Drug exposure and susceptibility of second-line drugs correlate with treatment response in patients with multidrug-resistant tuberculosis: a multicentre prospective cohort study in China

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Drug exposure and susceptibility were proved to be associated with treatment responses during multidrug-resistant tuberculosis treatment, and identified thresholds may serve as targets for dose adjustment in future clinical studies to improve treatment efficacy https://bit.ly/3pZQbFU

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Abstract

Background Understanding the impact of drug exposure and susceptibility on treatment response of multidrug-resistant tuberculosis (MDR-TB) will help to optimise treatment. This study aimed to investigate the association between drug exposure, susceptibility and response to MDR-TB treatment.

Methods Drug exposure and susceptibility for second-line drugs were measured for patients with MDR-TB. Multivariate analysis was applied to investigate the impact of drug exposure and susceptibility on sputum culture conversion and treatment outcome. Probability of target attainment was evaluated. Random Forest and CART (Classification and Regression Tree) analysis was used to identify key predictors and their clinical targets among patients on World Health Organization-recommended regimens.

Results Drug exposure and corresponding susceptibility were available for 197 patients with MDR-TB. The probability of target attainment was highly variable, ranging from 0% for ethambutol to 97% for linezolid, while patients with fluoroquinolones above targets had a higher probability of 2-month culture conversion (56.3% *versus* 28.6%; adjusted OR 2.91, 95% CI 1.42–5.94) and favourable outcome (88.8% *versus* 68.8%; adjusted OR 2.89, 95% CI 1.16–7.17). Higher exposure values of fluoroquinolones, linezolid and pyrazinamide were associated with earlier sputum culture conversion. CART analysis selected moxifloxacin area under the drug concentration–time curve/minimum inhibitory concentration ($AUC_{0–24h}$/MIC) of 231 and linezolid $AUC_{0–24h}$/MIC of 287 as best predictors for 6-month culture conversion in patients receiving identical Group A-based regimens. These associations were confirmed in multivariate analysis.

Conclusions Our findings indicate that target attainment of TB drugs is associated with response to treatment. The CART-derived thresholds may serve as targets for early dose adjustment in a future randomised controlled study to improve MDR-TB treatment outcome.

Background

Multidrug-resistant tuberculosis (MDR-TB) is a global public health crisis and its poor treatment outcome is threatening achieving the World Health Organization (WHO) End TB Strategy targets by 2035 [1]. The

MDR-TB treatment success rate was 54% in China in 2019, while it has been reported up to 80–85% in less burdened countries [1]. Adequate drug exposure is key for effective therapy as suboptimal exposures of anti-TB drugs are correlated with delayed sputum culture conversion and poor treatment outcome [2]. Well-designed studies linking drug exposure to treatment outcome are urgently needed to guide dose optimisation and implementation of therapeutic drug monitoring (TDM) [3–5].

TDM is a tool considered in the American Thoracic Society, US Centers for Disease Control and Prevention, European Respiratory Society, and Infectious Diseases Society of America clinical practice guideline [3] and WHO consolidated guidelines [4] to individualise drug doses to maximise the therapeutic effects while minimising the risk of adverse events, particularly for drugs with narrow therapeutic windows such as linezolid. Although variability in pharmacokinetics and drug susceptibility has been reported for second-line TB drugs [5, 6], clinical targets are predominantly based on pre-clinical models [7–13]. Large clinical studies establishing targets for drug exposure and susceptibility are still lacking due to logistical and financial hurdles, including the need for long-term follow-up, variability in drug regimens, and inability to integrate both drug concentrations and susceptibility for *Mycobacterium tuberculosis* [14, 15]. Such barriers prevented implementation of individualised, TDM-based therapy [16].

Although new anti-TB drugs and shorter treatment regimens demonstrate improved treatment outcome, there is still a long way to go before all patients will benefit from these drugs. Besides, new drugs are not free of variability in drug exposure [17]. Improving treatment should consider variability in *M. tuberculosis* susceptibility and drug exposure [5] in addition to other factors such as treatment adherence.

Our international TB research consortium previously showed that treatment outcomes in patients with drug-susceptible TB could be explained by drug exposure and susceptibility [18]. Thus, we designed a multicentre, prospective, population-based study to determine the association between drug exposure/susceptibility targets and MDR-TB treatment responses.

Materials and methods

Study design and participants

A multicentre prospective cohort study was conducted between June 2016 and June 2019 in five hospitals from Guizhou, Henan and Jiangsu Province in China. Eligible patients had an MDR-TB (*M. tuberculosis* simultaneously resistant to rifampicin and isoniazid) diagnosis confirmed by bacterial culture and phenotypic drug susceptibility testing (DST), and were aged between 18 and 70 years. Patients were excluded if they were clinically abnormal in liver or kidney function, were pregnant or infected with HIV, hepatitis B or C virus, or had received MDR-TB treatment previously for >1 day, or refused to participate. The study was approved by the Ethics Committee of the School of Public Health, Fudan University (Shanghai, China; 2015-08-0568) and written informed consent was obtained from all subjects.

MDR-TB treatment and information collection

The patients with MDR-TB were routinely transferred to designated hospitals for 2-week inpatient treatment followed by outpatient treatment. A standardised oral regimen of fluoroquinolones, bedaquiline, linezolid, clofazimine and cycloserine for 6 months, followed by fluoroquinolones, linezolid, clofazimine and cycloserine for 18 months, was used [4, 19]. Treatment modification was made according to phenotypic DST results, clinical characteristics of patients and drug availability. Directly observed therapy was implemented daily by study nurses during inpatient treatment and by community healthcare workers during the outpatient phase [19]. Missing doses and/or treatment interruption and the reasons for these were recorded. Patients were routinely examined once a month during the intensive phase and once every 2 months during the consolidation phase. A questionnaire was used to collect demographic data, while medical and laboratory data were extracted from hospital records. Sputum samples were collected at each visit and were sent to the up-level quality controlled prefectural TB reference laboratory for analysis [20].

