b | Reduced chance of hearing loss associated with Therapeutic Drug Monitoring of Aminoglycosides in the treatment of Multidrug Resistant Tuberculosis

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Submitted

Keywords: pharmacokinetics, pharmacodynamics, amikacin, TDM, tuberculosis
Key points: the occurrence of nephrotoxicity and ototoxicity was not significantly correlated with therapeutic parameters in the treatment with aminoglycosides, yet the extent of ototoxicity was correlated with the dose per kg bodyweight.

ABSTRACT

Hearing loss and nephrotoxicity are associated with prolonged treatment duration and higher dosage of amikacin and kanamycin. In our Tuberculosis Center, we have employed therapeutic drug monitoring (TDM) targeting pre-set pharmacokinetic/pharmacodynamic (PK/PD) surrogate endpoints in an attempt to maintain efficacy while preventing (oto-)toxicity. To evaluate this strategy, we retrospectively evaluated medical charts of TB patients treated with amikacin or kanamycin in the period 2000 - 2012.

Patients with culture-confirmed multi- or extensively drug resistant tuberculosis (MDR/XDR-TB) receiving amikacin or kanamycin as part of their TB treatment for at least 3 days were eligible for inclusion in this retrospective study. Clinical data, including C_{max}, C_{min} and audiometry data were extracted from the patients' medical charts.

80 patients met the inclusion criteria. The mean weighted C_{max}/MIC ratio obtained from 57 patients was 31.2 for amikacin and 12.3 for kanamycin. The extent of hearing loss was limited and correlated with the cumulative drug dose per kg body weight during daily administration. At follow-up, 35 (67.3%) of all patients had successful outcome; there were no relapses.

At a median dose of 6.5 mg/kg a correlation was found between the dose per kg bodyweight during daily dosing and the extent of hearing loss in dB at 8000 Hz. This study suggests that the efficacy at this lower dosage is maintained with limited toxicity. A randomized controlled trial should provide final proof of the safety and efficacy of TDM-guided use of aminoglycosides in MDR-TB treatment.

BACKGROUND

Amikacin and kanamycin are almost similar aminoglycosides and are both are considered very useful as second line injectable drugs for the treatment of multidrug resistant tuberculosis (MDR-TB)(1). MDR-TB is caused by *Mycobacterium tuberculosis* resistant to at least isoniazid and rifampicin. Although *in vitro* activity of amikacin and kanamycin appeared high against *M. tuberculosis* (2, 3), early bactericidal activity was low (4). In addition, extremely resistant TB (XDR-TB) is resistant to at least one aminoglycoside and any fluoroquinolone.

According to World Health Organization (WHO) guidelines, aminoglycosides are administered in a dose of 15 mg/kg/day with a maximum of 1000 mg daily in the treatment of patients with MDR-TB (5). Although cross resistance between amikacin and kanamycin is thought to be nearly complete (6-7) isolates resistant to one may still be susceptible to the other aminoglycoside and in vitro susceptibility should therefore be evaluated for each drug (8). Toxicity of aminoglycosides is profound and permanent, and hearing loss and nephrotoxicity have been observed in 8-37% of the patients receiving these drugs for any period of time (9-11). These adverse effects may aggravate with prolonged treatment and higher dosage (1). In a study based on data of 28 TB patients in Botswana treated with 15-25 mg/kg amikacin daily, 7 patients developed hearing loss. The cumulative area under the curve (AUC) and duration of amikacin treatment were predictors of hearing loss (12).

Aminoglycosides are not metabolised – renal excretion is the only elimination pathway. Patients with increased serum creatinine values and/or nephrotoxic co-medication run a higher risk for encountering nephrotoxicity (1). Because of these serious adverse events monitoring is advised and should consist of a baseline evaluation (audiogram, vestibular testing, Romberg testing and serum creatinine measurement) and a monthly evaluation during treatment (questionnaire regarding auditory or vestibular symptoms and serum creatinine) (1). Aminoglycoside-related ototoxicity generally manifests first at high frequencies, sometimes without the patients noticing their hearing loss (13). Regular monitoring gives the opportunity to alter the provided therapy in order to prevent more extensive hearing loss.

