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Aminoglycosides, such as amikacin, are used to treat multidrug-resistant tuberculosis. However, ototoxicity is a common problem and is monitored using peak and trough amikacin concentrations based on World Health Organization recommendations. Our objective was to identify clinical factors predictive of ototoxicity using an agnostic machine learning method. We used classification and regression tree (CART) analyses to identify clinical factors, including amikacin concentration thresholds that predicted audiometry-confirmed ototoxicity among 28 multidrug-resistant pulmonary tuberculosis patients in Botswana. Amikacin concentrations were measured for all patients. The quantitative relationship between predictive factors and the probability of ototoxicity were then identified using probit analyses. The primary predictors of ototoxicity on CART analyses were cumulative days of therapy, followed by cumulative area under the concentration-time curve (AUC), which improved on the primary predictor by 87%. The area under the receiver operating curve was 0.97 on the test set. Peak and trough were not predictors in any tree. When algorithms were forced to pick peak and trough as primary predictors, the area under the receiver operating curve fell to 0.46. Probit analysis revealed that the probability of ototoxicity increased sharply starting after 6 months of therapy to near maximum at 9 months. A 10% probability of ototoxicity occurred with a threshold cumulative AUC of 87,232 days · mg · h/liter, while that of 20% occurred at 120,000 days · mg · h/liter. Thus, cumulative amikacin AUC and duration of therapy, and not peak and trough concentrations, should be used as the primary decision-making parameters to minimize the likelihood of ototoxicity in multidrug-resistant tuberculosis.

Multidrug-resistant tuberculosis (MDR-TB) is a major source of public health concern (1–3). The mainstay for treatment of MDR-TB has been fluoroquinolones and aminoglycosides such as amikacin (4). However, aminoglycosides can cause both ototoxicity and nephrotoxicity. A potential way to decouple the toxicity from efficacy is to determine if the pharmacokinetic parameters that drive toxicity differ from those driving efficacy, as has been the case with several other antibacterial agents (5–12). Therefore, we measured serum amikacin concentrations in patients being treated for MDR-TB and determined aspects of pharmacokinetic parameters that predicted toxicity.

Amikacin is an important component of MDR-TB treatment in Botswana and is also recommended by the World Health Organization (WHO) for most countries. In 437 MDR-TB patients in Botswana, 62% developed amikacin-associated hearing loss, while the therapeutic success rate was 73% (13). Thus, therapeutic success is approximately as common as ototoxicity, leading to the commonly used and flippant statement by patients and caregivers, "Better deaf than dead." Currently, peak and trough concentrations are used to adjust dosing to minimize chances of toxicity under the hypothesis that toxicity is concentration dependent (14). Here, we performed a formal literature search to assess this relationship and found no studies that specifically addressed this in MDR-TB. To address this possibility, we utilized classification and regression tree analysis (CART), an algorithm of machine learning and, hence, artificial intelligence, to identify potential predictors of ototoxicity in MDR-TB patients and interactions among them. CART is a nonparametric method that can examine both linear and nonlinear interactions simultaneously, and it was designed to handle missing data by identifying and using surrogate variables to minimize ascertainment bias, as is the case in data collected for routine clinical care (9, 15–17). The classification and ranking subroutines also yield intuitive predictors by ranking predictors in order of importance and, more importantly, calculating threshold decision-making values above which patients would likely have higher toxicity rates.

MATERIALS AND METHODS

Literature search. First, we searched PubMed for articles in any language published before 1 December 2014 with search terms "amikacin," "ototoxicity," and "tuberculosis." This was in order to systematically identify clinical studies performed in the past on amikacin ototoxicity in MDR-TB and the relationship to drug concentration.

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Ethics approval. The clinical study was approved by the Human Research Development Committee at the Ministry of Health, Botswana, and the University of Pennsylvania Institutional Review Board.