Drug susceptibility testing

Bacterial culture, phenotypic DST and minimum inhibitory concentration (MIC) values for the studied drugs were performed using the BACTEC MGIT 960 system (Becton Dickinson, Franklin Lakes, NJ, USA). Critical concentrations were used for the classification of drug susceptibility of the isolates [21]. The following concentrations were used for MIC testing: levofloxacin 0.06–32 $mg \cdot L^{-1}$, moxifloxacin 0.03–16 $mg \cdot L^{-1}$, linezolid 0.06–4 $mg \cdot L^{-1}$, bedaquiline 0.015–4 $mg \cdot L^{-1}$, cycloserine 2–64 $mg \cdot L^{-1}$, clofazimine 0.03–4 $mg \cdot L^{-1}$, prothionamide 0.3–20 $mg \cdot L^{-1}$, pyrazinamide 16–1024 $mg \cdot L^{-1}$ and ethambutol 0.5–32 $mg \cdot L^{-1}$. The MIC was defined as the lowest concentration of a drug that inhibited bacterial growth. For details, see the supplementary material.

Drug exposure

After 2 weeks of inpatient treatment, blood samples were collected *via* a venous catheter at pre-dose and at 1, 2, 4, 6 and 8 h after witnessed intake of anti-TB drugs (steady state) [22]. Additional blood samples at 12 and 18 h post-dose were collected in patients receiving bedaquiline. Samples were measured using a validated liquid chromatography tandem mass spectrometry method previously established (for details, see the supplementary material) [23]. Noncompartmental analysis was applied to calculate the area under the concentration–time curve (AUC_{0-24h}) for all drugs and the percentage of time that the concentration persisted above the MIC ($\%T_{>MIC}$) for cycloserine.

Response to treatment and main definitions

The response to treatment in this study was evaluated by: 2-month sputum culture conversion as a marker of early treatment response, 6-month culture conversion (previously reported to be predictive of treatment outcome [24]), time to culture conversion using time-to-event analysis and final treatment outcome. Sputum culture conversion was defined as two consecutive negative cultures of samples taken at least 30 days apart [25]. Treatment outcome was defined according to the WHO guidelines [25]. Cure and treatment completion were considered as a successful treatment outcome, while failure, death and lost to follow-up were considered as unfavourable outcomes. Severe disease was defined as TBscore ⩾8 [26]. The Timika score was used to assess chest radiograph severity and a score ⩾71 was defined as extensive pulmonary disease [27]. Effective drugs were defined as those with confirmed susceptibility by phenotypic DST or no previous exposure history.

Statistical analyses

The statistics for patient characteristics and treatment responses were presented in line with the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) statement for observational cohort studies (www.strobe-statement.org). Between-group differences were evaluated by the Chi-squared test, Fisher's exact test or Mann–Whitney U-test as appropriate. A p-value <0.05 was considered statistically significant and 95% confidence intervals were calculated. Due to the lack of clinical pharmacokinetic/pharmacodynamic targets, the probability of target attainment for drug exposure/susceptibility (AUC_{0-24h}/MIC) ratios was based on previous *in vitro* studies (the targets were moxifloxacin 56, levofloxacin 160, linezolid 119, cycloserine 25.8, pyrazinamide 11.3, prothionamide 56.2 and ethambutol 119 [7–13]). Patients were grouped into quartiles based on the quartiles of AUC_{0-24h}/MIC ratio. The association between these groups and time to sputum culture conversion was investigated by Kaplan–Meier survival analysis and then adjusted for potential confounders in Cox proportional hazards regression models with death and lost to follow-up as censored data, while the association with sputum culture conversion at 2 and 6 months and treatment outcome was investigated in univariate and multivariate logistic regression models.

In the subgroup analysis of treatment arms of WHO-recommended Group A-based regimens, Random Forest analysis was used to rank variable importance for all demographic characteristics, clinical features and drug AUC_{0-24h}/MIC ratios. The top 10 variables were selected for subsequent analysis. To detect interactions and deal with missing values, CART (Classification and Regression Tree) analysis was used to identify the AUC_{0-24h}/MIC ratio thresholds predictive of treatment response using Salford Predictive Miner System software (Salford Systems, San Diego, CA, USA). The association of derived targets with

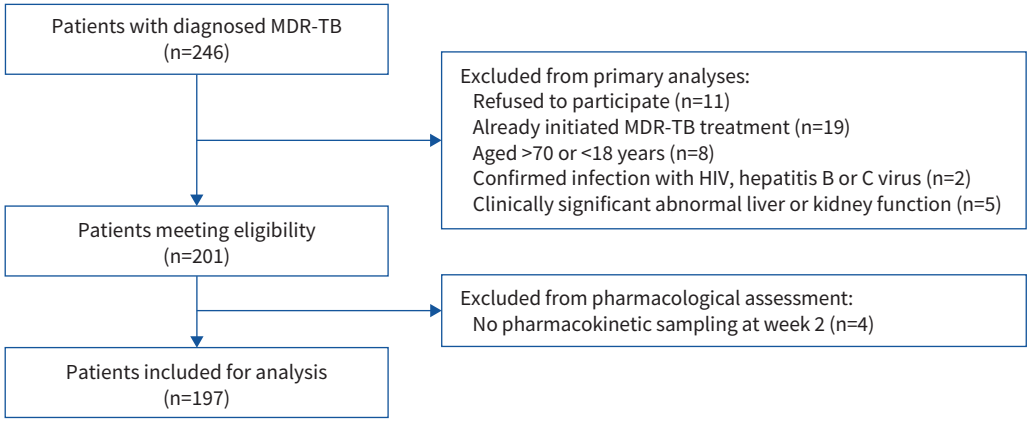

FIGURE 1 Enrolment of patients with multidrug-resistant tuberculosis (MDR-TB).

treatment response was further investigated in modified Poisson regression and Cox proportional hazards regression models. Further statistical analyses are summarised in the supplementary material.

Results

Study patients

In total, 246 patients were newly diagnosed with MDR-TB in the study hospitals during the study period and 201 of them were included; data were available for analysis for 197 patients (figure 1). Of the 197 patients, 71.1% were male, and the mean±SD age and median (interquartile range (IQR)) weight were 42.0 ±9.9 years and 54 (48–66) kg, respectively (table 1). Baseline DST identified 37 (18.8%) *M. tuberculosis* strains with additional resistance to fluoroquinolones.

Treatment regimens and procedures

111 (56.3%) patients received an all-Group A+B drug regimen; 86 (43.7%) patients received a personalised regimen. All patients received a treatment regimen consisting of at least four effective drugs based on susceptibility testing.

