



Sequential analysis as a tool for detection of amikacin ototoxicity in the treatment of multidrug-resistant tuberculosis

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INTRODUCTION

Worldwide, tuberculosis is the leading cause of death among infectious diseases and is associated with population clusters, poor housing and food conditions, alcohol abuse, tobacco abuse, and other comorbidities, such as HIV infection and diabetes mellitus, all of which contribute to the dissemination of the disease.⁽¹⁻³⁾

The increase in the number of reported cases of multidrug-resistant tuberculosis (MDR-TB) is considered by the World Health Organization a worldwide threat to tuberculosis control. In 2014, the estimated global prevalence of MDR-TB was 3.3% for new cases and 20% for previously treated tuberculosis cases.⁽²⁾ In Brazil, the incidence rate ranges from 11.0 to 68.4/100,000 population among the states, with the lowest and highest rates being observed in the states of Goiás and Amazonas, respectively. Rio de Janeiro has an incidence rate of 60.9/100,000 population and is the state with the highest mortality rate in the country (5.0 deaths/100,000 population). In 2013 in Brazil, a national system known as the *Sistema de Informação de Tratamentos Especiais de Tuberculose* (System of Information on Special Treatment for Tuberculosis) was implemented, and, since then, it has been possible to classify and monitor cases of drug-resistant tuberculosis.

ABSTRACT

Objective: To investigate early detection of amikacin-induced ototoxicity in a population treated for multidrug-resistant tuberculosis (MDR-TB), by means of three different **tests:** pure-tone audiometry (PTA); high-frequency audiometry (HFA); and distortion-product otoacoustic emission (DPOAE) testing. **Methods:** This was a longitudinal prospective cohort study involving patients aged 18-69 years with a diagnosis of MDR-TB who had to receive amikacin for six months as part of their antituberculosis drug regimen for the first time. Hearing was assessed before treatment initiation and at two and six months after treatment initiation. Sequential statistics were used to analyze the results. **Results:** We included 61 patients, but the final population consisted of 10 patients (7 men and 3 women) because of sequential analysis. Comparison of the test results obtained at two and six months after treatment initiation with those obtained at baseline revealed that HFA at two months and PTA at six months detected hearing threshold shifts consistent with ototoxicity. However, DPOAE testing did not detect such shifts. **Conclusions:** The statistical method used in this study makes it possible to conclude that, over the six-month period, amikacin-associated hearing threshold shifts were detected by HFA and PTA, and that DPOAE testing was not efficient in detecting such shifts.

Keywords: Tuberculosis; Hearing loss; Aminoglycosides/toxicity.

The complexity of clinical management of MDR-TB is explained by the high treatment default rates.⁽³⁾

The disappearance of symptoms at the beginning of treatment contributes to default and to the emergence of strains that are resistant to various drugs.^(4,5) With the increase in the number of cases of MDR-TB, it becomes necessary to adopt second-line treatment regimens, with the use of aminoglycosides.⁽⁵⁾

Aminoglycosides are cost-effective and are widely used in patients with MDR-TB treated in low- and medium-income countries.^(6,7) These drugs have ototoxicity as an important adverse effect,⁽⁸⁾ and their toxicity predominantly affects one portion of the inner ear: the hair cells in the cochlea and labyrinth.^(9,10) Data on the incidence of this event in humans remain controversial.^(10,11) Incidence rates range from 7% to 90%,^(8,11-22) and, according to Brumett et al.,⁽⁹⁾ the discrepancy between the clinical evidence and laboratory findings of ototoxicity is due to two primary issues. The first is the fact that aminoglycosides initially affect higher frequencies (above 8 kHz), outside the range of human speech perception. The second issue is related to the different study models and different criteria established for ototoxicity.

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Adequate monitoring of patients on aminoglycosides is essential in order to detect hearing impairment affecting the human speech frequency range and thereby prevent psychosocial changes associated with difficulty in communication. The choice of a test for an appropriate hearing assessment is essential,^(10,12,13,15,17,18,20) as is the choice of a data analysis method.

The objective of the present study was to investigate early detection of amikacin ototoxicity in patients treated for MDR-TB, by means of three different tests: pure-tone audiometry (PTA); high-frequency audiometry (HFA); and distortion-product otoacoustic emission (DPOAE) testing.

METHODS

This was a longitudinal prospective cohort study. We included patients aged 18-69 years with a diagnosis of pulmonary MDR-TB who were treated at the Professor Hélio Fraga Referral Center, located in the city of Rio de Janeiro, Brazil, and had to receive amikacin as part of their antituberculosis drug regimen for the first time.