Pharmacokinetic (PK) and pharmacodynamic (PD) parameters have increasingly gained attention in the development of drugs and treatment of TB in recent years (14). Data regarding PK and PD parameters in TB are however scarce. For other bacterial infections, predominantly Gram-negative infections, e.g., caused by *Pseudomonas aeruginosa*, the maximum concentration (C_{max}) to mean inhibitory concentration (MIC) ratio is the most relevant PK/PD parameter to assess the efficacy of aminoglycosides (15-16). Additionally, it was shown that PK parameters of aminoglycosides may vary and the patients may benefit from individualized treatment (17-21). In our TB Center, we have used PK/PD parameters

targeting a surrogate endpoint of a C_{max}/MIC ratio >20, in an attempt to maintain efficacy while preventing (oto-)toxicity Therefore we performed a retrospective survey to evaluate the PK parameters of amikacin and kanamycin to detect predictors for PK parameters, as well as efficacy and toxicity.

PATIENTS, MATERIALS AND METHODS

In this retrospective study we evaluated all patients with culture-confirmed MDR-TB or XDR-TB, either pulmonary or extrapulmonary, receiving amikacin or kanamycin as part of their TB treatment for at least three days (steady state) who were hospitalized at the Tuberculosis Centre Beatrixoord between 1st of August 2000 and 16st of May 2012. Only patients older than 17 years were included. As retrospective data were collected the Institutional Review Board of the University Medical Center Groningen waived the requirement for research subjects to give informed consent (METc 2013/492).

Data collection

Medical history, age, sex, weight, length, ethnicity, co-morbidity, type of diagnosis, localisation of TB, MIC of amikacin and kanamycin, resistance pattern, dose and duration of TB treatment, creatinine levels at baseline and adverse events (hearing loss and renal dysfunction) were collected from the patients' medical records. Parameters such as the cumulative dose and the dose per kg bodyweight were calculated based on the gathered data. Serum levels of routine TDM of amikacin and kanamycin and the MIC of the sputum isolates were also retrieved from the patients' records. Adverse events were monitored using audiometric monitoring and the determination of the serum creatinine as described below.

Serum level measurements

C_{max} samples, obtained 30 min after a one-h infusion, and C_{min} samples obtained immediately before infusion were collected. Amikacin concentrations were determined by fluorescence polarization immunoassay (TDx or Architect, Abbott laboratories, Illinois, USA) with a lower limit of quantitation (LOQ) of 1.5 mg/L. Kanamycin concentrations were determined using a validated analytical method using liquid chromatography with coupled tandem mass spectrometry (TSQ Quantum, Thermo Fisher, San Jose, CA, USA) with a LOQ of 0.1 mg/L (22).

Drug susceptibility testing

The sputum isolates were subjected to drug susceptibility testing for amikacin and kanamycin at the Dutch National Mycobacteria Reference Laboratory (National institute

for Public Health and the Environment; RIVM). The Middlebrook 7H10 agar dilution method was applied for drug susceptibility testing of the isolate(s) (23). Drug susceptibility testing was not repeated during the treatment, except when the physicians expected the development of drug resistance based on clinical non-improvement. Sputum samples for microscopy (fluorescent staining) and culture were collected weekly and were sent to the national reference laboratory for analysis.

PK/PD analysis

The C_{max}/MIC ratio and time to sputum and culture conversion was calculated and considered to be a proxy parameter for efficacy. The aminoglycoside dose was adjusted based on the amikacin and kanamycin concentration and MIC.