Study population. All MDR-TB patients 15 years of age or older who were started on MDR-TB treatment between 1 January 2011 and 30 December 2012 and who had serum amikacin concentrations measurements reported were included. We included all patients who had at least one creatinine measurement within 1 month prior to or after the start of amikacin and one follow-up creatinine measurement (within the last 6 months prior to censoring).

Patient follow-up and definitions of ototoxicity. MDR-TB patients were treated with a standardized MDR-TB regimen composed of amikacin, levofloxacin, ethionamide, cycloserine, and pyrazinamide. The treatment was administered daily at the observation clinic under directly observed therapy. Amikacin dosage was calculated according to the WHO recommendations and adjusted for renal function (4). Doses of 15 to 25 mg per kg of body weight (with a maximum dose of 1,000 mg per day) were administered intramuscularly. Amikacin was administered once daily, 7 days per week, and changed to three times per week after culture conversion in patients who had hearing loss. The injections were discontinued 4 months after culture conversion.

Hearing assessments, including subjective self-reports by patients plus audiograms using a GSI 61 audiometer, were performed routinely as part of clinical care provided to all patients treated for MDR-TB in Botswana. Both self-reported hearing loss and audiometry panel tests were used to ascertain ototoxicity, in accordance with the Botswana National Tuberculosis Program guidelines and American Speech-Language-Hearing Association (ASHA) criteria. Based on these criteria, audiometry-determined neurosensory hearing loss was defined as a shift or loss of 15 to 25 dB relative to the baseline averaged at two or more contiguous frequencies in at least one ear or loss of response at 3 consecutive frequencies where responses had previously been obtained. High-frequency audiometry was performed on some patients who reported hearing loss; follow-up on those patients with audiometry was performed every 3 months until 3 months after amikacin treatment. Audiometry tests were performed by trained and experienced personnel at baseline prior to starting antituberculosis therapy and was repeated at least at monthly intervals during treatment. Self-reported hearing was ascertained by crude functional clinical examination and defined as the absence of usable hearing acquired during or soon after (within 6 months) amikacin treatment. Subjective measures of hearing loss, including tinnitus, were not included in defining ototoxicity, and henceforth audiometry-confirmed hearing is synonymous with ototoxicity. Clinical examinations of the vestibular functions are routinely done during follow-up; however, the results of those tests are not presented in this study.

Pharmacokinetic sampling and modeling. We analyzed amikacin plasma concentration levels that had been collected prospectively as part of standard clinical monitoring in the adult MDR-TB patients. Patients had blood drawn at 1 h and 23.5 h after an intramuscular amikacin dose. The blood concentration measurements were made on the COBAS INTEGRA systems, for which drug assay parameters were previously described (18). All amikacin concentrations were comodeled in ADAPT 5 software using methods described before (9, 19, 20).

CART. Classification and regression tree (CART) analyses were performed in three steps (9, 16, 21, 22). In all steps, confirmed hearing loss was the dependent variable. First, potential predictors of ototoxicity were examined *in toto* in CART models, using Salford Predictive Miner System software (San Diego, CA). Potential predictors examined included patients' ages, gender, initial weight, HIV infection status, receipt of antiretroviral therapy, and pharmacokinetic measures of drug exposures, including amikacin dose, cumulative dose, time on amikacin therapy, and amikacin dose (in milligrams per kilogram, both as daily dose and cumulative dose), area under the concentration-time curve from 0 to 24 h (AUC_{0-24}), cumulative AUC, peak concentration, and trough concentration. Many of these measures are highly correlated, but CART is designed to break such colinearity. Second, we performed CART analyses focused solely on the pharmacokinetic and treatment factors identified in the first step for better identification of threshold values that predicted hearing loss. Third, we performed CART analyses in which we forced a pick of peak and trough concentrations as predictors, given the wide use of these parameters for clinical care. Priors were set to equal, i.e., all categories had equal probability assignment, with no penalty sets for misclassification. Gini methods were used for building trees together with 5-fold cross-validation of each tree, restricting to >3 subjects per node (15). Area under the receiver operating characteristic curve (AUROC) and parsimony were used to assess and choose trees during pruning.

