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Population Pharmacokinetic Model and Limited Sampling Strategies for Personalized Dosing of Levofloxacin in Tuberculosis Patients

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ABSTRACT Levofloxacin is an antituberculosis drug with substantial interindividual pharmacokinetic variability; therapeutic drug monitoring (TDM) could therefore be helpful to improve treatment results. TDM would be more feasible with limited sampling strategies (LSSs), a method to estimate the area under the concentration curve for the 24-h dosing interval (AUC_{0-24}) by using a limited number of samples. This study aimed to develop a population pharmacokinetic (popPK) model of levofloxacin in tuberculosis patients, along with LSSs using a Bayesian and multiple linear regression approach. The popPK model and Bayesian LSS were developed using data from 30 patients and externally validated with 20 patients. The LSS based on multiple linear regression was internally validated using jackknife analysis. Only clinically suitable LSSs (maximum time span, 8 h; minimum interval, 1 h; 1 to 3 samples) were tested. Performance criteria were root-mean-square error (RMSE) of <15%, mean prediction error (MPE) of <5%, and r^2 value of >0.95. A one-compartment model with lag time best described the data while only slightly underestimating the AUC_{0-24} (mean, −7.9%; standard error [SE], 1.7%). The Bayesian LSS using 0- and 5-h postdose samples (RMSE, 8.8%; MPE, 0.42%; $r^2 = 0.957$) adequately estimated the AUC_{0-24}, with a mean underestimation of −4.4% (SE, 2.7%). The multiple linear regression LSS using 0- and 4-h postdose samples (RMSE, 7.0%; MPE, 5.5%; $r^2 = 0.977$) was internally validated, with a mean underestimation of −0.46% (SE, 2.0%). In this study, we successfully developed a popPK model and two LSSs that could be implemented in clinical practice to assist TDM of levofloxacin. (This study has been registered at ClinicalTrials.gov under identifier NCT01918397.)

KEYWORDS levofloxacin, pharmacodynamics, pharmacokinetics, sampling strategy, therapeutic drug monitoring, tuberculosis

Tuberculosis (TB) is the leading killer from a single infectious pathogen worldwide, and poor outcomes are more frequent among patients with rifampin-resistant (RR) and multidrug-resistant (MDR) TB. In 2016, approximately 10.4 million TB cases were identified, including 490,000 cases with MDR-TB and 600,000 with RR-TB (1). MDR-TB and RR-TB are treated with a combination of at least five anti-TB drugs to which a *Mycobacterium tuberculosis* strain is likely to be susceptible (2). Under programmatic conditions, the worldwide success rate of MDR-TB and RR-TB treatment is low, at 54% (1). Recently, in fluoroquinolone (FQ)-susceptible patients, a shorter 9- to 12-month MDR-TB regimen was proposed, reducing the burden for patients and the associated costs of treatment (2). Levofloxacin is a FQ frequently included in MDR-TB treatment because of its high efficacy and a favorable safety profile (2, 3). The World Health Organization (WHO) just released a revised grouping of drugs in the treatment of MDR-TB and RR-TB that prioritizes FQ together with bedaquiline and linezolid and thereby confirms the key position of FQ (4).

In general, the optimal FQ efficacy depends on the ratio of the area under the concentration-time curve from 0 to 24 h (AUC_{0-24}) to MICs with reported target values of >100 to 125 for Gram-negative bacteria and >40 for Gram-positive bacteria (5–7). Levofloxacin target AUC_{0-24}/MIC values for other pathogens cannot be extrapolated to *M. tuberculosis* due to its unique characteristics (8). Recently, a hollow-fiber study indicated a levofloxacin target AUC_{0-24}/MIC in MDR-TB treatment for the first time. The target AUC_{0-24}/MIC of 146 against *M. tuberculosis* was proposed based on the concentration associated with 80% effective concentration (EC_{80}), and an AUC_{0-24}/MIC of 360 was associated with suppression of acquired drug resistance (9). Additionally, a levofloxacin target AUC_{0-24}/MIC is being prospectively studied using linear regression of AUC_{0-24}/MIC and log-transformed time to sputum conversion in TB patients receiving various levofloxacin doses (11 to 20 mg/kg of body weight) in addition to an optimized background regimen (10). This study is expected to provide a conclusive levofloxacin target AUC_{0-24}/MIC and make a statement on the optimal levofloxacin dose to be used in TB treatment; the results from this study are expected in March 2019 (ClinicalTrials.gov identifier NCT01918397).

Adequate drug exposure of FQ, as key drugs in MDR-TB/RR-TB treatment, is important to prevent acquired FQ resistance, even more so in the shorter MDR-TB regimen (11). Acquired FQ resistance can be caused by interpatient variability in pharmacokinetic parameters or *M. tuberculosis* strains with increasing resistance, leading to insufficient attainment of the pharmacokinetic/pharmacodynamic target (12–14). Standard doses of 750 or 1,000 mg levofloxacin (10 to 15 mg/kg of body weight) have shown to achieve suboptimal drug exposures and an increased risk of acquired FQ resistance (7, 15). Levofloxacin doses of 17 to 20 mg/kg of body weight are suggested based on target attainment analysis, although additional data on efficacy and toxicity are still needed (15). With the recent findings of a higher target AUC_{0-24}/MIC (146) than assumed in these studies (53 and 100), the evidence for optimal levofloxacin doses above 15 mg/kg has grown even stronger.