Based on the peak and through levels, the $AUC_{0\text{-}24h}$ was estimated with the use of a validated population pharmacokinetic model using MW/Pharm 3.81 (Mediware, The Netherlands) (24). The C_{max}/MIC was consecutively calculated by dividing the C_{max} by the median MIC of 1 mg/L (amikacin) and 2.5 mg/L (kanamycin). A weighted C_{max}/MIC was calculated for each patient by the following formula:

$$weighted\ \frac{C_{max}}{MIC} = \frac{\sum days\ of\ treatment\ with\ dose\ X * \frac{C_{max}\ attained\ using\ dose\ X}{MIC}}{total\ treatment\ duration\ (days)}$$

Adverse events and clinical outcome

Adverse events of the aminoglycosides were assessed by evaluation of ototoxicity and renal function at baseline and during treatment. Audiometry was performed monthly at 250, 500, 1000, 2000, 4000 and 8000 Hz. Hearing loss was defined as 20 dB reduction in hearing threshold from baseline irrespective of side (right or left ear) or frequency (25). Audiometry was usually performed every 3 to 4 weeks during aminoglycoside treatment. Renal function was evaluated at least once a week by measuring creatinine in serum. Renal toxicity was defined as more than 50% increase in the baseline serum creatinine concentration at any moment during the treatment, in accordance with the common toxicity criteria (CTC) (26). Treatment outcome was evaluated two years after completion of treatment using common WHO criteria (27).

Statistics

All statistics were performed using SPSS 20 (SPSS, Virginia, IL). *M. tuberculosis* isolates showing no growth at <1 mg/L were statistically analysed as 1 mg/L. Differences in gender and type of aminoglycoside were assessed using Mann-Whitney U-tests. Determinants in nephrotoxicity and ototoxicity were also assessed using Mann-Whitney U-tests, except

for the gender (Chi-squared test), and the use of other co-medication (Fisher's Exact Test). Correlations between the extent of nephrotoxicity and ototoxicity and continuous or categorical factors were calculated using Spearman's coefficient. The correlation between clearance and distribution volume and the occurrence of side effects was assessed using Spearman's coefficient. The relation between the nephrotoxicity, classified by the CTC criteria as binary or categorical and demographic data was determined by Spearman's rank-order correlation test. Relations between the weighted C_{max}/MIC and time to sputum and culture conversion was assessed using simple linear regression and CART (CHAID) tree classification analysis. All P-values below 0.05 were considered significant.

RESULTS

Patient characteristics

Eighty patients with a median age of 30.5 (IQR; 25.0 – 39.0) years met the inclusion criteria; 37 (46.3%) patients were female and 43 (53.8%) were male. Patient characteristics at baseline are presented in table 1. Drug susceptibility testing was performed for all patients. All except three patients had a favorable outcome. One patient stopped due to drug addiction related problems and two patients were transferred to other hospitals without follow-up. Blood levels of 57 patients (71%) were retrievable from the patient files.

TABLE 1: Patient characteristics at baseline (total n = 80)

	N (%) or median (IQR)	
Common parameters	**Amikacin**	**Kanamycin***
Male (%)	26 (48.1)	17 (65.4)
Female (%)	28 (51.9)	9 (34.6)
Age (yr)	30 (25 – 39)	31 (25 – 40)
Weight (kg)	61.4 (55.2 – 68.4)	57.2 (50.0 – 68.2)
BMI (kg/m^2)	21.2 (19.4 – 23.6)	20.5 (18.5 – 22.4)
Ethnicity (%)		
-European	7 (13.0)	2 (7.7)
-Asian	17 (31.5)	4 (15.4)
-African	14 (25.9)	12 (46.2)
-Other	14 (25.9)	7 (26.9)
-Unknown	2 (3.7)	1 (3.8)

	N (%) or median (IQR)	
Tuberculosis		
Localisation		
Pulmonary (%)	42 (77.8)	19 (73.1)
Extra-pulmonary (%)	6 (11.1)	3 (11.5)
Both pulmonary and extra-pulmonary (%)	6 (11.1)	4 (15.4)
Drug Susceptibility		
MDR (%)	52 (96.3)	26 (100)
XDR (%)	2 (3.7)	0
Comorbidity		
Diabetes Mellitus type 1 (%)	3 (5.6)	1 (3.8)
Diabetes Mellitus type 2 (%)	3 (5.6)	1 (3.8)
HIV co-infection (%)	4 (7.4)	4 (15.4)
Creatinine level at baseline	64.0 (50.8 – 77.3)	69.5 (51.3 – 77.3)

Results are presented as median with interquartile range between brackets or as number of patients (n) with the percentage between brackets (%). BMI = body mass index; MDR = Multi Drug Resistant; XDR = extensively drug resistant; HIV = human immunodeficiency virus.

