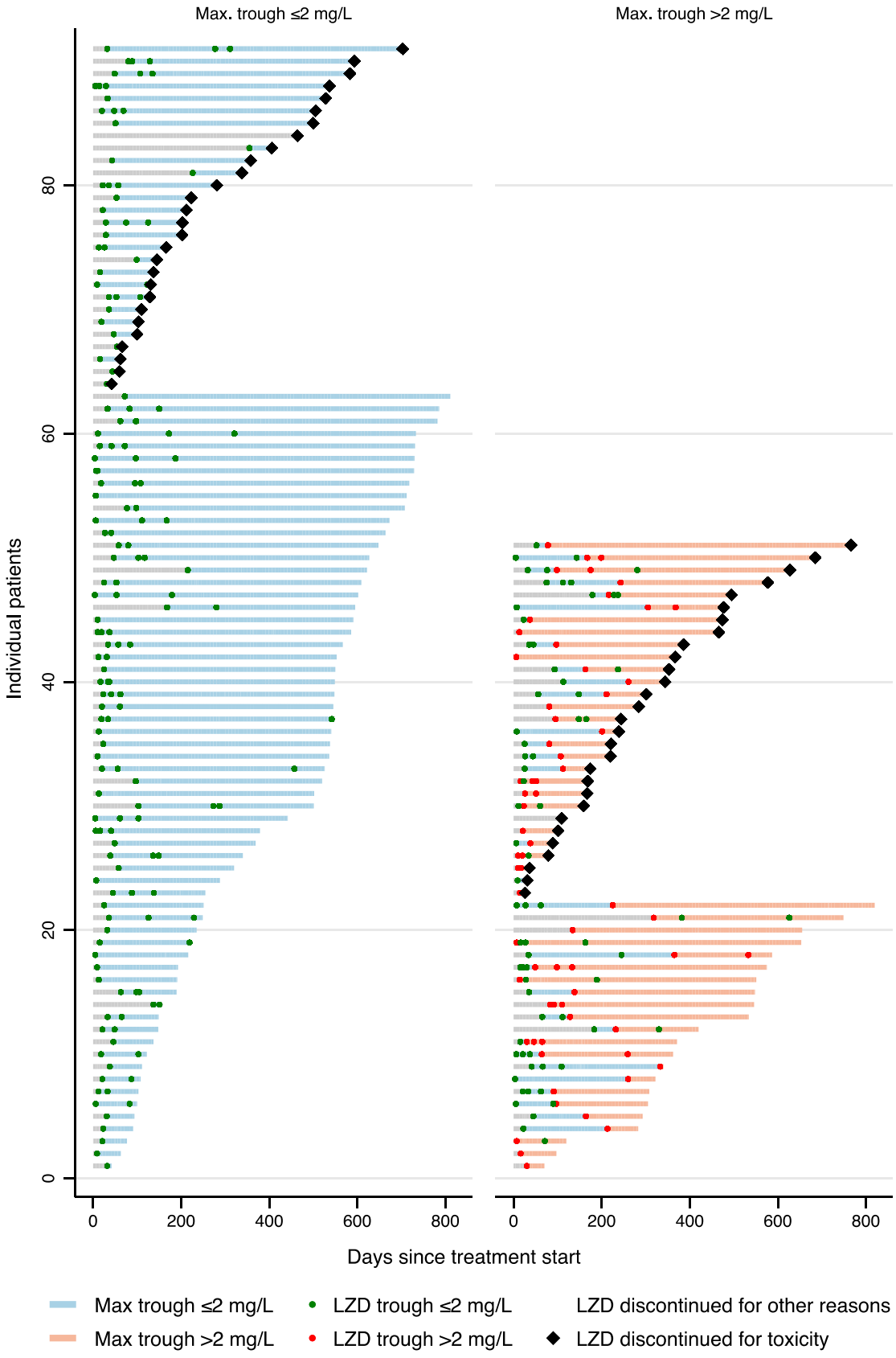

Max. trough ≤2 mg/L
Max. trough >2 mg/L
Individual patients
0
20
40
60
80
0
200
400
600
800
Days since treatment start
Max trough ≤2 mg/L
Max trough >2 mg/L
LZD trough ≤2 mg/L
LZD trough >2 mg/L
LZD discontinued for other reasons
LZD discontinued for toxicity