We excluded patients with a history of exposure to high sound pressure levels during the study, those who were receiving other ototoxic drugs, and those who, at any time during the study, had results consistent with impairment of the outer or middle ear. Impairment was assessed by otoscopic examination of the external auditory meatus and immittance testing. The data obtained from these tests were not included in the analysis of the present study. We included patients who had a normal, type A tympanogram curve exclusively and who participated in an initial interview after giving written informed consent. Data collection was carried out between January 2015 and January 2016.

Hearing assessment consisted of the following tests: DPOAE testing; PTA; and HFA. The tests were performed before initiation of antituberculosis treatment (M_0); at two months after treatment initiation (M_2)—time at which the weekly dose of amikacin is reduced; and at six months after treatment initiation (M_6)—time of completion of amikacin therapy. The baseline assessment served as a reference for the others.

Hearing tests

All tests were performed in a calibrated sound-treated booth in accordance with the Brazilian Federal Council for Speech Therapy (ISO 8253-1 standard).

PTA and HFA

PTA and HFA were performed as described by Katz.⁽¹⁰⁾ In PTA, air conduction was measured at 0.25 kHz, 0.5 kHz, 1 kHz, 2 kHz, 3 kHz, 4 kHz, 6 kHz, and 8 kHz. Bone conduction was measured at 0.5 kHz to 4 kHz. Speech recognition thresholds were determined in all tests in order to confirm the hearing thresholds at

speech frequencies. In HFA, responses were measured at 9 kHz, 10 kHz, 11.2 kHz, 12.5 kHz, 14 kHz, and 16 kHz. Ototoxicity was defined (on the basis of air-conduction thresholds) as a 20-dB increase in threshold at any single frequency or a 10-dB increase in threshold at two or more adjacent frequencies for PTA results, as well as a 10-dB increase in threshold at one or more frequencies between 9 kHz and 14 kHz.^(23,24)

The equipment used was a Madsen Itera II A audiometer (GN Otometrics A/S, Taastrup, Denmark) with TDH-39 headphones (Telephonics Corporation, Farmingdale, NY, USA). The results were expressed as dB hearing level.

DPOAE testing

In DPOAE testing, simultaneous stimulation with two pure tones (f_1/f_2) was presented. These frequencies were expressed at a ratio of 1.22 ($f_1/f_2 = 1.22$). DPOAE responses were recorded at $2f_1/f_2$. The intensity ratios used were 65 dB/55 dB sound pressure level (SPL).^(25,26) The frequencies tested were 1 kHz, 1.5 kHz, 2 kHz, 3 kHz, 4 kHz, and 6 kHz.^(5,11) DPOAE responses with values greater than or equal to 6 dB above the noise level at each frequency were considered present. The maximum noise level permitted for analysis of responses was 6 dB SPL.^(25,26) Testing was performed in the acoustic booth in order to attain the maximum reduction in recorded noise levels.

The criterion used for assessing cochlear damage by testing DPOAEs was the same as that described by Reavis et al.,⁽²⁴⁾ according to which DPOAE amplitude reductions of 4 dB or more at two or more adjacent frequencies, on the basis of results obtained at M_0 , are considered an ototoxic drug effect.

The equipment used was an adult probe (ILO 292 USB II module; Otodynamics Ltd., Hatfield, UK) connected to a laptop (Hewlett-Packard Brasil, Barueri, Brazil).

Statistical analysis

Results were assessed by sequential analysis. This method meets the methodological rigor that ensures reproducibility, validity, and reliability, providing time and consumable savings. This is due to the fact that the sample size required for decision making is a random variable, in contrast with statistical tests commonly used in health care. Decisions are made immediately after each piece of information is obtained over the course of the study, that is, the H_0 hypothesis is rejected or accepted or the experiment continues with a larger number of parameters. The experiment ends with the H_0 hypothesis being accepted or rejected, thus reducing the number of observations required. In order to determine decision regions, we proposed the following hypothesis: H_0 , there is no hearing loss; and H_1 , there is hearing loss. With α errors set to 5% and β errors set to 10% and assuming $p_0 = 1\%$ ⁽²⁷⁾ and p_1 (the probability of people exposed to amikacin developing hearing loss),⁽²⁸⁾ and considering the reference range established for each test, we have

the following: for PTA, $p_1 = 18\%$ ⁽¹²⁾; b) for HFA, $p_1 = 67\%$ ⁽¹³⁾; and c) for DPOAE testing, $p_1 = 78\%$ ⁽²⁴⁾. For each calculation, a plot was constructed with H_0 rejection and acceptance lines, with the y axis representing "s" and the x axis representing "n - s". On the basis of these parameters, decision lines were calculated using the following formulas:

$$R = \frac{\log \frac{1 - \beta}{\alpha}}{\log \frac{p_1}{p_0}} + (n - s) \times \frac{\log \frac{1 - p_0}{1 - p_1}}{\log \frac{p_1}{p_0}}$$

$$A = \frac{\log \frac{\beta}{1 - \alpha}}{\log \frac{p_1}{p_0}} + (n - s) \times \frac{\log \frac{1 - p_0}{1 - p_1}}{\log \frac{p_1}{p_0}}$$

where R represents the H_0 rejection line and A represents the H_0 acceptance line.^(29,30) The ears were assessed separately to check for damage to each cochlea.