Pharmacokinetic and pharmacodynamics

All patients but one started with a daily dosing regimen with a median dose of 400.0 (IQR; 400.0 – 568.2) mg with a median duration of 85 (IQR; 60 – 111) days. From these patients, 36 patients continued their aminoglycoside treatment – after the initial daily treatment – in a 5 times-weekly regimen with a median dose of 400.0 (IQR; 387.5 – 500.0) mg and a median duration of 61 (IQR; 56 - 78) days. One patient did not receive the first daily dosing schedule and was treated with the 5 times-weekly regimen from start. After this 5 times-weekly regimen, 27 patients received a 3 times-weekly regimen with a median dose of 400.0 (IQR; 350.0 – 500.0) mg with a median duration of 61 (IQR; 54 - 82) days. Four patients immediately received the three times weekly regimen after the daily regimen. Co-medication used is displayed in table 2.

TABLE 2: Anti-tuberculosis medication (total n = 80)

Fluoroquinolones	**N (%)**
Levofloxacin	21 (26.3%)
Moxifloxacin	57 (71.3%)
Second line injectable agents	
Amikacin	54 (67.5%)
Kanamycin	25 (31.3%)
Both amikacin and kanamycin	1 (1.3%)
Capreomycin	2 (2.5%)
Other core second-line agents	
Linezolid	62 (77.5%)
Protionamide	52 (65.0%)
Clofazimine	64 (80.0%)
Cycloserine	8 (10.0%)
Add-on agents (D1)	
Pyrazinamide	32 (40.0%)
Ethambutol	58 (72.5%)
Add-on agents (D3)	
Thioacetazone	7 (8.8%)
Others / not classified	
Rifabutin	11 (13.8%)
Clarithromycin	11 (13.8%)
Azithromycin	3 (3.8%)
Co-trimoxazole	7 (8.8%)
Ciprofloxacin	4 (5.0%)
Ertapenem	7 (8.8%)

Treatment details are displayed in table 3. All C_{max} levels and AUCs are displayed in figure 1a and 1b. The trough level was below 3 mg/L in all patients. The C_{max} and AUC correlated both with the dose per kg bodyweight ($r = 0.53$ and 0.25, $p < 0.05$). The C_{max} and AUC were both not significantly different between both aminoglycosides ($P = 0.86$ and 0.61). The median dose per kg bodyweight was slightly, yet significantly, higher in male (6.7 mg/kg) in comparison to female patients (6.0 mg/kg; $P = 0.025$) for both aminoglycosides.