Of the 197 patients, one patient died due to cardiovascular disease after 12 months of treatment, while two patients were lost to follow-up. During the treatment, 125 patients reported 219 adverse events, including gastrointestinal disorders (33.5%), psychiatric disorders (14.7%) and anaemia (13.2%). Dose reductions

TABLE 1 Demographic characteristics, clinical features and treatment outcome of study participants with multidrug-resistant tuberculosis (MDR-TB) (n=197)

Age (years)	42.0±9.9
Male	140 (71.1)
Weight (kg)	54 (48–66)
Current smoker	107 (54.3)
Diabetes mellitus type 2	40 (20.3)
Pulmonary cavities	78 (39.6)
Extensive pulmonary disease	38 (19.3)
Severe disease	56 (28.4)
Time to positivity (days)	11.9±3.0
Drug susceptibility profile	
MDR-TB alone	160 (81.2)
Pre-XDR-TB	37 (18.8)
Drug intake (mg·kg^{-1})	
Levofloxacin (500 mg, once daily)	7.7 (6.7–8.4)
Moxifloxacin (400 mg, once daily)	8.6 (7.2–10.4)
Linezolid (600 mg, once daily)	11.3 (9.2–12.8)
Bedaquiline (400 mg, once daily)	7.5 (6.2–8.3)
Clofazimine (100 mg, once daily)[#]	9.3 (7.6–10.4)
Cycloserine (500 mg, twice daily)	3.8 (3.2–4.3)
Prothionamide (600 mg, three times a day)	10.8 (9.3–12.0)
Pyrazinamide (1500 mg, three times a day)	26.8 (21.7–30.0)
Ethambutol (750 mg, once daily)	13.9 (11.3–15.6)
Number of drugs[¶] in	
Group A	2.0±0.6
Group B	1.6±0.5
Group C	2.0±1.1
Effective drugs	5.6±0.7
2-month culture conversion	88 (44.7)
6-month culture conversion	128 (65.0)
Time to culture conversion (months)	4 (2–14)
Treatment outcome	
Success	156 (79.2)
Failure	38 (19.3)
Death	1 (0.5)
Lost to follow-up	2 (1.0)

Data are presented as mean±SD, median (interquartile range) or n (%). XDR-TB: extensively drug-resistant tuberculosis. [#]: loading dose 200 mg twice daily for 2 months; [¶]: Group A, B and C drugs refer to the World Health Organization MDR-TB treatment guidelines [4].

were performed for cycloserine (n=5), linezolid (n=4) and bedaquiline (n=2), while cycloserine was discontinued in 10 patients after a median (range) of 7 (6–10) months of treatment (supplementary table S2).

Treatment responses and risk factors

Sputum culture conversion was achieved in 88 (44.7%) patients after 2 months of MDR-TB treatment, 128 (65.0%) achieved 6-month culture conversion while 156 (79.2%) finally had a favourable outcome during follow-up (table 1). The median (IQR) time to culture conversion was 4 (2–14) months. As shown in supplementary table S1, baseline time to culture positivity (TTP) was found to be significantly associated with 2- and 6-month culture conversion and treatment outcome (p<0.001). Patients who had diabetes mellitus type 2 (50.0% *versus* 68.8%; p=0.026) or currently smoked (57.9% *versus* 73.3%; p=0.024) were less likely to achieve 6-month culture conversion. Sex, severe disease and extensive pulmonary disease were not associated with any treatment responses (p>0.05). Compared with patients receiving at least two Group A drugs, patients taking only one Group A drug had a lower probability of 2-month culture conversion (23.1% *versus* 50.0%; p=0.002) and 6-month culture conversion (48.7% *versus* 69.0%; p=0.017) as well as a lower probability of a favourable outcome (53.8% *versus* 85.4%; p<0.001). Patients receiving three Group A drugs had a higher treatment success rate compared with others (100.0% *versus* 73.9%; p<0.001) (supplementary figure S1).

Association between drug exposure/susceptibility ratio and treatment response

Patients with a higher exposure/susceptibility ratio for fluoroquinolones, linezolid and pyrazinamide had a better treatment response (p<0.05), while prothionamide and ethambutol had little impact (table 2 and supplementary table S3). A more favourable exposure/susceptibility ratio for bedaquiline (2890.1 *versus* 1527.2; p=0.001), cycloserine (111.0 *versus* 79.2; p<0.001) and clofazimine (101.1 *versus* 51.7; p=0.005) was strongly associated with 6-month culture conversion. As shown in figure 2, time to sputum culture conversion was observed to be significantly shorter in patients with a higher exposure/susceptibility ratio for fluoroquinolones, linezolid, cycloserine and pyrazinamide (p<0.001). Similar effects were not observed for bedaquiline, clofazimine and prothionamide (p>0.05). These associations were confirmed in a multivariate analysis (tables 3 and 4). For details of MIC and $AUC_{0–24h}$ distribution, see supplementary figures S2 and S3.

TABLE 2 Distribution of drug exposure/susceptibility ratios in patients with multidrug-resistant tuberculosis

	2-month sputum culture result		6-month sputum culture result		Treatment outcome	
	Positive (n=109)	Negative (n=88)	Positive (n=69)	Negative (n=128)	Unfavourable (n=41)[#]	Success (n=156)
Levofloxacin (n=78)	93.3 (50.6–114.3)	108.7 (90.0–249.1)*	63.3 (48.7–107.4)	108.8 (90.1–136.8)*	52.9 (44.3–96.6)	108.8 (90.4–134.0)*
Moxifloxacin (n=79)	177.1 (68.5–268.1)	742.9 (478.7–887.5)*	119.6 (51.6–192.7)	625.8 (387.4–834.7)*	159.0 (91.5–194.7)	411.5 (184.3–763.3)*
Linezolid (n=168)	472.2 (372.5–569.7)	492.4 (432.5–715.4)*	429.6 (273.6–503.1)	497.7 (439.0–715.4)*	373.4 (129.7–503.8)	492.4 (433.7–617.9)*
Bedaquiline (n=70)	2107.5 (914.7–3275.4)	2901.0 (2103.2–3765.2)*	1527.2 (852.3–2383.1)	2890.1 (2163.8–3867.6)*	2382.0 (1441.4–3120.0)	2383.1 (1627.0–3743.4)
Clofazimine (n=136)	64.4 (30.2–141.3)	115.7 (45.8–235.1)*	51.7 (26.8–138.1)	101.1 (45.0–235.6)*	102.7 (34.4–154.3)	94.8 (36.8–228.6)
Cycloserine (n=186)	86.2 (46.7–152.4)	130.0 (65.5–219.3)*	79.2 (33.4–119.0)	111.0 (65.0–214.6)*	90.7 (33.6–144.6)	99.4 (59.3–201.0)
Cycloserine (n=186)[¶]	100.0 (100.0–100.0)	100.0 (100.0–100.0)	100.0 (93.6–100.0)	100.0 (100.0–100.0)*	100.0 (95.2–100.0)	100.0 (100.0–100.0)
Prothionamide (n=86)	38.0 (28.8–77.4)	51.4 (37.1–72.4)	39.5 (18.7–75.9)	51.1 (36.0–73.4)	37.7 (16.4–69.5)	51.0 (35.5–76.0)
Pyrazinamide (n=99)	4.2 (3.1–5.6)	7.7 (4.6–11.9)*	4.1 (3.4–5.4)	5.9 (3.5–9.3)*	3.9 (3.4–5.2)	5.6 (3.5–9.3)*
Ethambutol (n=123)	19.1 (12.7–23.5)	21.5 (13.9–31.8)*	19.3 (12.9–23.5)	20.2 (13.5–27.7)	19.2 (12.7–23.1)	20.1 (13.5–27.6)