Therapeutic drug monitoring (TDM) of second-line anti-TB drugs, e.g., levofloxacin, is recommended by the American Thoracic Society (ATS)/Centers for Disease Control and Prevention (CDC)/Infectious Diseases Society of America (IDSA) guidelines and could therefore be used to adjust individual FQ doses based on obtained pharmacokinetic data to ensure adequate drug exposure (13, 16, 17). To calculate AUC_{0-24} for use in TDM, one requires a full pharmacokinetic curve with multiple blood draws throughout the 24-h dosing interval. This is not only time-consuming and expensive, but it is unacceptable to patients and therefore unfeasible in clinical practice. A limited sampling strategy (LSS) is a method that requires fewer, usually one to three, optimally timed samples to accurately estimate the AUC. LSSs can be determined using both multiple linear regression and the Bayesian approach (18). The ease of multiple linear regression is that the resulting equation can estimate AUC with the obtained drug concentrations, although the samples should be timed exactly. The Bayesian approach is less rigid with timing of the samples and will generally result in more accurate

TABLE 1 Patient characteristics of the study population used for development of pharmacokinetic model versus external validation[a]

Parameter	Data set 1 (n = 10)	Data set 2 (n = 20)	Pharmacokinetic model (data sets 1 and 2) (n = 30)	External validation (data set 3) (n = 20)	P value (model versus validation)
Sex (no. [%])					
Male	8 (80)	12 (60)	20 (67)	12 (60)	0.765[b]
Female	2 (20)	8 (40)	10 (33)	8 (40)	
Age (yr)	43.5 (41.5–47.0)	38.5 (31.3–48.0)	41.5 (33.5–48.0)	30.5 (25.5–34.8)	0.002[c]
Ht (m)	1.69 (1.60–1.76)	1.68 (1.63–1.74)	1.69 (1.61–1.75)	1.74 (1.66–1.82)	0.038[c]
Wt (kg)	55.5 (50.1–60.8)	51.5 (43.7–59.7)	54.6 (47.9–59.9)	63.4 (53.8–78.5)	0.001[c]
Dose (mg/kg of body wt)	18.0 (16.5–20.0)	14.6 (12.8–17.2)	15.7 (13.6–18.1)	15.8 (12.8–16.6)	0.348[c]
BMI (kg/m²)	19.4 (18.7–21.2)	18.3 (16.1–21.4)	18.9 (17.5–21.2)	20.6 (18.9–25.6)	0.016[c]
Serum creatinine concn (μmol/liter)	80 (67–93)	73 (67–80)	74 (68–87)	66 (59–72)	0.014[c]

[a]Data are presented as the median (interquartile range [IQR]) unless otherwise stated.
[b]Fisher's exact test.
[c]Mann-Whitney U test.

estimates of the AUC, since it includes the population pharmacokinetic model, patient characteristics, sampling errors, and assay errors (15, 18). However, the Bayesian method requires pharmacokinetic modeling software that is not available to all clinical centers in settings endemic for MDR-TB and RR-TB. So far, only one study has described an LSS for levofloxacin. Alsultan et al. developed an LSS based on Bayesian approach and multiple linear regression using 4-h and 6-h postdose samples to estimate AUC_{0-24} (15). Pharmacokinetic data of only 10 TB patients were used, and no external validation was performed to determine whether the population pharmacokinetic model and LSSs were suitable for other groups of patients.

The aim of this study was to develop and validate a population pharmacokinetic (popPK) model of levofloxacin in TB patients and LSSs using the Bayesian approach as well as multiple linear regression to facilitate levofloxacin TDM in daily practice.

RESULTS

Study population. In total, the pharmacokinetic curves from data from 30 TB patients were used to develop the popPK model, and 20 curves of TB patients were used as external validation of the model and Bayesian LSS. Baseline characteristics of age, height, weight, body mass index (BMI), and serum creatinine levels of the patients included in the development of the model were significantly different ($P < 0.05$) from those included in the external validation (Table 1). The reference AUC_{0-24} ($AUC_{0-24,ref}$) and dose-corrected $AUC_{0-24,ref}$ of patients in data set 1 were significantly different ($P < 0.05$) from those of data set 3 as well (Table 2). An overview of the median (interquartile range [IQR]) levofloxacin concentrations of the pharmacokinetic curves is provided in Table 3.