The reference values used in each test were obtained from studies in which aminoglycoside-induced hearing impairment was assessed with the same test and technique, as well as with the same criteria for defining hearing loss.^(24,27)

The study was approved by the Research Ethics Committee of the Clementino Fraga Filho University Hospital, Federal University of Rio de Janeiro (Protocol no. 75676/12).

RESULTS

Sixty-one patients were included on the basis of the inclusion criteria. Our final analysis population comprised 10 patients because sample size was determined using sequential analysis. This population consisted of 7 men (70%), with a mean age of 45.4 years, and 3 women (30%), with a mean age of 49.0 years. All had MDR-TB and used amikacin for six months as part of their drug regimen.

Regarding otological history, there were self-reports only of previous sensorineural hearing loss (in 1, 10%) and dizziness (in 1, 10%; Table 1).

There were no concomitant diseases (HIV, diabetes mellitus, or systemic arterial hypertension). Among habits and dependences, we found that 8 patients (80%) reported alcohol dependence, 2 (20%) reported frequent use of illicit drugs, and 7 (70%) reported smoking dependence, 2 of whom described themselves as former smokers (Table 1).

When comparing the test response values obtained at M_2 and M_6 with those obtained at M_0 , we found that, on the basis of PTA, none of the patients had hearing threshold shifts consistent with ototoxicity

Table 1. Characteristics of the study population.

	Male		Female	
	n	%	n	%
History of ototoxic drugs				
Yes	0	0.0	0	0.0
No	7	100.0	3	100.0
History of tinnitus				
Yes	0	0.0	0	0.0
No	7	100.0	3	100.0
History of hypoacusis				
Yes	1	14.3	0	0.0
No	6	85.7	3	100.0
History of dizziness				
Yes	0	0.0	1	33.3
No	7	100.0	2	66.7
History of exposure to noise				
Yes	2	28.6	2	66.7
No	5	71.4	1	33.3
Otologic surgery				
Yes	0	0.0	0	0.0
No	7	100.0	3	100.0
HIV positive				
Yes	0	0.0	0	0.0
No	7	100.0	3	100.0
Diabetes mellitus				
Yes	0	0.0	0	0.0
No	7	100.0	3	100.0
Arterial hypertension				
Yes	0	0.0	0	0.0
No	7	100.0	3	100.0
Smoking				
Yes	5	71.4	2	66.7
No	2	28.6	1	33.3
Alcoholism				
Yes	7	100.0	1	33.3
No	0	0.0	2	66.7
Illicit drug use				
Yes	2	28.6	0	0.0
No	5	71.4	3	100.0
Level of education				
Illiterate	1	14.3	1	33.3
Elementary school	3	42.9	2	66.7
High school	2	28.6	0	0.0
College	1	14.3	0	0.0

criteria at M_2 . However, at M_6 , we found threshold shifts consistent with ototoxicity in 20% and 30% of the sample, respectively, in the right and left ears. On the basis of HFA, we found hearing threshold shifts in 50% and 60% of the patients, respectively, in the right and left ears at M_2 , whereas, at M_6 , these were found in 70% of the patients in both ears. On the basis of DPOAEs, we found impairment only in the right ear in 20% of the patients at M_2 ; however, no impairment was observed in the patients at M_6 .