Data are presented as median (interquartile range) area under the drug concentration–time curve/minimum inhibitory concentration ($AUC_{0–24h}$/MIC) ratio. [#]: unfavourable outcome was defined as the sum of failure, death and lost to follow-up; [¶]: percentage of time that the concentration persisted above the MIC ($\%T_{>MIC}$). The Mann–Whitney U-test was applied for comparisons. *: p<0.05.

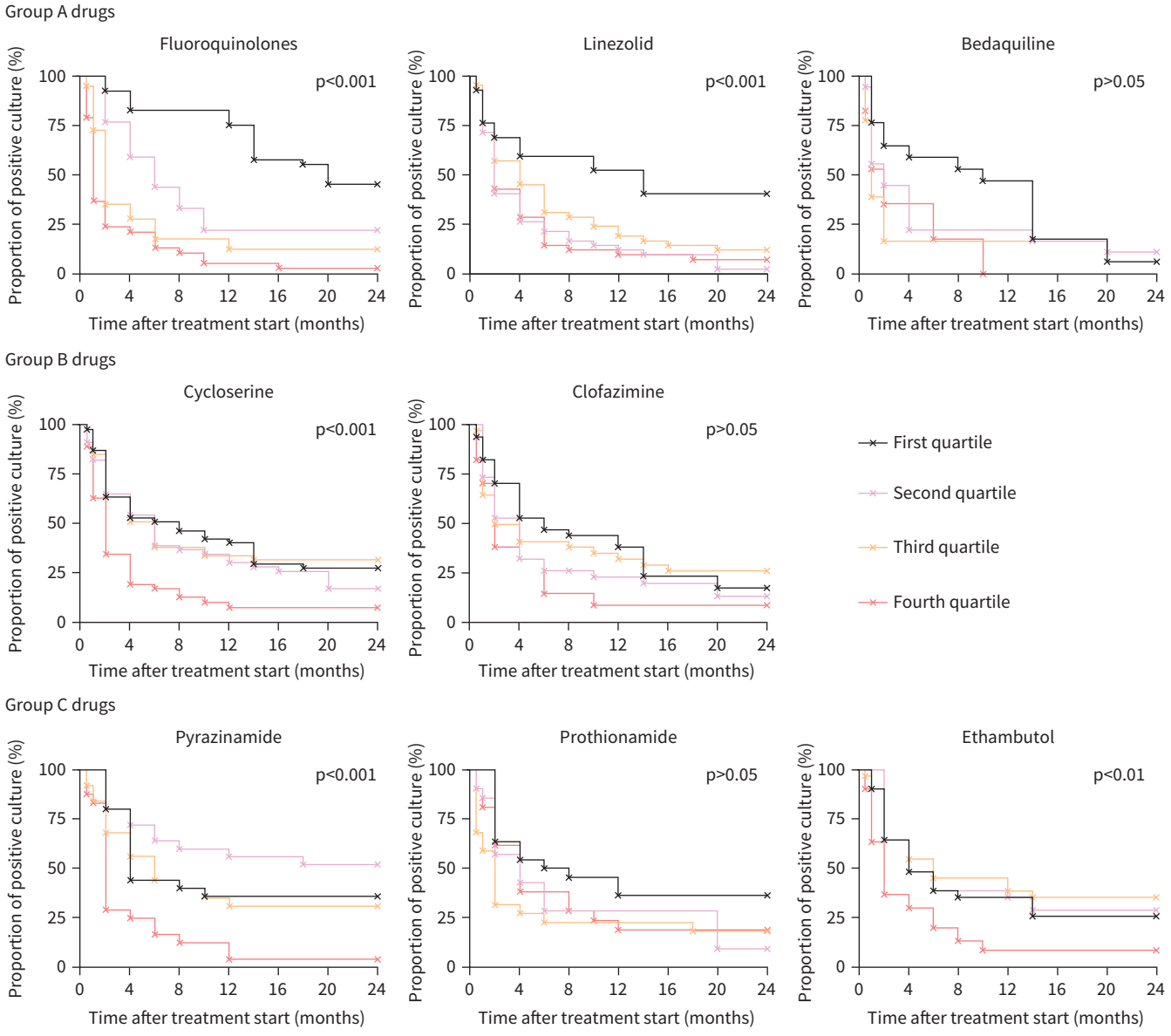

FIGURE 2 Time to culture conversion among patients with multidrug-resistant tuberculosis grouped by drug exposure/susceptibility ratio quartiles.

The probabilities of target attainment for moxifloxacin, linezolid and cycloserine were >85%, while they were <45% for levofloxacin, pyrazinamide, prothionamide and ethambutol (table 5 and supplementary figure S4). Multivariate analysis showed that patients with fluoroquinolone exposure above the previously suggested targets had a higher probability of 2-month culture conversion (adjusted OR 2.91, 95% CI 1.42–5.94) and treatment success (adjusted OR 2.89, 95% CI 1.16–7.17). Patients with moxifloxacin exposure above target were more likely to achieve 6-month culture conversion (adjusted OR 15.6, 95% CI 1.48–165.0).