Population pharmacokinetic model. The default models resulted in an Akaike information criterion (AIC) value of 9,950 for one-compartment and AIC value of 4,933 for two compartments. Based on AIC, a one-compartment pharmacokinetic model with lag time best described the data (AIC = 574). A two-compartment model was not favorable (AIC = 765 without lag time, AIC = 592 with lag time), possibly due to too

TABLE 2 Noncompartmental parameters of data sets 1 and 2 versus 3[a]

Parameter	Data set 1 (n = 10)	Data set 2 (n = 20)[b]	Pharmacokinetic model (data sets 1 and 2) (n = 30)	External validation (data set 3) (n = 20)	P value (model versus validation)
$AUC_{0-24,ref}$ (mg h/liter)	129 (118–191)	NA	129 (118–191)[c]	105 (86–128)	0.028[d]
$AUC_{0-24,ref}$/dose (h/liter)	0.129 (0.121–0.143)	NA	0.129 (0.121–0.143)[c]	0.109 (0.088–0.127)	0.035[d]
C_{max} (mg/liter)	15.6 (11.8–18.5)	8.9 (7.2–12.2)	10.3 (7.9–15.4)	10.5 (7.9–13.0)	0.649[d]
T_{max} (h)	1 (1–2)	2 (2–5)	2 (1–2)	1 (1–2)	0.073[d]

[a]Data are presented as the median (interquartile range [IQR]).
[b]NA, not applicable.
[c]Only available for data set 1 (n = 10).
[d]Mann-Whitney U test.

TABLE 3 Overview of included pharmacokinetic curves

Time (h)	No. of samples	Levofloxacin concn (median [IQR]) (mg/liter)
0	30	1.36 (0.95–1.58)
1	50	8.36 (5.74–12.79)
2	50	9.20 (7.63–11.31)
3	20	8.35 (7.08–9.95)
4	30	8.81 (7.23–10.34)
6	19	6.47 (5.38–8.10)
7	20	6.50 (4.70–7.08)
8	10	6.67 (6.10–7.55)
12	50	4.30 (2.88–5.08)
18	10	2.54 (2.34–3.41)
24	10	1.50 (1.30–1.71)

few data points during the elimination phase (19). The popPK parameters of the final model are summarized in Table 4.

External validation of the popPK model (Fig. 1) showed that AUC_{0-24} was slightly underestimated, with a mean of -7.9% (range, -25.1% to -1.6%; standard error [SE], 1.7%). Correlation of the Bayesian fitted AUC_{0-24} ($AUC_{0-24,fit}$) and $AUC_{0-24,ref}$ with an r^2 of 0.977 was found in Passing-Bablok regression.

LSS development using the Bayesian approach. The three best-performing strategies are displayed in Table 5, including root-mean-square error (RMSE), mean prediction error (MPE), and r^2. All strategies using 2 and 3 samples, except at $t = 0$ and 7 h, met the acceptance criteria (RMSE, $<15\%$; MPE, $<5\%$; $r^2 > 0.95$). Overall, the LSS with samples at 0, 2, and 8 h postdose was the best-performing strategy, with an RMSE of 7.1%, MPE of -0.70%, and r^2 of 0.972. However, the LSS with 0- and 5-h samples (RMSE, 8.8%; MPE, 0.42%; r^2, 0.957) was chosen for further evaluation because of its clinical suitability in addition to its relatively good performance. The results of the external evaluation (Fig. 2) showed a mean underestimation of -4.4% (range, -38.4% to 6.1%; SE, 2.7%) and r^2 of 0.821.

LSS development using multiple linear regression. The three best-performing LSSs with and without an 8-h sample are displayed (Table 6), including the number of included curves (n), RMSE, MPE, and r^2. Again sampling at 0, 2, and 8 h postdose was the best-performing LSS, with an RMSE of 1.7%, MPE of 1.4%, and r^2 of 0.997. LSS of 4- and 8-h samples was the best-performing strategy with two time points, with an RMSE of 2.5%, MPE of 2.1%, and r^2 of 0.997. The LSS using 0- and 4-h postdose samples showed a good performance as well, with an RMSE of 7.0%, MPE of 5.5%, and r^2 of 0.977. This LSS was chosen for further evaluation because of clinical suitability in addition to good performance. AUC_{0-24} (mg h/liter) can be estimated ($AUC_{0-24,est}$) using the equation $AUC_{0-24,est} = 4.96 + 18.12 \times C0 + 10.04 \times C4$, where C0 and C4 are the levofloxacin concentrations (mg/liter) at 0 and 4 h after drug intake, respectively. The results of the internal validation showed a mean underestimation of -0.46% (range, -46.5% to 11.8%; SE, 2.0%) and r^2 of 0.966 (Fig. 3).

DISCUSSION

In this study, we successfully developed and validated a population pharmacokinetic model of levofloxacin in TB patients. Furthermore, we developed and validated an

TABLE 4 Pharmacokinetic parameters of the population pharmacokinetic model of levofloxacin