Analysis using frequentist statistics. Comparisons of proportions were made using Fisher's exact χ tests, while Kruskal-Wallis or Student's *t* tests were used to compare the medians or means. Estimates of potential predictors of ototoxicity identified in CART, such as cumulative AUC, initial patient weight, and the time on amikacin therapy associated with ototoxicity in 50% of the maximal proportion of patients with ototoxicity (50% toxic concentration, or TC_{50}) and the 95% confidence intervals (CI), were calculated by an analysis of probits. All analyses were two sided with alpha set at 0.05 and performed with STATA software, version 13 (StataCorp LP, College Station, TX).

Design of optimal sampling times for clinical decision making. In order to identify a sampling strategy that would give clinicians a good description of the entire AUC_{0-24}, we applied optimal sampling theory to the amikacin pharmacokinetic parameters we identified in the MDR-TB patients. This allows blood draws to be performed at particular information-rich time points, after at least 3 half-lives, to allow for identification of unbiased pharmacokinetic parameter estimates with a minimum number of samples. We ran this application in the subroutine SAMPLE of ADAPT 5 and set upper and lower limits of 0 and 24 h, a maximum of 999 iterations, and selected D-optimality criterion.

RESULTS

Our systematic literature search identified three clinical studies that examined the question of amikacin ototoxicity in patients (23–25). Two were retrospective studies that did not measure drug concentrations. Renal dysfunction, old age, and male gender were identified as risk factors on logistic regression (23, 24). The third study measured amikacin concentrations in 22 patients, but only four of the patients had tuberculosis (25). Thus, no prior studies formally investigated the role of amikacin concentration in tuberculosis.

Twenty-eight patients in Botswana who had MDR-TB had amikacin concentration measurements and audiometry assessments. The clinical and demographic characteristics of these patients are shown in Table 1. The mean age and mean body weight on initiating therapy did not significantly differ by gender ($P = 0.301$ and $P = 0.172$, respectively). All HIV-infected patients were on combination antiretroviral treatments, either nevirapine with zidovudine-lamivudine or efavirenz with zidovudine-lamivudine or tenofovir-emtricitabine. Of the 10 patients with prior aminoglycoside exposure, 6 had been on streptomycin for category II treatments and 4 for category IV MDR-TB treatments. Four of 10 patients had prior aminoglycoside exposure, whereas 3/18 patients without prior exposure developed ototoxicity ($P = 0.172$).

Amikacin pharmacokinetics were best explained by a 2-compartment model, with the model predicted versus observed concentrations shown in Fig. 1. The pharmacokinetic parameter estimates of amikacin are shown in Table 2. We used the parameter estimates in each patient to identify the AUC_{0-24}, to calculate the cumulative AUC, and to identify the distribution of these parameters and the observed drug concentrations, with results shown in Fig. 2. As expected, cumulative dose (days · milligrams per kilo-

TABLE 1 Demographic and clinical characteristics on 28 patients enrolled in the study

Clinical variable	Value ($n = 28$)
No. (%) female	12 (43)
No. (%) HIV infected	12 (43)
No. (%) on antiretroviral therapy	12 (43)
No. (%) with hearing loss	11 (39)
Subjective	11 (39)
Tinnitus	9 (32)
Audiometry confirmed	7 (25)
Mean (SD) age (yr)	44 (18)
Mean (SD) initial wt (kg)	50.57 (10.34)
No. (%) with prior aminoglycoside exposure	10 (36)
Median (range) amikacin dose (mg)	875 (400–1,000)
Median (range) therapy duration (days)	183.5 (28–866)
Median (range) cumulative dose (mg)	94,914 (17,864–601,394)

grams) correlated closely with cumulative AUC ($r^2 = 0.87$) and duration of therapy ($r^2 = 0.95$), given that duration of therapy is a common variable for the two other parameters. The other concentration measures did not closely correlate with each other, including peak versus AUC_{0-24} ($r^2 = 0.27$), peak versus trough ($r^2 = 0.08$), trough versus AUC_{0-24} ($r^2 = 0.16$), peak versus cumulative AUC ($r^2 = 0.31$), and trough versus cumulative AUC ($r^2 = 0.10$).