CART analysis for clinical drug exposure/susceptibility targets

Random Forest and CART analysis was performed among subgroups of patients receiving "moxifloxacin+linezolid±bedaquiline" (n=67)-based or "levofloxacin+linezolid±bedaquiline" (n=61)-based regimens. The results showed that the primary node for the "moxifloxacin+linezolid±bedaquiline"-based regimen was moxifloxacin AUC_{0-24h}/MIC of 231, where 97.6% of patients exceeding this target achieved 6-month culture conversion compared with 3.8% in those below the target (figure 3). For the "levofloxacin+linezolid±bedaquiline"-based regimen, linezolid was selected as the primary node with an AUC_{0-24h}/MIC cut-off value of 287 and patients with linezolid above the target had a higher probability of sputum culture

TABLE 3 Univariate and multivariate analysis for drug exposure/susceptibility ratio quartiles with 2- and 6-month culture conversion

	2-month culture results			6-month culture results		
	Negative (%)[#]	OR (95% CI)	Adjusted OR (95% CI)[¶]	Negative[#] (%)	OR (95% CI)	Adjusted OR (95% CI)[¶]
Fluoroquinolones (n=157)						
First quartile	3 (7.5)	1	1	7 (17.5)	1	1
Second quartile	9 (23.1)	3.70 (0.92–14.9)	4.02 (0.91–17.7)	22 (56.4)	6.10 (2.17–17.1)	5.92 (1.93–18.2)
Third quartile	26 (65.0)	22.9 (5.97–87.8)	31.3 (6.61–148.0)	33 (82.5)	22.2 (7.01–70.4)	23.4 (6.38–85.6)
Fourth quartile	29 (76.3)	39.7 (9.86–160.2)	53.3 (10.6–268.8)	34 (89.5)	40.1 (10.7–149.8)	36.4 (8.31–159.3)
Linezolid (n=168)						
First quartile	13 (31.0)	1	1	17 (40.5)	1	1
Second quartile	25 (59.5)	3.28 (1.34–8.06)	2.91 (1.11–7.64)	33 (78.6)	5.39 (2.06–14.1)	4.92 (1.74–13.9)
Third quartile	18 (42.9)	1.67 (0.68–4.10)	1.36 (0.52–3.57)	30 (71.4)	3.68 (1.48–9.13)	2.85 (1.06–7.64)
Fourth quartile	24 (57.1)	2.97 (1.22–7.28)	2.59 (0.99–6.78)	36 (85.7)	8.82 (3.05–25.5)	8.52 (2.74–26.4)
Bedaquiline (n=70)						
First quartile	6 (35.3)	1	1	7 (41.2)	1	1
Second quartile	10 (55.6)	2.29 (0.59–8.94)	2.70 (0.62–11.8)	14 (77.8)	5.00 (1.15–21.8)	7.71 (1.43–41.6)
Third quartile	15 (83.3)	9.17 (1.87–44.9)	9.74 (1.64–57.9)	15 (83.3)	7.14 (1.48–34.4)	6.38 (1.04–39.1)
Fourth quartile	11 (64.7)	3.36 (0.82–13.7)	2.69 (0.52–14.0)	15 (88.2)	10.7 (1.84–62.5)	8.35 (1.11–63.0)
Cycloserine (n=186)						
First quartile	17 (36.2)	1	1	23 (48.9)	1	1
Second quartile	16 (34.8)	0.94 (0.40–2.20)	0.93 (0.37–2.30)	28 (60.9)	1.62 (0.71–3.70)	1.58 (0.65–3.83)
Third quartile	17 (36.2)	1.00 (0.43–2.32)	1.07 (0.44–2.64)	30 (63.8)	1.84 (0.81–4.20)	2.05 (0.84–5.01)
Fourth quartile	30 (65.2)	3.31 (1.41–7.74)	3.45 (1.38–8.61)	38 (82.6)	4.96 (1.91–12.9)	5.26 (1.88–14.7)
Clofazimine (n=136)						
First quartile	10 (29.4)	1	1	18 (52.9)	1	1
Second quartile	16 (47.1)	2.13 (0.79–5.79)	2.23 (0.79–6.27)	25 (73.5)	2.47 (0.89–6.83)	2.66 (0.91–7.76)
Third quartile	17 (50.0)	2.40 (0.89–6.51)	2.34 (0.83–6.59)	20 (58.8)	1.27 (0.49–3.31)	1.13 (0.41–3.13)
Fourth quartile	21 (61.8)	3.88 (1.41–10.7)	3.81 (1.34–10.9)	30 (88.2)	6.67 (1.93–23.1)	6.57 (1.81–23.8)
Pyrazinamide (n=99)						
First quartile	5 (20.0)	1	1	14 (56.0)	1	1
Second quartile	5 (20.0)	1.00 (0.25–4.00)	1.21 (0.27–5.47)	9 (36.0)	0.44 (0.14–1.38)	0.39 (0.11–1.35)
Third quartile	8 (32.0)	1.88 (0.52–6.84)	2.05 (0.51–8.15)	14 (56.0)	1.00 (0.33–3.06)	0.96 (0.30–3.12)
Fourth quartile	17 (70.8)	9.71 (2.60–36.3)	12.5 (2.81–55.3)	20 (83.3)	3.93 (1.04–14.9)	3.30 (0.81–13.5)
Prothionamide (n=86)						
First quartile	8 (36.4)	1	1	11 (50.0)	1	1
Second quartile	9 (42.9)	1.31 (0.39–4.47)	1.08 (0.25–4.64)	15 (71.4)	2.50 (0.71–8.84)	3.16 (0.78–12.8)
Third quartile	15 (68.2)	3.75 (1.08–13.1)	5.88 (1.33–25.9)	17 (77.3)	3.40 (0.93–12.5)	4.08 (1.00–16.7)
Fourth quartile	8 (38.1)	1.08 (0.31–3.71)	1.11 (0.27–4.53)	13 (61.9)	1.63 (0.48–5.47)	1.77 (0.47–6.60)
Ethambutol (n=123)						
First quartile	11 (35.5)	1	1	19 (61.3)	1	1
Second quartile	11 (35.5)	1.00 (0.35–2.83)	0.77 (0.25–2.42)	19 (61.3)	1.00 (0.36–2.78)	0.70 (0.23–2.17)
Third quartile	11 (35.5)	1.00 (0.35–2.83)	0.83 (0.27–2.53)	17 (54.8)	0.77 (0.28–2.11)	0.52 (0.17–1.60)
Fourth quartile	19 (63.3)	3.14 (1.10–8.93)	2.38 (0.76–7.40)	24 (80.0)	2.53 (0.80–7.98)	1.47 (0.42–5.17)

[#]: percentage calculated by dividing number of patients with culture conversion by number of patients in each quartile; [¶]: adjusted according to current smoking, diabetes mellitus type 2, time to culture positivity at baseline and effective drug numbers at the onset of treatment.

conversion at 6 months of treatment (82.4% *versus* 10.0%). After adjusting for current smoking, diabetes mellitus type 2, baseline TTP and number of effective drugs, patients with moxifloxacin or linezolid exposure above target had a greater probability of 6-month culture conversion and showed earlier culture conversion (supplementary table S4).