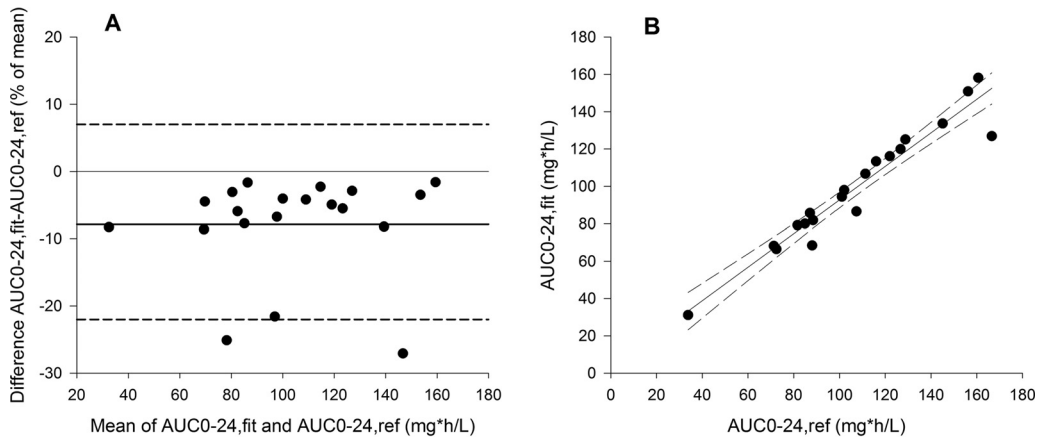| Parameter	Geometric (mean ± SD) ($n = 30$)
CL/F (liters/h)	7.1710 ± 3.0503
V_d/F (liters/kg of body weight)	1.5148 ± 0.2970
K_a (/h)	4.2922 ± 5.8764
T_{lag} (h)[a]	0.7693 ± 0.1277

[a]T_{lag}, lag time.

FIG 1 Bland-Altman plot (A) and Passing-Bablok regression (B) of external validation of a population pharmacokinetic model of levofloxacin ($n = 20$).

LSS based on multiple linear regression using 0- and 4-h samples and an LSS based on the Bayesian approach using 0- and 5-h samples.

The popPK model was able to estimate the AUC_{0-24} of TB patients, with significant differences in age, height, weight, BMI, serum creatinine, and levofloxacin exposure in the external validation, with a mean underestimation of only -7.9% (Tables 1 and 2 and Fig. 1). The popPK parameters of the developed model were comparable to those of the prior one-compartment model in healthy volunteers (20).

Second, we developed two LSSs that can be used in clinical practice to estimate levofloxacin drug exposure. In this analysis, we considered an LSS clinically feasible if it required 1 to 3 samples with a maximal time span of 8 h postdosing. However, we feel that a smaller time span between the samples is more favorable in daily practice. Both LSSs, multiple linear regression LSS using the equation and Bayesian LSS using the popPK model, were able to adequately estimate the AUC_{0-24}. We expect no problems concerning 0-h concentrations below the limit of quantification of assays, since in our data sets, the median levofloxacin concentration at 0 h was 1.36 mg/liter (IQR, 0.95 to 1.58 mg/liter), and no data were missing due to low concentrations.

We developed an LSS based on multiple linear regression because it is a straight-forward method that can be used at any clinical center. It only requires the equation and the levofloxacin concentrations at 0 and 4 h after drug intake to estimate AUC_{0-24}. The 8-h single-sample LSS was not chosen for validation despite its remarkably good performance, due to the limited number of included curves. Moreover, this time point may be unfeasible in combination with directly observed treatment (DOT) at 0 h, and it may be challenging to obtain a precisely timed 8-h sample.

Bayesian LSSs, on the other hand, can only be used in centers that have access to pharmacokinetic modeling software. The Bayesian LSS resulted in other, more optimal sampling time points (0 and 5 h) than the multiple linear regression-based LSS (0 and

TABLE 5 LSSs of levofloxacin using the Bayesian approach

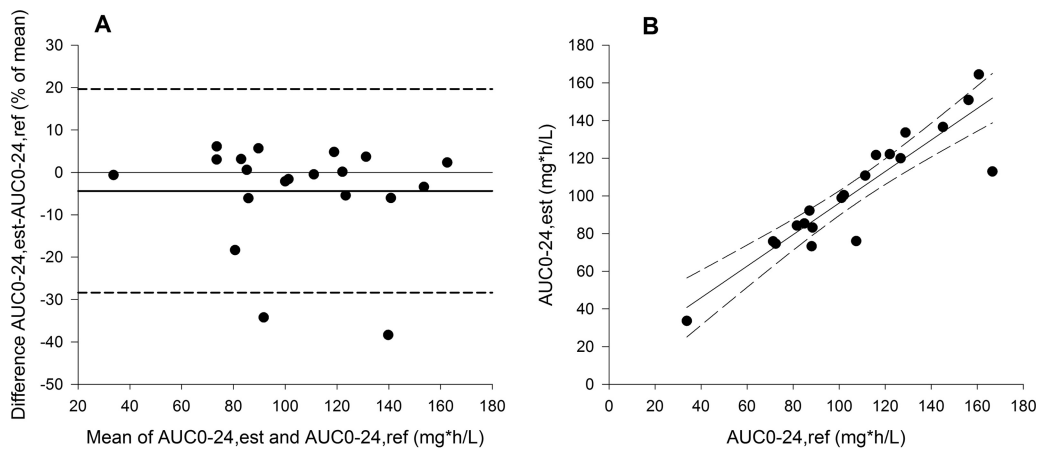| First sampling time point (h)	Second sampling time point (h)	Third sampling time point (h)	r^2	MPE (%)	RMSE (%)
6			0.847	−0.62	16.5
7			0.883	−0.29	14.4
8			0.906	0.88	12.9
0	7		0.949	0.43	9.5
0	6		0.952	0.36	9.2
0	5		0.957	0.42	8.8
0	2	7	0.970	−1.13	7.4
0	3	8	0.970	−0.93	7.3
0	2	8	0.972	−0.70	7.1

FIG 2 Bland-Altman plot (A) and Passing-Bablok regression (B) of external validation of the Bayesian LSS using $t = 0$ and $t = 5$ h sampling ($n = 20$).