Hearing loss was encountered in 11/28 (39%) patients based on subjective reporting but in only 7/28 (25%) confirmed by audiograms. The median duration of amikacin therapy to confirmed hearing loss (range) was 177 (126 to 896) days, showing that the hearing loss observed in these patients was chronic onset instead of the reversible form, which tends to be acute onset. Three patients with confirmed hearing loss did not report any tinnitus, but out of the 9 patients who reported tinnitus, only 4 (44%) were confirmed by audiometry (Tables 1 and 3). Table 3 compares the pharmacokinetic parameters and demographics in patients with and without hearing loss, based on frequentist statistical inferences. This standard statistical approach, which averages out concentrations between the groups, obscures pharmacokinetic variability effects shown in Fig. 2. No statistically significant differences between patients with hearing loss and those without were observed using this statistical approach.

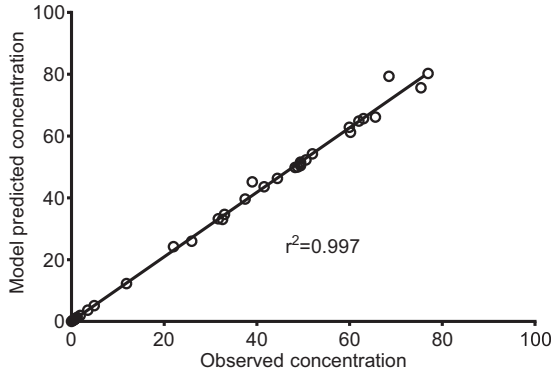

FIG 1 Observed concentrations versus model prediction. A two-compartment model (central compartment or serum versus peripheral compartment) described the amikacin pharmacokinetics in MDR-TB patients in Botswana well, with an r^2 of 0.997.

TABLE 2 Population pharmacokinetic parameter estimates in 28 MDR-TB patients[a]

Pharmacokinetic parameter	Mean	% RSE	SD (as %CV)
Total clearance (liter/h)	1.47	245	23.6
Volume of central compartment (liter)	2.10	562	171
Intercompartmental clearance (liter/h)	7.17	614	29
Volume of peripheral compartment	0.25	245	13.6
Absorption constant	2.89	114	4.43

[a] CV, coefficient of variation; RSE, relative standard error; SD, standard deviation.

CART identified deep trees of predictors for confirmed hearing loss for which the primary node was duration of therapy, which was followed closely by several of the measures of cumulative drug exposures, including cumulative AUC, cumulative dose, and cumulative dose divided by weight, followed by AUC_{0-24} and patient weight. Peak and trough were not identified as significant predictors. We next ran a CART model that focused on drug dose factors and pharmacokinetics, including drug measurements such as peak and trough. CART analysis identified the primary node as cumulative days of therapy, while cumulative AUC was ranked second and improved on the primary node by another 87%, followed by AUC_{0-24} with a score of 34% and initial weight with a score of 29%. The model AUROC for the test sets was 0.97. This was confirmed in cross-validation, performed by the algorithm randomly splitting the data set into training and test data sets multiple times; predictive power is the performance of the training set-derived tree on the test data set. Figure 3 shows the CART analysis output when drug concentration measures were examined solely as potential predictors in order to more accurately identify the concentration cutoff thresholds. Finally, prior to discarding peak and trough, we ran yet another CART model that was forced to pick the trough and peak as predictors. The AUROC on the test set was 0.46; thus, it was inferior to the 0.97 in the model that picked the duration of therapy and cumulative AUC. Thus, single-snapshot measures of drug exposures such as peak or trough amikacin concentration were unequivocally poorer predictors of ototoxicity in MDR-TB patients compared to duration of therapy, cumulative AUC, and AUC_{0-24}.