Discussion

In this large multicentre study, higher drug exposure in relation to susceptibility for all drugs, except prothionamide and ethambutol, was found to be associated with favourable treatment responses in patients with MDR-TB. This is the first study demonstrating that adequate exposure to fluoroquinolones, bedaquiline and linezolid is strongly associated with sputum culture conversion at various time-points during MDR-TB treatment in programmatic regimens.

It is well known that current Group A drugs contribute to improved treatment response. Our study demonstrates that adequate exposure to these drugs translated to higher 2- and 6-month culture conversion

TABLE 4 Univariate and multivariate analysis for drug exposure/susceptibility ratio quartiles with treatment outcome and time to culture conversion

	Treatment outcome			Time to culture conversion		
	Success (%)[#]	OR (95% CI)	Adjusted OR (95% CI)[¶]	Months[+]	HR (95% CI)	Adjusted HR (95% CI)[¶]
Fluoroquinolones (n=157)						
First quartile	22 (55.0)	1	1	20 (12.5–24)	1	1
Second quartile	30 (76.9)	2.73 (1.03–7.20)	2.09 (0.73–6.04)	6 (4–10)	2.14 (1.22–3.74)	1.97 (1.10–3.52)
Third quartile	35 (87.5)	5.73 (1.86–17.6)	4.16 (1.19–14.5)	2 (1–6)	3.62 (2.10–6.26)	3.82 (2.14–6.83)
Fourth quartile	37 (97.4)	30.3 (3.78–242.7)	24.8 (2.68–229.6)	1 (1–2.5)	6.26 (3.61–10.9)	6.49 (3.53–12.0)
Linezolid (n=168)						
First quartile	25 (59.5)	1	1	14 (1.8–24)	1	1
Second quartile	41 (97.6)	27.9 (3.49–222.5)	30.3 (3.51–261.6)	2 (1–6)	2.71 (1.63–4.49)	2.45 (1.45–4.14)
Third quartile	37 (88.1)	5.03 (1.64–15.4)	4.93 (1.45–16.7)	4 (1.8–10.5)	1.98 (1.19–3.30)	1.79 (1.06–3.03)
Fourth quartile	39 (92.9)	8.84 (2.35–33.3)	8.94 (2.25–35.6)	2 (1.8–6)	2.58 (1.54–4.31)	2.30 (1.35–3.90)
Bedaquiline (n=70)						
First quartile	16 (94.1)	1	1	10 (1.5–14)	1	1
Second quartile	16 (88.9)	0.50 (0.04–6.08)	0.92 (0.06–15.2)	2 (1–6.5)	1.31 (0.65–2.64)	1.57 (0.76–3.26)
Third quartile	16 (88.9)	0.50 (0.04–6.08)	0.85 (0.06–12.2)	1 (0.9–2)	1.67 (0.83–3.37)	1.96 (0.89–4.33)
Fourth quartile	17 (100)	NA	NA	2 (1–6)	1.98 (0.98–4.02)	1.76 (0.76–4.11)
Cycloserine (n=186)						
First quartile	34 (72.3)	1	1	8 (2–24)	1	1
Second quartile	38 (82.6)	1.82 (0.67–4.91)	1.84 (0.62–5.40)	6 (2–20)	1.22 (0.77–1.94)	1.17 (0.73–1.87)
Third quartile	32 (68.1)	0.82 (0.34–1.98)	0.90 (0.34–2.38)	6 (2–24)	0.98 (0.61–1.60)	0.99 (0.61–1.61)
Fourth quartile	42 (91.3)	4.01 (1.20–13.4)	4.87 (1.28–18.5)	2 (1–4)	2.18 (1.38–3.45)	2.06 (1.30–3.27)
Clofazimine (n=136)						
First quartile	28 (82.4)	1	1	6 (2–15.5)	1	1
Second quartile	29 (85.3)	1.24 (0.34–4.54)	0.83 (0.20–3.45)	4 (1–8.5)	1.29 (0.76–2.17)	1.22 (0.72–2.06)
Third quartile	25 (73.5)	0.60 (0.19–1.91)	0.48 (0.13–1.76)	3 (1–24)	1.03 (0.60–1.77)	0.98 (0.57–1.69)
Fourth quartile	31 (91.2)	2.21 (0.51–9.70)	2.14 (0.43–10.7)	2 (1–6)	1.70 (1.01–2.84)	1.65 (0.98–2.78)
Pyrazinamide (n=99)						
First quartile	16 (64.0)	1	1	4 (4–24)	1	1
Second quartile	12 (48.0)	0.52 (0.17–1.61)	0.44 (0.13–1.51)	24 (4–24)	0.65 (0.31–1.38)	0.64 (0.30–1.37)
Third quartile	17 (68.0)	1.20 (0.37–3.86)	1.15 (0.34–3.94)	6 (2–24)	1.17 (0.59–2.32)	1.14 (0.57–2.26)
Fourth quartile	23 (95.8)	12.9 (1.49–112.4)	9.51 (1.04–87.1)	2 (2–5.5)	2.47 (1.30–4.72)	2.24 (1.14–4.39)
Prothionamide (n=86)						
First quartile	14 (63.6)	1	1	7 (2–24)	1	1
Second quartile	19 (90.5)	5.43 (1.00–29.6)	8.16 (1.20–55.3)	4 (2–20)	1.69 (0.85–3.37)	1.48 (0.73–3.00)
Third quartile	18 (81.8)	2.57 (0.64–10.3)	3.36 (0.70–16.1)	2 (0.5–9)	2.12 (1.05–4.27)	2.46 (1.18–5.09)
Fourth quartile	17 (81.0)	2.43 (0.60–9.78)	2.72 (0.58–12.8)	4 (2–11)	1.57 (0.77–3.19)	1.49 (0.72–3.08)
Ethambutol (n=123)						
First quartile	23 (74.2)	1	1	4 (2–24)	1	1
Second quartile	22 (71.0)	0.85 (0.28–2.60)	0.64 (0.19–2.19)	4 (2–24)	0.92 (0.51–1.64)	0.78 (0.43–1.43)
Third quartile	20 (64.5)	0.63 (0.21–1.88)	0.48 (0.15–1.58)	6 (2–24)	0.84 (0.46–1.52)	0.75 (0.41–1.39)
Fourth quartile	27 (90.0)	3.13 (0.74–13.2)	1.70 (0.36–8.05)	2 (1–6)	1.92 (1.10–3.36)	1.58 (0.88–2.84)