4 h). This discrepancy is most likely caused by an unlimited choice of time points, more patients being included in LSS development due to inclusion of data set 2, and the influence of the popPK model. The Bayesian strategy using 0- and 4-h samples was not among the three best-performing two sample strategies shown in Table 5 but still had a performance within acceptable limits (RMSE, 9.5%; MPE, 0.04%; $r^2 = 0.949$). Therefore, it would be possible to take 0- and 4-h samples and use both the Bayesian estimation and multiple linear regression to estimate the AUC_{0-24}.

AUC_{0-24} estimated by LSS produced a slight underestimation which is acceptable and expected to be clinically irrelevant. In a comparison of $AUC_{0-24,ref}$ with $AUC_{0-24,est}$, the underestimation resulted in a different decision whether to increase the levofloxacin dose or not in only 1 out of 30 patients for the LSS based on multiple linear regression and in 1 out of 20 TB patients for the Bayesian LSS. The arget AUC_{0-24} was set at >150 mg h/liter (9) based on an MIC of 1 mg/liter (13). In MDR-TB treatment practice, the precise AUC_{0-24} is not as important to the clinician as whether or not the TDM result triggers a dose increase. Dose increments will be based on available tablets, and these are expected to account for a dose-proportional 25% (1,000 to 1,250 mg) to 33% (750 to 1,000 mg) increase in AUC_{0-24} (21). Moreover, the risks of treatment failure and acquired antibiotic resistance are more relevant than the potential for relatively mild adverse drug reactions compared to other anti-TB drugs and other FQ (13). The performance of an LSS has to be balanced against its alternatives, i.e., the collection of

TABLE 6 LSSs of levofloxacin using multiple linear regression

| Maximum time span (h)	Sampling time point (h)			$AUC_{0-24,est}$ calculation	n	r^2	MPE (%)	RMSE (%)
	1st	2nd	3rd					
8	0			$65.71 + 35.59 \times C0$	30	0.849	14.8	18.5
8	4			$-22.43 + 16.51 \times C4$	30	0.892	11.2	15.6
8	8			$-16.40 + 21.93 \times C8$	10	0.996	2.6	3.1
7	0	2		$27.84 + 23.87 \times C0 + 5.50 \times C2$	30	0.923	9.1	12.9
7	1	7		$-5.43 + 3.00 \times C1 + 13.88 \times C7$	20	0.939	5.9	7.1
7	0	4		$4.96 + 18.12 \times C0 + 10.04 \times C4$	30	0.977	5.5	7.0
8	2	8		$-18.79 + 0.99 \times C2 + 20.60 \times C8$	10	0.996	2.5	2.9
8	0	8		$0.11 + 6.48 \times C0 + 18.05 \times C8$	10	0.997	2.2	2.5
8	4	8		$-4.28-4.76 \times C4 + 26.98 \times C8$	10	0.997	2.1	2.5
7	0	2	7	$-3.01 + 10.58 \times C0 + 2.91 \times C2 + 11.31 \times C7$	20	0.979	3.0	4.1
7	0	3	7	$-2.98 + 10.69 \times C0 + 3.99 \times C3 + 10.18 \times C7$	20	0.986	2.6	3.3
7	0	4	7	$3.10 + 11.79 \times C0 + 5.63 \times C4 + 7.12 \times C7$	20	0.987	2.2	3.2
8	1	2	8	$-16.34-1.33 \times C1 + 2.11 \times C2 + 21.05 \times C8$	10	0.997	1.8	2.3
8	2	4	8	$-6.51 + 1.04 \times C2 - 4.87 \times C4 + 25.70 \times C8$	10	0.997	1.8	2.1
8	0	2	8	$= 1.18 + 8.35 \times C0 + 1.53 \times C2 + 14.86 \times C8$	10	0.997	1.4	1.7
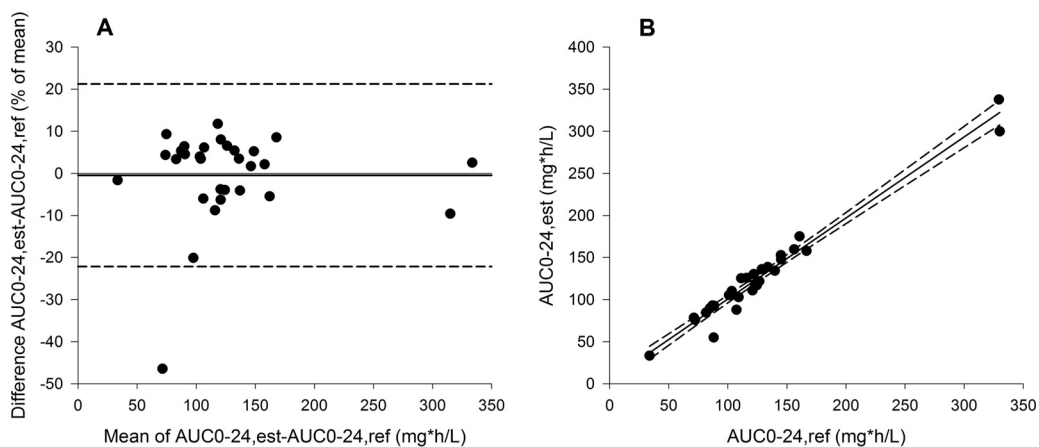
FIG 3 Bland-Altman plot (A) and Passing-Bablok regression (B) of internal validation ($n - 3$) of the multiple linear regression based LSS using $t = 0$ and $t = 4$ h sampling ($n = 30$).