We next examined the relationships between duration of therapy, cumulative AUC, and weight using probit models. Figure 4 shows that the probability of ototoxicity can be reliably estimated mathematically as a function of each of these 3 parameters. The relationship between each of these parameters and probability of ototoxicity was sigmoidal, as described by equations shown in Fig. 4. The amikacin cumulative AUC TC_{50} was 87,323 (CI, 81,248 to 93,216) days · mg · h/liter, while the TC_{80} was 120,238 days · mg · h/liter. Similarly, Fig. 4C shows that by day 166.7 (CI, 152.8 to 180.6), the probability for ototoxicity starts to increase steeply and approached near certainty by day 280 (~9 months).

Four patients had subjective hearing loss that was not confirmed by audiometry: two had cumulative AUCs of 156,423.3 and 144,217.7 days · mg · h/liter. Of the other two, one HIV-infected patient had a cumulative AUC of 36,466.56 days · mg · h/liter but was concurrently on tenofovir, efavirenz, and emtricitabine, which have the potential for ototoxicity (26).

Finally, given the role of cumulative AUC identified by CART and probit analysis, concentrations for clinical decision making will need to include enough time points to better calculate AUCs for each patient. The application of optimal sampling theory in

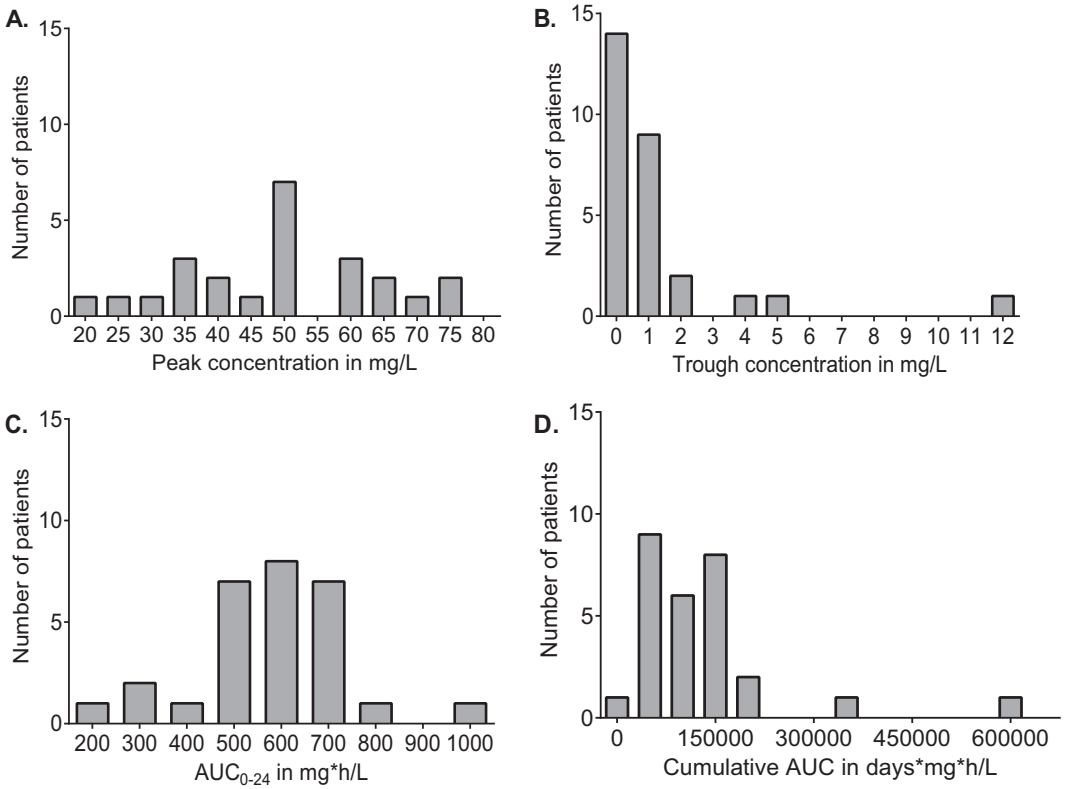

FIG 2 Distribution of amikacin concentrations in the 28 patients. (A) Peak concentrations were normally distributed based on the D'Agostino and Pearson omnibus normality test ($P = 0.774$). The ratio of the lowest to highest peak concentration was 3.49. (B) Trough concentrations were not normally distributed ($P < 0.001$). Fifty percent of patients had a trough below the limits of detection. (C) The AUCs were normally distributed ($P = 0.223$); the ratio of the lowest to highest AUC was 4.09. (D) The cumulative AUCs were not normally distributed ($P < 0.001$) and had an even wider range, with a ratio of highest to lowest cumulative AUC of 33.67.