NA: not available (95% CI was infinite due to the small number of patients in the subgroup). [#]: percentage calculated by dividing number of patients with culture conversion by number of patients in each quartile; [¶]: adjusted according to current smoking, diabetes mellitus type 2, time to culture positivity at baseline and effective drug numbers at the onset of treatment; [+]: median (interquartile range).

rates compared with patients below these targets. By showing that targets established in *in vitro* studies are associated with improved treatment response across 2- and 6-month culture conversion, time to culture conversion, and overall treatment outcome, our study is the first to bridge the gap between pre-clinical studies and clinical trials evaluating treatment outcome [28–31]. Although the efficacy of levofloxacin and moxifloxacin is believed to be comparable in MDR-TB treatment [4], moxifloxacin was found to play a more important role in driving treatment response in our study. However, the observed difference may well be attributed to underdosing of levofloxacin [5]. Although a standard levofloxacin dose of 750 mg was recommended [4, 19], physicians still tended to prescribe 500 mg, due to the lack of clear dose recommendations for domestically manufactured levofloxacin, as well as concerns about potential adverse events. Underdosing of fluoroquinolones should be avoided and there is room for treatment optimisation using TDM. We urgently request that no country uses 500 mg of levofloxacin as standard dose, as this has been shown to lead to subtherapeutic drug levels [5, 32, 33].

TABLE 5 Association between drug exposure/susceptibility targets and treatment response in patients with multidrug-resistant tuberculosis[#]

AUC_{0-24h}/MIC	Overall	2-month sputum culture result		6-month sputum culture result		Treatment outcome	
		Negative (%)	Adjusted OR (95% CI)[¶]	Negative (%)	Adjusted OR (95% CI)[¶]	Success (%)	Adjusted OR (95% CI)[¶]
Moxifloxacin (n=79)							
⩽56	10 (12.7)	0 (0.0)	NA	1 (10.0)	1	8 (80.0)	1
>56	69 (87.3)	34 (49.3)	NA	45 (65.2)	15.6 (1.48–165.0)*	60 (87.0)	1.15 (0.08–6.95)
Levofloxacin (n=78)							
⩽160	67 (85.9)	22 (32.8)	NA	39 (58.2)	NA	45 (67.2)	NA
>160	11 (14.1)	11 (100.0)	NA	11 (100.0)	NA	11 (100.0)	NA
Fluoroquinolones (n=157)							
⩽target	77 (49.0)	22 (28.6)	1	40 (51.9)	1	53 (68.8)	1
>target	80 (51.0)	45 (56.3)	2.91 (1.42–5.94)*	56 (70.0)	1.63 (0.79–3.36)	71 (88.8)	2.89 (1.16–7.17)*
Linezolid (n=168)							
⩽119	5 (3.0)	0 (0.0)	NA	0 (0.0)	NA	0 (0.0)	NA
>119	163 (97.0)	80 (49.1)	NA	116 (71.2)	NA	142 (87.1)	NA
Cycloserine (n=186)							
⩽25.8	18 (9.7)	7 (38.9)	1	8 (44.4)	1	11 (61.1)	1
>25.8	168 (90.3)	73 (43.5)	1.03 (0.36–2.96)	111 (66.1)	2.21 (0.78–6.32)	135 (80.4)	2.37 (0.78–7.21)
Pyrazinamide (n=99)							
⩽11.3	88 (88.9)	25 (28.4)	1	46 (52.3)	NA	57 (64.8)	NA
>11.3	11 (11.1)	10 (90.9)	31.4 (3.50–281.1)*	11 (100.0)	NA	11 (100.0)	NA
Prothionamide (n=86)							
⩽56.2[+]	48 (55.8)	21 (43.8)	1	30 (62.5)	1	37 (77.1)	1
>56.2[+]	38 (44.2)	19 (50.0)	1.63 (0.60–4.45)	26 (68.4)	1.36 (0.51–3.61)	31 (81.6)	1.49 (0.46–4.79)
Ethambutol (n=123)							
⩽119	123 (100.0)	52 (42.3)	NA	79 (64.2)	NA	92 (74.8)	NA
>119	0 (0.0)						

AUC_{0-24h}: area under the drug concentration–time curve; MIC: minimum inhibitory concentration; NA: not available (95% CI was infinite due to the small number of patients in the subgroup). [#]: no AUC_{0-24h}/MIC targets were found for prothionamide, bedaquiline and clofazimine in published studies (as an alternative, the AUC_{0-24h}/MIC target of ethionamide was applied for prothionamide in this study); [¶]: adjusted according to current smoking, diabetes mellitus type 2, time to culture positivity at baseline and effective drug numbers at the onset of treatment; [+]: prothionamide referred to the target for ethionamide. *: p<0.05 and 95% CI of OR did not include 1.