a full pharmacokinetic curve or not performing TDM at all. Considering the current poor MDR-TB treatment results, we realize that the added value of TDM using LSSs may be substantial.

Apparently, the popPK model and therefore also the Bayesian LSS did not correctly fit three curves of data set 3, resulting in outliers (Fig. 1 and 2). Two of these outliers showed slow drug absorption (T_{max}, 4 and 7 h), causing difficulties in fitting. Food likely did not play a role in this slow absorption, since all patients fasted before drug intake (22). The third outlier had a relatively high concentration at 12 h postdose, possibly due to a measurement error, and was recognized by the model as an outlier. This caused a considerable difference in the $AUC_{0-24,ref}$ and $AUC_{0-24,est}$, as the 12-h sample was the last sample of the curve and for that reason had a major influence on the trapezoid of 12 to 24 h and $AUC_{0-24,ref}$.

This study had other limitations. Due to the low number of concentrations collected during the elimination phase, we were unable to develop a two-compartment model. Due to a small range of serum creatinine values, we were unable to determine the renally and nonrenally cleared fractions, as well as the influence of creatinine clearance on total body clearance using Fr, which is defined as ratio of creatinine clearance to renal clearance. It must be noted that the AUC_{0-24} of patients with impaired renal function might not be adequately estimated by our model and LSS due to this limitation, as creatinine clearance is known to be associated with levofloxacin clearance (23). The popPK model as well as the LSSs included only data of patients without renal insufficiency. The results obtained using our model in patients with renal insufficiency should be interpreted carefully. However, moxifloxacin is preferred to levofloxacin in MDR-TB treatment in case of kidney failure, because moxifloxacin is mostly eliminated by hepatic metabolism (24). Despite these limitations, we developed a model and LSSs that were able to adequately predict the AUC_{0-24} of a study population with significantly variable age, height, weight, BMI, and levofloxacin exposures, indicating general suitability in a heterogeneous population of TB patients. Last, the use of retrospective data resulted in a limited number of included curves and less variability in sampling times for the LSSs using multiple linear regression. We still succeeded in developing two LSSs to adequately estimate levofloxacin drug exposure in clinical practice using just two blood samples.

The ATS/CDC/IDSA guidelines recommend TDM for patients treated with second-line anti-TB drugs, e.g., levofloxacin (16). A validated LSS is capable of simplifying the procedure of TDM by limiting the number of required blood samples and therefore reducing the burden for patients, decreasing the impact on daily schedules in the clinic and reducing sampling costs. Using the described LSSs, it is possible to adequately predict levofloxacin exposure with only 2 plasma samples and if necessary adjust the
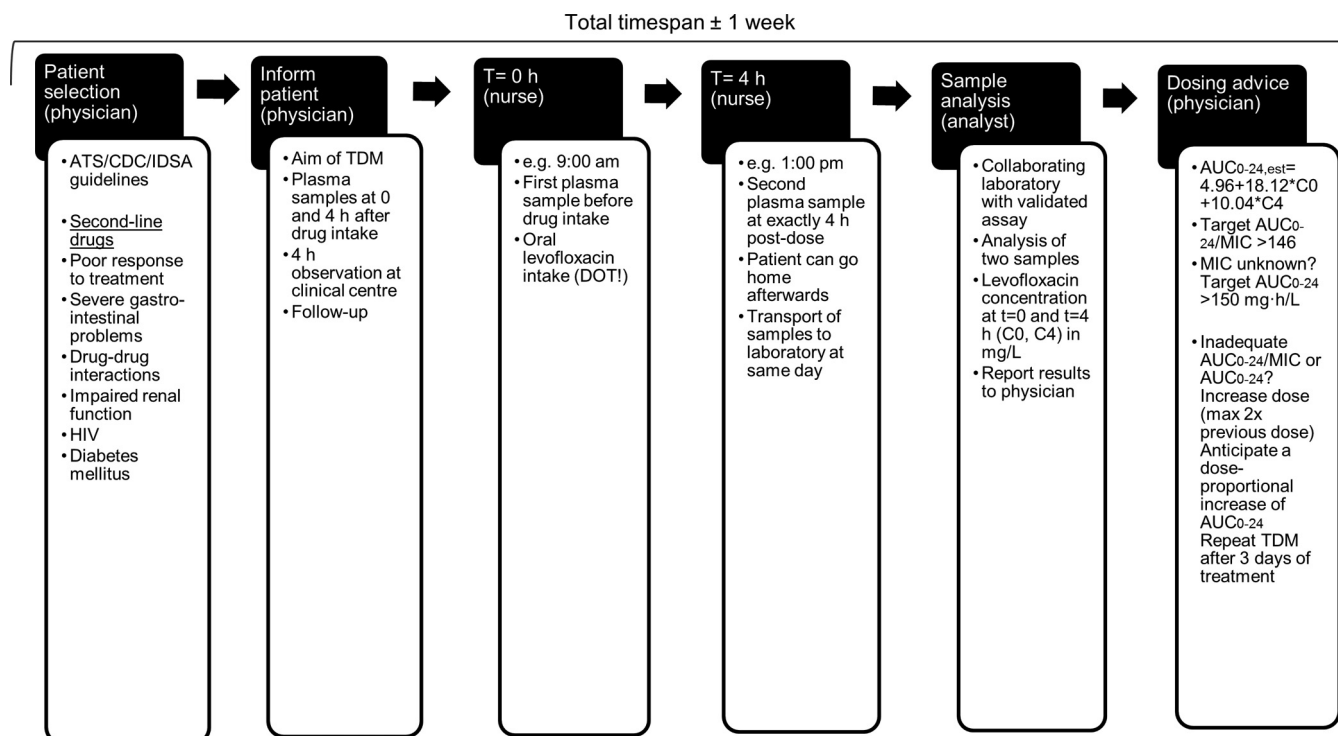
Total timespan ± 1 week