ADAPT identified 0, 15 min, 2.5 h, and 18.7 h as the best times for blood draw. If the currently used 0.5-h sampling time was fixed, the model chose 0, 2.7 h, and 18.7 h, which are virtually the same as those for the nonfixed analysis. Thus, for therapeutic drug monitoring of amikacin during MDR-TB treatment, the best time points are 0, 15 min, 2.5 h, and 18.7 h, not peak and trough.

DISCUSSION

We identified amikacin compartmental pharmacokinetic parameters in patients on long-term treatment for MDR-TB. The amikacin clearance was relatively uniform with minimum between-patient variability, consistent with renal clearance in patients with normal renal function. However, the volume of the central compartment had a large between-patient variability, with a 57-fold range difference from highest to lowest, higher than that seen in studies of short-term amikacin therapy duration (27, 28). This means that there will be a wide variability in peak concentrations even if patients were given the same amikacin dose in Botswana. The reasons are unclear. We utilized these individual pharmaco-

TABLE 3 Comparison of clinical and pharmacokinetic characteristics in patients with or without hearing loss

Variable	Value(s) for patients with:			*P* value
	Confirmed hearing loss ($n = 7$)	Subjective hearing loss without confirmation ($n = 4$)	No hearing loss ($n = 17$)	
Demographics				
No. (%) female	5 (71)	0	7 (41)	0.069
No. (%) HIV infected (%)	2 (29)	2 (50)	8 (47)	0.789
Age (yr; range)	52 (28–57)	44.5 (28–70)	40 (29–65)	0.978
Initial wt (kg; range)	47.5 (41.2–57.8)	50.13 (45.0–59.8)	53.1 (42.5–58.5)	0.904
Amikacin doses and concentrations				
Dose (mg; range)	750 (750–1,000)	750 (500–1,000)	1000 (750–1,000)	0.875
Cumulative dose (days · mg; range)	140,679 (91,251–182,502)	100,418 (45,626- 174,898)	91,251 (45,625–152,085)	0.344
AUC_{0-24} (mg · h/liter; range)	548 (486–672)	546 (364–725)	569 (507–680)	0.881
Cumulative AUC (days · mg · h/liter; range)	90,539 (77,254–159,482)	96,833 (33,186–127,452)	96,479 (56,729–151,080)	0.449
Peak (mg/liter; range)	44.92 (31.68–65.59)	44.19 (22.02–59.97)	49.42 (39.00–62.02)	0.662
Trough (mg/liter; range)	0.70 (0–1.23)	0.71 (0–1.87)	0.35 (0–0.76)	0.734