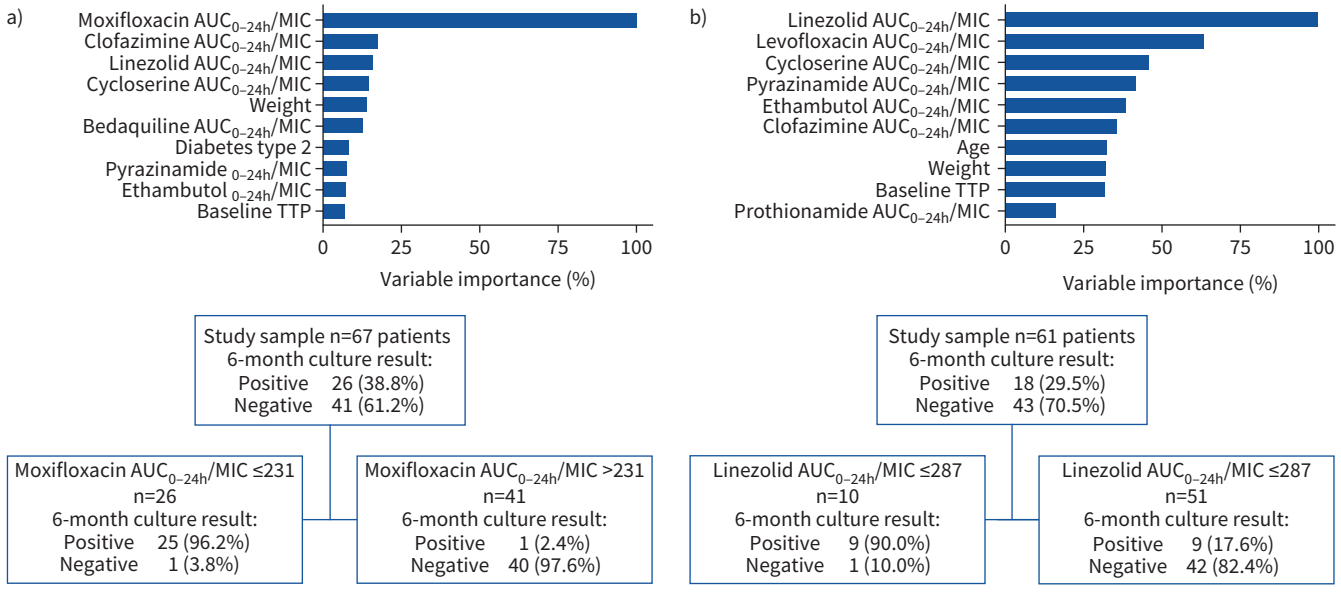

FIGURE 3 Random Forest and CART (Classification and Regression Tree) analysis for 6-month sputum culture conversion among patients receiving a) "moxifloxacin+linezolid±bedaquiline"- and b) "levofloxacin+linezolid±bedaquiline"-based regimens. AUC_{0-24h}: area under the drug concentration–time curve; MIC: minimum inhibitory concentration; TTP: time to culture positivity.

Having a higher exposure/susceptibility ratio for linezolid and bedaquiline was also associated with a better treatment response. This underpins the critical importance of interpretation of the highly variable exposures of bedaquiline and linezolid in relation to baseline drug susceptibility [17, 34]. Although the mean bedaquiline exposure after 2 weeks of MDR-TB treatment ($AUC_{0–24h}$ 41.5 $mg·h·L^{-1}$) in our study was higher (p=0.04) than in a previous study (33.0 $mg·h·L^{-1}$) [29], the clinical relevance is unclear. The study by Conradie *et al.* [35] has fuelled the discussion on linezolid dosing as >80% of the patients in that study experienced toxicity, prompting a dose reduction or interruption of treatment when receiving a dose of 1200 mg daily. In our study, the linezolid dose was reduced in four patients. Meanwhile, most patients (92.9% (156 out of 168)) were eligible for dose reduction while maintaining adequate drug exposure. Clearly there is some room for linezolid dose individualisation to balance efficacy and toxicity [6, 30].

Adequate exposure to cycloserine and clofazimine contributed to improved treatment response in our study. A high probability of target attainment for cycloserine supports the use of the agent with the currently recommended dosage of 10–15 $mg·kg^{-1}$ [12]. Regarding treatment optimisation in the Chinese setting, more efforts are needed to promote the use of these Group B drugs since nearly a third of patients received only one of them. The main reason was that the two drugs were not covered by medical insurance in China and needed to be paid for by the patient. When susceptibility is proven, our study showed that pyrazinamide is a valuable addition for composing an MDR-TB treatment regimen as it increased the probability of sputum culture conversion and reduced the time to culture conversion, confirming previous studies [36, 37]. However, as only 11.1% of patients reached the target for pyrazinamide [10], the need of higher dosing of pyrazinamide (40 $mg·kg^{-1}$) should be considered to increase the benefits without compromising its tolerability [38]. Prothionamide and ethambutol had little impact on treatment responses, reflecting their limited bactericidal and/or sterilising effect compared with other second-line drugs.

This study has some important implications for future randomised controlled studies on personalised dosing. Although TDM is recommended to optimise MDR-TB treatment in guidelines [3, 4], our study is the first to identify clinical targets for moxifloxacin and linezolid. The CART-derived clinical targets are higher compared with the targets reported in *in vitro* studies [11, 13]. However, these differences need to be viewed in a clinical context and in terms of the methodology of MIC determination, as MIC determination has inherent variability due to laboratory and strain variability [39], and with each two-fold change in the MIC, the target as calculated by the $AUC_{0–24h}$/MIC ratio will double. Moreover, free drug concentrations and tissue penetration to the site of infection (*e.g.* cavitary disease) needs to be considered when applying $AUC_{0–24h}$/MIC targets in clinical practice. Considering the delay and complexity of phenotypic testing in routine care, we foresee that genotypic testing to determine drug susceptibility in combination with drug exposure assessment would allow for early treatment modifications. Establishing $AUC_{0–24h}$ targets based solely on clinical breakpoints would result in significant overexposure in many patients as most isolates have an MIC lower than the breakpoint.

Our study has some limitations. We excluded patients aged >70 years and patients co-infected with HIV, hepatitis B or C virus in order to reduce the heterogeneity of study participants. Therefore, our results cannot be extrapolated to these patients. Sputum culture conversion was used to assess treatment response but more sensitive biomarkers should be considered in future studies evaluating interventions on drug dosing in relation to treatment response. We assessed drug exposure after 2 weeks of treatment (steady state) and we assumed that intra-patient variability in drug exposure was limited compared with inter-patient variability, as sputum culture conversion at 2 and 6 months was comparable. It is important to realise that analysing the interaction between drug concentrations, pathogen susceptibility and treatment outcome is complex, and thresholds derived from a population depend on the distribution of different variables in that population [40]. This must be considered when comparing or translating study results.

In conclusion, our findings indicate that targets based on drug exposure/susceptibility are associated with response to treatment for most TB drugs used in MDR-TB treatment, especially for Group A drugs and pyrazinamide. For fluoroquinolones, linezolid and pyrazinamide, there is a clear opportunity for dose optimisation in general, in addition to individualisation. We recommend clinical targets for efficacy to be evaluated in a randomised controlled study as a strategy to improve MDR-TB treatment outcome, adjusted for differences in susceptibility testing.

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