FIG 4 Practical guideline to perform TDM of levofloxacin using an LSS based on multiple linear regression. max, maximum.

dose based on the recently proposed target AUC_{0-24}/MIC of >146 (9). If the MIC is unknown, the target AUC_{0-24} would be approximately >150 mg h/liter, since levofloxacin MIC values of 1.0 mg/liter were most frequently reported for drug-resistant *M. tuberculosis* strains (13).

By determining the individualized levofloxacin dose, treatment failure and development of antibiotic resistance may be minimized (12, 13, 25). A helpful practical guideline for performing TDM of levofloxacin using the described multiple linear regression LSS is provided in Fig. 4 to encourage physicians to implement TDM in their clinic (26). We feel that TDM of anti-TB drugs should be available to most (if not all) TB patients, even in high-TB-burden areas, to support the end-TB strategy worldwide (27).

In conclusion, this study successfully developed a population pharmacokinetic model of levofloxacin in TB patients. Levofloxacin drug exposure can be adequately estimated with LSSs using 0- and 4-h postdose samples (multiple linear regression) or 0- and 5-h postdose samples (Bayesian approach).

MATERIALS AND METHODS

Study population. Three different data sets were included in this study. Data set 1 included data from a study on the pharmacokinetics of 1,000 mg levofloxacin in 10 Brazilian TB patients (6, 15). Blood samples were taken at 0, 1, 2, 4, 8, 12, 18, and 24 h after the fifth dose of levofloxacin. Data set 2 consisted of levofloxacin concentrations from 20 MDR-TB patients in Kibong'oto Infectious Diseases Hospital in Tanzania. Patients received either 750-mg or 1,000-mg levofloxacin doses based on body weight. Two weeks after initiating treatment, samples were taken at 1, 2, 6, and 12 h. Data set 3 included data from a pharmacokinetic study of levofloxacin in 20 MDR-TB and extensively drug-resistant TB (XDR-TB) patients in Republic Scientific and Practical Center for Pulmonology and Tuberculosis in Minsk, Belarus (7). The data set included 750-mg and 1,000-mg levofloxacin dosing regimens based on body weight. Following 7 days of levofloxacin treatment, plasma samples were drawn at 0, 1, 2, 3, 4, 7, and 12 h after drug intake.

Levofloxacin was administered to all patients under fasting conditions. As steady-state concentrations are reached on day 3, we selected data obtained at steady state (28). Because of steady-state conditions, levofloxacin concentrations at 0 and 24 h were assumed to be equal. Informed consent was not required for this study due to the retrospective analysis of anonymous data.

Noncompartmental parameters of $AUC_{0-24,ref}$ (calculated using trapezoidal rule), dose-corrected $AUC_{0-24,ref}$ ($AUC_{0-24,ref}$ divided by levofloxacin dose in milligrams), C_{max}, and T_{max} were determined. C_{max} was defined as the highest observed concentration and T_{max} as the corresponding time to C_{max}.