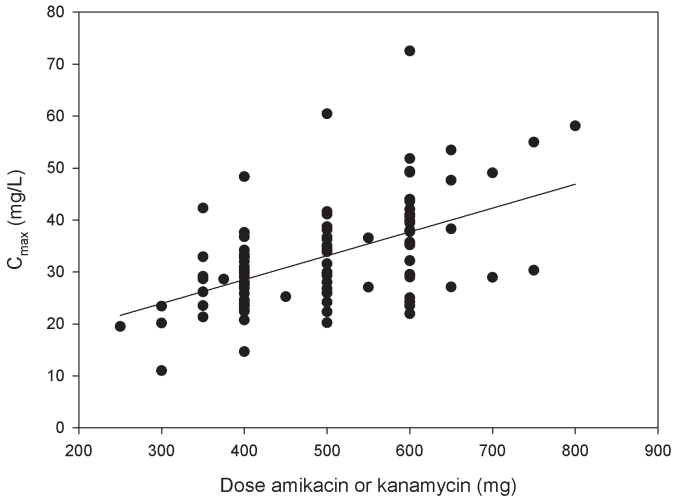

FIGURE 1A

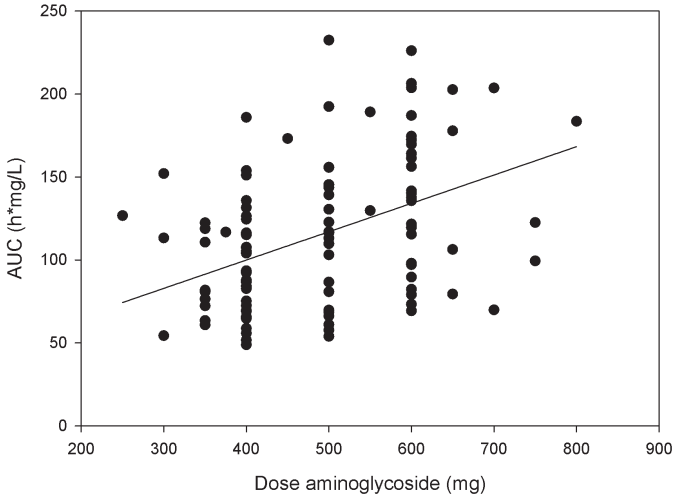

FIGURE 1B

The median treatment duration with amikacin was 166 days (IQR; 78 - 202 days) with a median cumulative dose of 791.0 (IQR; 522.0 – 1,281.6) mg/kg. With kanamycin, the median treatment duration was 124 (IQR 82 – 193) days with a median cumulative dose of 860.7 (IQR; 569.2 – 1,337.5) mg/kg. Treatment duration and cumulative dose were not significantly different between both aminoglycosides (P = 0.650 and P = 0.945) or between genders (P = 0.813 and P = 0.265).

TABLE 3: Treatment details and side effects

	n (%) / median (IQR)	
	Amikacin	**Kanamycin***
Common parameters		
Duration of hospital stay (days)	92.5 (67.3 – 162.3)	110.0 (90.5 – 186.5)
Duration of treatment with aminoglycosides (days)	138.0 (69.8 – 187.0)	104.0 (82.0 – 179.8)
Creatinine (µmol/L) after 90 days of treatment	80.0 (66.0 – 93.0)	77.0 (62.0 – 100.5)
Creatinine (µmol/L) l after 180 days of treatment	82.0 (70.0 – 95.0)	83.5 (67.8 – 101.5)
Observed side effects		
Nephrotoxicity *1	11 (22.9)	9 (34.6)
Ototoxicity *2	4 (9.1)	5 (21.7)

*1 Nephrotoxicity is defined as a serum creatinine of more than 1.5 times the baseline serum creatinine at any time during treatment

*2 Ototoxicity defined as reduced hearing at any frequency >20dB by audiometry, any time during treatment compared to baseline.

*The patient is included in the kanamycin results, as this aminoglycoside represented the largest treatment period.

The median MIC value for amikacin and kanamycin was, with and without resistant cases (MIC > 5 mg/L) 1.0 mg/L (range 1 – 20 mg/L, n = 67) and 2.5 mg/L (range 1 – 20 mg/L, n = 12), respectively. The achieved mean weighted C_{max}/MIC was 25.0 for both aminoglycosides. With amikacin, the mean weighted C_{max}/MIC was 31.2, while a mean weighted C_{max}/MIC of 12.3 was obtained using kanamycin. The mean cumulative $AUC_{0\text{-}24h}$ was 15,205 mg/L*h*days for amikacin and 15,518 mg/L*h*days for kanamycin.

Adverse events and clinical outcome

Serum creatinine levels of 20 patients (25.0%) were considered elevated as displayed in table 3. All except six patients were classified as a grade 1 toxicity, five patients as grade 2 toxicity and 1 patient as grade 3 toxicity according to the CTC (26).