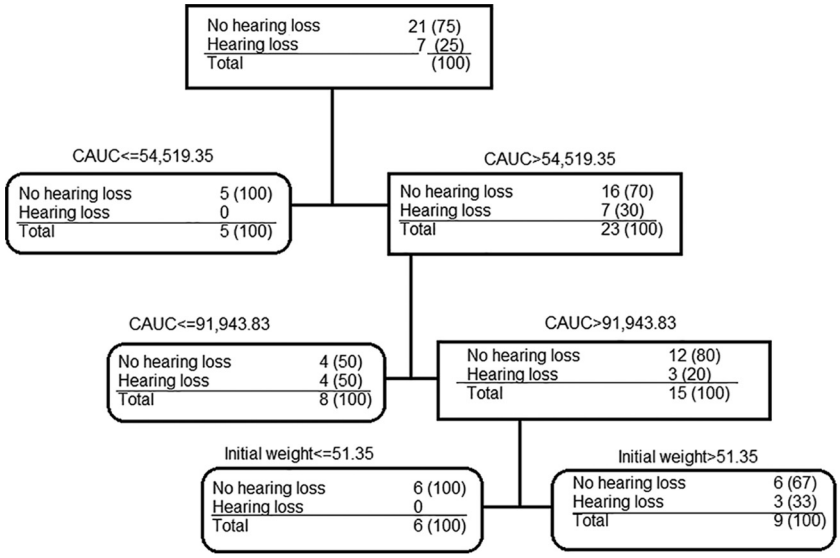

FIG 3 Optimum classification and regression analysis tree to identify concentration thresholds. The tree identified cumulative AUC thresholds of 54,519 and 91,914 days · mg · h/liter.

kinetic factors, *in toto* with all other clinical factors, with no prior assumptions of predictors or their distribution (i.e., agnostic), in machine learning algorithms to identify predictors of ototoxicity. Standard frequentist statistical inferences, which use measures of central tendency, failed to identify any associations, as shown in Table 3. On the other hand, despite the sample size, a pharmacometrics-based approach in tandem with nonparametric CART identified the main predictors of ototoxicity as duration of therapy and cumulative amikacin AUC. This relationship also was identified using parametric probit models; thus, it likely will hold even as sample size increases. Peak and trough concentrations were poor predictors of ototoxicity and also correlated poorly with AUC_{0-24} and cumulative AUC.

Our results are in accordance with animal model studies by Beaubien et al., who examined amikacin ototoxicity in albino guinea pig experiments (29, 30). They found that ototoxicity best correlated with both plasma cumulative AUC and duration of therapy and not peak or trough concentrations. The relationship between plasma cumulative AUC and hearing loss in the guinea pig experiments was also sigmoidal. They demonstrated that the rate of amikacin entry into perilymph and endolymph was proportional to the plasma cumulative AUC; it was this high cumulative amikacin AUC in the organ of Corti that damaged hair cells (29–31). Thus, our findings using an agnostic artificial intelligence approach that examined all clinical factors as potential predictors identified factors similar to those found in the animal experiments. These concordant animal results give a biological basis for, and mechanistic consistency with, our clinical findings.

We identified the cumulative AUC and therapy duration threshold values that clinicians treating pulmonary tuberculosis could use for monitoring patients. The decision making depends on the probability of ototoxicity that clinicians are willing to tolerate: a 10% probability occurs with a threshold cumulative AUC of 87,232, while that of 20% is ~120,000 (Fig. 4). This decision needs to be balanced versus the 75% therapeutic cure rate, which also could be concentration dependent. While the AUC is the speed toward target (cumulative AUC), the duration of therapy constitutes the distance to that target. The inflection point is at about 6 months of therapy (Fig. 4C), suggesting a limit for the amikacin therapy duration with regard to ototoxicity. On the other hand, patients with higher AUCs will have a higher incidence of ototoxicity earlier than the 6 months (higher so-called speed toward the cliff), so that if concentrations can be measured the optimal duration of therapy for safety then can be calculated during the first week of therapy. We identified the best time points as just prior to dose and 15 min, 2.5 h, and 18.7 h after dose for blood draws in tuberculosis programs for amikacin therapeutic drug monitoring.