Population pharmacokinetic model. Data sets 1 and 2 were used to develop the popPK model to ensure a proportional number of patients in model development versus external validation (30 versus 20) and because data set 2 could not be used for external validation due to a lack of 0- and 24-h data. The KinPop module of MWPharm 3.82 (Mediware, The Netherlands) was used to create a population pharmacokinetic model using an iterative two-stage Bayesian procedure. Bioavailability (F) was fixed at 1, as only oral data were available and F is known to be almost complete for levofloxacin (21). The popPK parameters were related to this fixed F and assumed to be log normally distributed. A residual error with a concentration-dependent SD was applied (SD = 0.1 + 0.1 × C, where C is the levofloxacin concentration). Levofloxacin is mainly eliminated renally (79.6%) as unchanged drug, but it is also metabolized to desmethyl levofloxacin (1.75%) and levofloxacin-N-oxide (1.63%) in the liver (21). Total body clearance is the composite of metabolic clearance (CL_M) and renal clearance (Fr × CL_{CR}, where Fr is the ratio of creatinine clearance [CL_{CR}] to renal clearance) (29) Due to a small spectrum of creatinine clearance values in our data set, we were unable to determine the exact Fr and renal elimination. One-compartment and two-compartment models of levofloxacin have been described (6, 15, 20, 30, 31). First, a default one-compartment model (15) with fixed values of CL, volume of distribution (V), and absorption rate constant (K_a) was tested, and subsequently, Bayesian estimations of V, CL, and K_a were added one by one. Additionally, a default two-compartment model (30) with fixed values of distribution rate constants (k_{12} and k_{21}), elimination rate constant (k_{10}), and central volume of distribution (V_1) was tested. K_a could not be fixed due to an unknown population estimation of K_a because of intravenous administration in the default model. Subsequently, Bayesian estimations of the other parameters were added one by one. Finally, Bayesian estimation of lag time was added to the one- and two-compartment models and evaluated because of the oral administration of levofloxacin. The final pharmacokinetic model was chosen by comparing the AIC values of each submodel as a measure for goodness of fit using likelihood penalization. An AIC decrease of 3 was considered significantly better (19, 32).

The final model based on data sets 1 and 2 was externally validated using data set 3. The Bayesian fitted AUC_{0-24} ($AUC_{0-24,fit}$) was compared with the noncompartmental AUC_{0-24} calculated with the trapezoidal rule ($AUC_{0-24,ref}$). The agreement of $AUC_{0-24,fit}$ and $AUC_{0-24,ref}$ was evaluated using a Bland-Altman plot and Passing-Bablok regression (Analyze-it 4.81; Analyze-it Software Ltd., Leeds, United Kingdom).

Patient characteristics and pharmacokinetic data of data set 3 used for external validation were compared with data sets 1 and 2 used to develop the pharmacokinetic model. The median (IQR) and number (%) data of the parameters were tested for significance by the Mann-Whitney U test and Fisher's exact test, respectively, using IBM SPSS Statistics 23 (IBM Corp., Armonk, NY). P values of <0.05 were considered significant.

LSS development using Bayesian approach. Monte Carlo simulation in MWPharm was used to create 1,000 virtual patients representing the data used to build the pharmacokinetic model. The reference patient for Monte Carlo simulation was chosen based on a well-fitting and representative pharmacokinetic curve in combination with representative patient characteristics (male, 50 years; BMI, 19.1 kg/m²; serum creatinine level, 80 μmol/liter; dose, 16.9 mg/kg of body weight). Steady-state AUC_{0-24} was chosen as a parameter for optimization by the LSS. Using this method, LSSs which were able to give the best estimation of AUC_{0-24}, and therefore are the best choice for levofloxacin TDM, could be selected. Only LSSs using 1, 2, or 3 samples with a minimum interval of 1 h and maximum time span of 8 h postdose were tested, because of clinical suitability. The performances of the LSSs were assessed using the RMSE as a measure of precision, MPE as a measure of bias, and adjusted r^2 (in declining order of relevance) with acceptance criteria of RMSE of <15%, MPE of <5%, and r^2 of >0.95. The LSS chosen was externally validated using data set 3 by comparing the AUC_{0-24} estimated by LSS ($AUC_{0-24,est}$) with $AUC_{0-24,ref}$ using Bland-Altman plot and Passing-Bablok regression.

LSS development using multiple linear regression. Data sets 1 and 3 were used for the development of LSSs. Data set 2 had to be excluded from these analyses, since both the 0- and 24-h samples were lacking, and we were unable to calculate the $AUC_{0-24,ref}$. For each LSS, pharmacokinetic curves without concentration data at the selected time points could not be included in the analysis. The levofloxacin concentrations at the sampling time points and the $AUC_{0-24,ref}$ were analyzed using multiple linear regression in Microsoft Office Excel 2010. Only clinically suitable LSSs were tested (maximum time span, 8 h; minimum interval, 1 h; 1 to 3 samples), and acceptance criteria were applied (RMSE < 15%, MPE < 5%, r^2 > 0.95). The chosen LSS was internally validated using jackknife analysis. Multiple linear regression analysis was repeated in 10 different ($n - 3$) subanalyses, each leaving out three randomly chosen patients. All 30 patients were excluded once (33). Each subanalysis resulted in a different equation to the estimate the AUC_{0-24} values using levofloxacin concentrations at the chosen sampling times. Per subanalysis, the AUC_{0-24} values of the 3 excluded curves were estimated by the corresponding equation ($AUC_{0-24,est}$). $AUC_{0-24,est}$ was compared to $AUC_{0-24,ref}$ using Bland-Altman plot and Passing-Bablok regression.

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