The total dose (P = 0.230), duration (P = 0.301), weighted C_{max} (P = 0.824), cumulative AUC (P = 0.970), age (P = 0.404), body weight at the start of the treatment (P = 0.121) and BMI were all non-significantly related to the occurrence of nephrotoxicity. All co-administrated drugs were also non-significantly related to nephrotoxicity (P > 0.05, Fischer's exact test), except for the drug co-trimoxazole (P = 0.01, n = 7), ethambutol (P = 0.034, n = 58) and levofloxacin (P = 0.044, n = 21). Cycloserine was also correlated with the occurrence of

nephrotoxicity (P = 0.02, Fishers' exact test). Five patients on cycloserine developed some extent of nephrotoxicity. Nephrotoxicity occurred already before the start of cycloserine.

Regression analysis on the different grades of nephrotoxicity and the factors mentioned did not reveal independent predictors for toxicity; see table 4. Furthermore, no significant increase of the incidence of nephrotoxicity was observed with diabetes mellitus type 2 (Mann-Whitney U-test p = 0.404). The relation between diabetes mellitus type 1 and nephrotoxicity showed a non-significant trend (P = 0.079). In addition, we performed several probit models in order to establish possible factors associated with the occurrence and extent of nephrotoxicity. However, the cumulative AUC, weighted trough and treatment duration did not correlate with the occurrence and extent of nephrotoxicity.

TABLE 4: Spearman correlations of different factors predicting nephrotoxicity

Classification		Total dose	Total duration	Dose mg/kg	Baseline serum creatinine
CTC > 50% binary*1	P =	0.226	0.313	0.159	0.000*
CTC >50% regression*2	P =	0.200	0.321	0.220	0.001*

* Significant at 95% significance level
*1 Serum creatinine above 50% of the baseline at any moment during treatment as defined by the common toxicity criteria [26]

Audiometry results were available in 70 patients (87.5%), generally at the start of the aminoglycoside treatment and thereafter every 3 – 4 weeks. The results of the audiometry showed hearing loss in 9 patients (11.3%, table 3), predominantly at higher frequencies (4000 and 8000 Hz). The mean hearing loss was 37.5 dB (range 25.0 – 50.0) at 4000 Hz and 46.1 dB (range 25.0 – 70.0) at 8000 Hz. Cumulative dose (P = 0.421), dose per kg bodyweight (P = 0.741), duration (P = 0.644), bodyweight (P = 0.978), gender (P = 0.386), age (P = 0.155) and BMI (P = 0.432) did not correlate with the occurrence of ototoxicity.

The $AUC_{0\text{-}24h}$, weighted C_{max} and duration of therapy did not relate to the occurrence or extent of ototoxicity using Probit models. Also, the weighted C_{max} was not related to the occurrence and extent of hearing loss (P > 0.05). Furthermore, none of all co-administrated drugs correlated with ototoxicity (P > 0.132). The administration of cycloserine was also not correlated with the occurrence of ototoxicity (P = 0.66, Fishers' exact test). In total, eight patients used cycloserine, of which one patient experienced hearing loss.

Regression analysis was performed on the extent of hearing loss at 8000 Hz in decibels (dB) of all patients with hearing loss (n = 9). The dose received during the daily regimen was correlated with hearing loss in dB at 8000 Hz (P = 0.004, R = 0.851).

Data on clinical outcome were available of 52 patients. Of all patients, 35 (67.3%) had successful outcome, fifteen patients were lost to follow-up (28.8%) and two patients (3.8%) died within the follow-up period of 2 years. None of the patients had a documented treatment failure or relapse. Simple linear regression between the weighted C_{max}/MIC and time to sputum and culture conversion did not reveal any linear relationship (P = 0.44 and 0.64, respectively). In addition, we performed a CART (CHAID) tree classification analysis to establish any links between Cmax/MIC, cumulative dosage and time to sputum and culture conversion. However, this did not yield any significant results.