Our study has several limitations. Amikacin has a long half-life in cochlear tissue; as a result, hearing loss has been known to occur long after cessation of therapy. Second, we only measured amikacin concentrations, even though MDR-TB patients receive several other drugs that could contribute to ototoxicity. Third, we did not have sufficient data on streptomycin exposure prior to receiving MDR-TB treatment in the study; therefore, we could not examine the relationship between this aminoglycoside exposure and ototoxicity. Fourth, defining ototoxicity is still an imprecise science. Most conventional audiometers test low frequencies up to 8 kHz, just enough to be within the regular hearing and speech ranges of 0.3 to 3 kHz. However, amikacin affects higher-frequency hearing loss, up to 20 kHz, earlier and more frequently than it does the lower ones (32). Some patients might not be aware that they have such high-tone deafness. In our study, one of the four patients with subjective hearing loss could have met that definition. Nonetheless, if all four patients were considered probable cases of ototoxicity, the computed incidence from this study, 11/28 (39%), would be within range of other widely reported estimates for aminoglycoside toxicities, including amikacin (23–25).

In summary, we identified cumulative AUC and duration-of-therapy thresholds as predictors of amikacin ototoxicity. Peak and trough concentrations which are currently used for clinical decision making were not predictors. We identified cumulative AUC

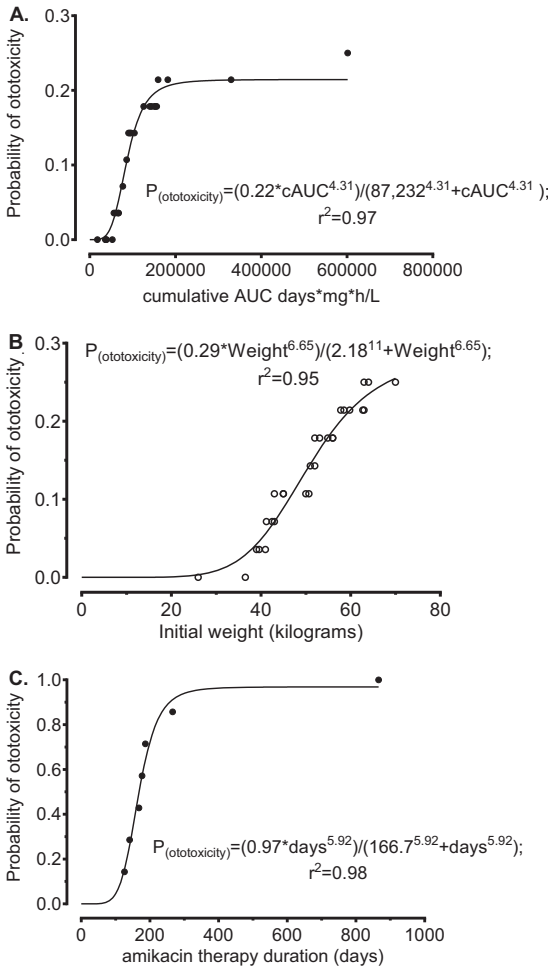

FIG 4 Probability of ototoxicity as a function of three different parameters. The probability of ototoxicity as a function of cumulative AUC is sigmoidal, with the TC_{50} of 87,232 days · mg · h/liter shown in the equation. This value is very close to the 91,914 days · mg · h/liter identified by CART. The more continuous relationship between concentration and toxicity can be used to calculate the cumulative AUC versus the probability and risk of toxicity that can be tolerated by clinicians and patients. (B) Probability of ototoxicity as a function of patient weight. Since this is a nonmodifiable patient factor at the time when a patient presents with MDR-TB, it would not factor much in the decision making. (C) Probability of ototoxicity as a function of duration of therapy. The risk increases at the inflection point of 6 months and is a near certainty at 9 months.

and duration of therapy thresholds predictive of the ototoxicity. These thresholds can be used for clinical decision making.

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