Figure 2

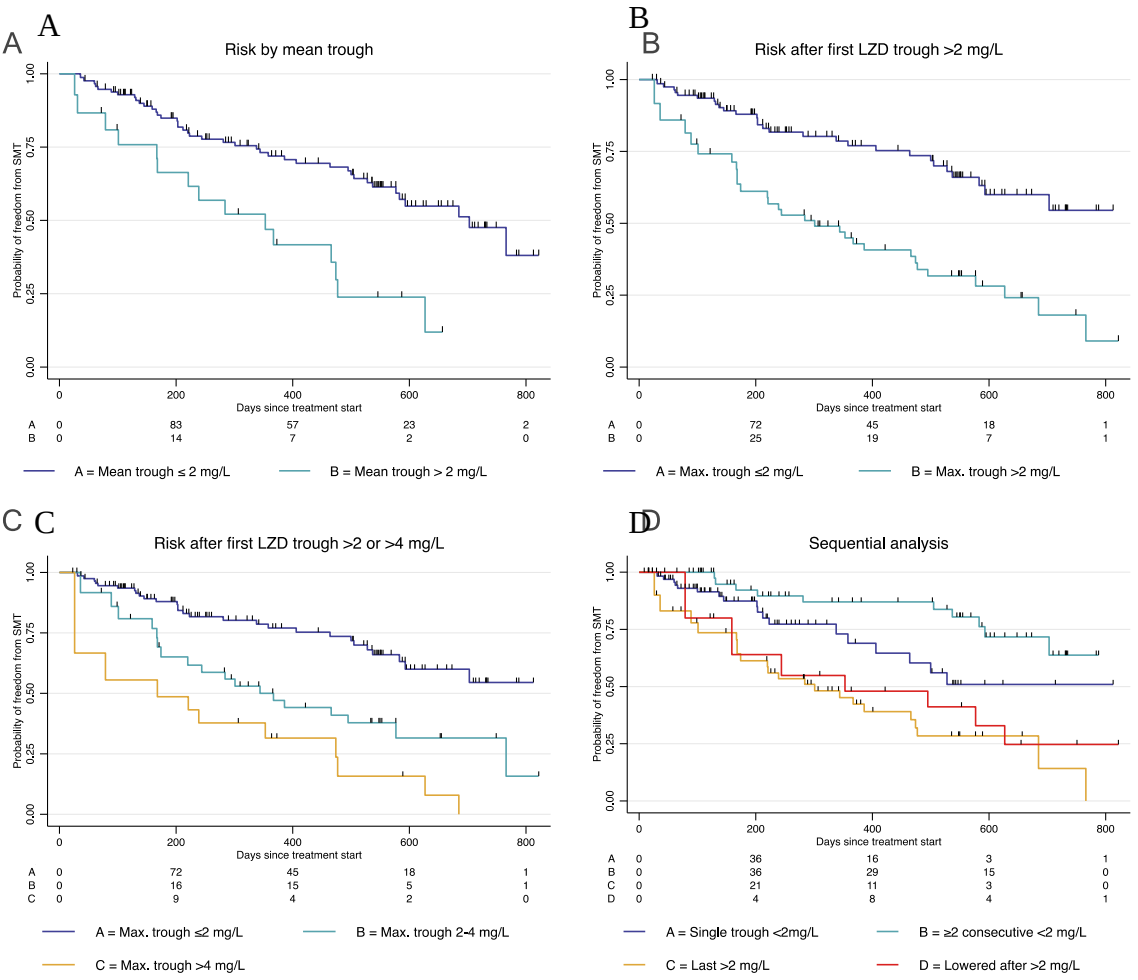

A
Risk by mean trough
Probability of freedom from SMT
1.00
0.75
0.50
0.25
0.00
0
200
400
600
800
Days since treatment start
A 0 83 57 23 2
B 0 14 7 2 0
A = Mean trough ≤ 2 mg/L
B = Mean trough > 2 mg/L
B
Risk after first LZD trough >2 mg/L
Probability of freedom from SMT
1.00
0.75
0.50
0.25
0.00
0
200
400
600
800
Days since treatment start
A 0 72 45 18 1
B 0 25 19 7 1
A = Max. trough ≤2 mg/L
B = Max. trough >2 mg/L
C
Risk after first LZD trough >2 or >4 mg/L
Probability of freedom from SMT
1.00
0.75
0.50
0.25
0.00
0
200
400
600
800
Days since treatment start
A 0 72 45 18 1
B 0 16 15 5 1
C 0 9 4 2 0
A = Max. trough ≤2 mg/L
B = Max. trough 2-4 mg/L
C = Max. trough >4 mg/L
D
Sequential analysis
Probability of freedom from SMT
1.00
0.75
0.50
0.25
0.00
0
200
400
600
800
Days since treatment start
A 0 36 16 3 1
B 0 36 29 15 0
C 0 21 11 3 0
D 0 4 8 4 1
A = Single trough <2mg/L
B = ≥2 consecutive <2 mg/L
C = Last >2 mg/L
D = Lowered after >2 mg/L

Figure 3