DISCUSSION

This study showed a low level of hearing loss in the investigated cohort, predominately in high frequencies as expected. Treatment outcome in patients receiving aminoglycosides given in a lower TDM guided dose, was good. This may be explained by the fact that C_{max} was related to the MIC in individual patients. Although of retrospective nature these findings are important as amikacin and kanamycin form the cornerstone of today's MDR-TB treatment.

A recent prospective study using classification and regression tree (CART) analysis showed that a cumulative AUC of amikacin above 87,232 mg/L*h*days significantly increases the probability of ototoxicity to 10% (12). This study in 28 patients, 10 of whom had earlier aminoglycoside exposure, found audiometry-confirmed hearing loss in 7 (25%) of the patients studied. The peak and trough concentration of amikacin did not correlate with the occurrence of ototoxicity. By using blood concentration guided dosing, our mean cumulative AUC was well below this threshold of 87,232 mg/L*h*days, which could explain the relatively low incidence of ototoxicity in our population. This should be an argument for minimizing the cumulative AUC during aminoglycoside treatment.

The occurrence of ototoxicity varies amongst different studies. According to the study of Peloquin *et al*. (28), the incidence of hearing loss after treatment with aminoglycosides was 37%. De Jager *et al*. found an incidence of 21.3% during treatment (9). This is higher than in our study, with an incidence of hearing loss in 11.3% of all patients. No difference in demographics was found between the group with and without ototoxicity. Therapeutic parameters, particularly dose, cumulative dose, duration and C_{max}, were all non-significantly correlated with ototoxicity; making ototoxicity prediction with these parameters not possible. The lack of relationship between C_{max} and the daily dose is consistent with a previous study (28). It has, however, previously been shown that the duration of treatment and the cumulative dose are associated with the occurrence of ototoxicity. However, the cumulative $AUC_{0\text{-}24h}$ in our population did not reach the threshold value of 87,232 mg/L*h*days (12), which could explain this difference.

Based on the above, regular audiometry should be common practice (29). This regular audiometry could be difficult in programmatic settings due to logistical problems or lack of equipment and trained personnel. However, it has been shown that audiological monitoring using a smartphone connected to headphones, preferable with passive noise cancelling, correlates well with professional audiometry (30, 31). This could be a viable option in developing countries. When there is evidence of ototoxicity, a possible solution could be to administer the aminoglycosides five times or even three times a week, according to WHO guidelines (32, 33). The effect of this dosing regimen on the clinical efficacy has however not been established. When reducing the dose, recommendations on the C_{max}/MIC ratio need to be taken into account to avoid loss of efficacy (34, 35).

The prevalence of nephrotoxicity in our study was comparable with an earlier report from our Center (16.8%) (9) and with the report by Peloquin *et al*. (11.6%) (28). No significant influence of different factors on either the occurrence or the extent of nephrotoxicity was found. This finding is in line with the earlier study of Peloquin *et al* (36). The results of the current cohort are in contrast with an earlier study from January 1995 to July 2000 performed in our center (9). In the earlier cohort, the total dose and duration of the aminoglycoside therapy were significantly correlated with nephrotoxicity. Applied doses in the earlier study were, however, more than a two-fold higher than the dose used in our study (750 – 1000 mg vs. 400 mg). It is, however, questionable whether the serum creatinine is the right tool to measure nephrotoxicity. A raise in serum creatinine could also be related with increased muscle mass and weight gain, which is often seen during successful TB treatment.

The use of co-trimoxazole was correlated with the occurrence of nephrotoxicity. Co-trimoxazole, a combination of trimethoprim and sulfamethoxazole, is known to increase the serum creatinine, since trimethoprim decreases the tubular secretion of creatinine (37, 38). This finding is supported by the fact that a clear time relationship between the co-trimoxazole administration and the elevation in serum creatinine was found in 5 out of 6 patients. The serum creatinine value has, however, limited predictive value during treatment with co-trimoxazole due to the specific inhibition of clearance of the creatinine molecule.

The dosage applied in our study is a two-fold lower than the 15 mg/kg recommended by the WHO, (5) yet outcome was favourable in the vast majority of patients, and in those with unfavourable outcome, aminoglycoside dosage was not a predictor of poor outcome. All but 3 patients completed their treatment and were well when discharged after a median of 150.5 days of treatment. This showed that the therapy provided was effective. This is

supported by the finding that of all patients with follow-up data, 35 (67.3%) did not have a relapse after 2 years. We therefore hypothesize that the dose of aminoglycosides can be decreased, taking into consideration that the C_{max}/MIC recommendations are met, when co-administrated with other highly active medication, such as linezolid, clofazimine and moxifloxacin, without apparent loss of efficacy.

Dosing based on the C_{max}/MIC of aminoglycosides should be used rather than dosing based on body weight in order to improve treatment outcomes, as the C_{max}/MIC is correlated with clinical outcome. This means that analytical techniques in order to analyse amikacin or kanamycin in serum with high throughput rates should be made available in all TB programmes to deliver fast and accurate results. In addition, simple drug susceptibility testing in order to establish a precise MIC value should also be available (39). Both PK and PD analysis requires trained and experienced personnel with equipment. However, it would be feasible to centralize these facilities in order to concentrate knowledge and reduce costs.

With accurate dosing based on the C_{max}/MIC, the cumulative AUC can be minimized in order to reduce ototoxicity. It should be noted that the cumulative AUC threshold value of 87,232 mg/L*h*days was established in a prospective study with only 28 patients (12) and its validity needs to be tested in larger cohorts. With our proposed limited sampling strategy, the $AUC_{0\text{-}24h}$ can be predicted with only 2 serum samples (24), which can be analysed in a centralized laboratory in order to estimate the $AUC_{0\text{-}24h}$. Treating physicians should be aware of the patients' cumulative $AUC_{0\text{-}24h}$ in order to reduce or possibly avoid hearing loss. It should be noted that the trough level of aminoglycosides should not be used to change the dose and to assess the risk of ototoxicity. In addition, there is a large variation in C_{max} and $AUC_{0\text{-}24h}$ (and thus efficacy and toxicity) as shown in figure 1a and 1b, which cannot be explained by the administered dose alone. This is an additional reason to use PK/PD guided dosing.

One limitation of this study was the rather imprecise method to determine the MIC. We analysed the MIC of amikacin <1 mg/L as 1 mg/L in our statistical analysis, however, it has been shown that many isolates have MICs below 1 mg/L for amikacin (40). Therefore, the weighted $C_{max/}$MIC could be higher for amikacin than reported, increasing its efficacy.

After more than 30 years of medical practice prescribing aminoglycosides in a dose of 15 mg/kg, we believe that a formal study is warranted between standard of care, and an individualised approach based on drug susceptibility and drug concentrations. With the dosage of 6.5 mg/kg used in this study and the old breakpoint MIC of 2 mg/L for amikacin

and 5 mg/L for kanamycin determined using the Middlebrook 7H10 agar method (41), the C_{max}/MIC ratio would be 12.5 and 5. However, the median MIC found in this study is lower than the breakpoint MIC found and sufficient C_{max}/MIC ratios were reached. In vitro testing using a hollow fiber infection model should be performed to detect the optimal C_{max}/MIC ratio as has already been done for other anti-TB drugs (42-45). Combining amikacin or kanamycin with other drugs in this setup seems rational since the treatment of MDR-TB is based on a treatment regimen with a combination of anti-TB drugs. Additional effect of single drugs in a multidrug regimen can therefore be evaluated. Based on these data a new MDR-TB dosing strategy can be designed to improve efficacy while toxicity may be reduced.

In conclusion, a lower, TDM-guided dosage of aminoglycosides resulted in an acceptable treatment outcome with relatively low percentages of hearing loss. However, this approach should be validated in a prospective randomized trial.

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