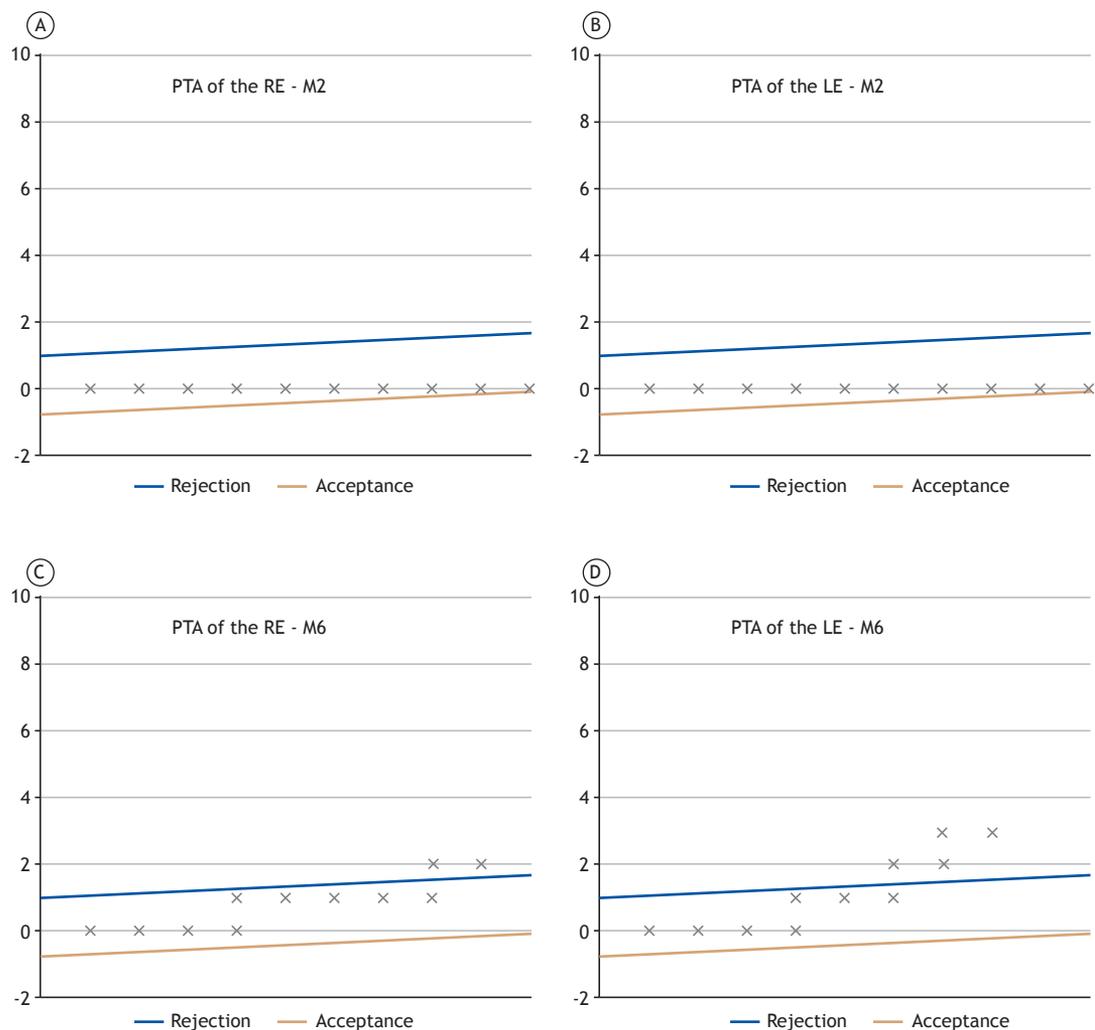


Figure 1. Sequential analysis of pure-tone audiometry (PTA) results for the right ear (RE) and the left ear (LE) at two months of treatment (M₂; in A and B) and at six months of treatment (M₆; in C and D).

The results were analyzed by comparing the results obtained at M₀ (baseline) with those obtained at M₂ (time at which the dose of amikacin is reduced) and those obtained at M₆ (time of completion of amikacin therapy). When considering the criteria for determining ototoxicity on the basis of PTA, we found, over the period of monitoring of auditory function, an association between amikacin use and hearing threshold shifts consistent with ototoxicity at M₆ (Figure 1). On the basis of HFA, we found an association between hearing threshold shifts and amikacin use already at M₂ (Figure 2). On the basis of DPOAE testing, H₀ was accepted already at M₂, that is, amikacin use was not associated with hearing impairment. Over the period of monitoring of auditory function, we found an increase in DPOAEs from M₂ onward (Figure 3).

DISCUSSION

Aminoglycoside-induced hearing impairment can consist of permanent hearing loss or tinnitus

secondary to degeneration of cochlear sensory hair cells.^(5,9) Dizziness or imbalance can occur as a result of damage to the sensory structures of the vestibular system. Damage to cochlear hair cells occurs as a result of oxidative stress, which begins in the basal portion of the cochlea.⁽²⁸⁾

Aminoglycosides are included in MDR-TB treatment regimens and are initially used for at least six months.⁽³¹⁾ In the present study, all patients were treated with the ototoxic drug amikacin. This drug is known to be cochleotoxic.^(8,21) The incidence of hearing loss varies greatly and may depend on genetic factors, individual susceptibility, the type of assessment used, and the criteria established to define hearing loss.⁽¹⁰⁾ Early detection of ototoxicity enables changes to be made to the drug regimen in order to stabilize damage to the structures of the ear and prevent further damage to them, thus reducing the chance of impaired psychosocial relationships due to impaired communication.⁽¹⁰⁾

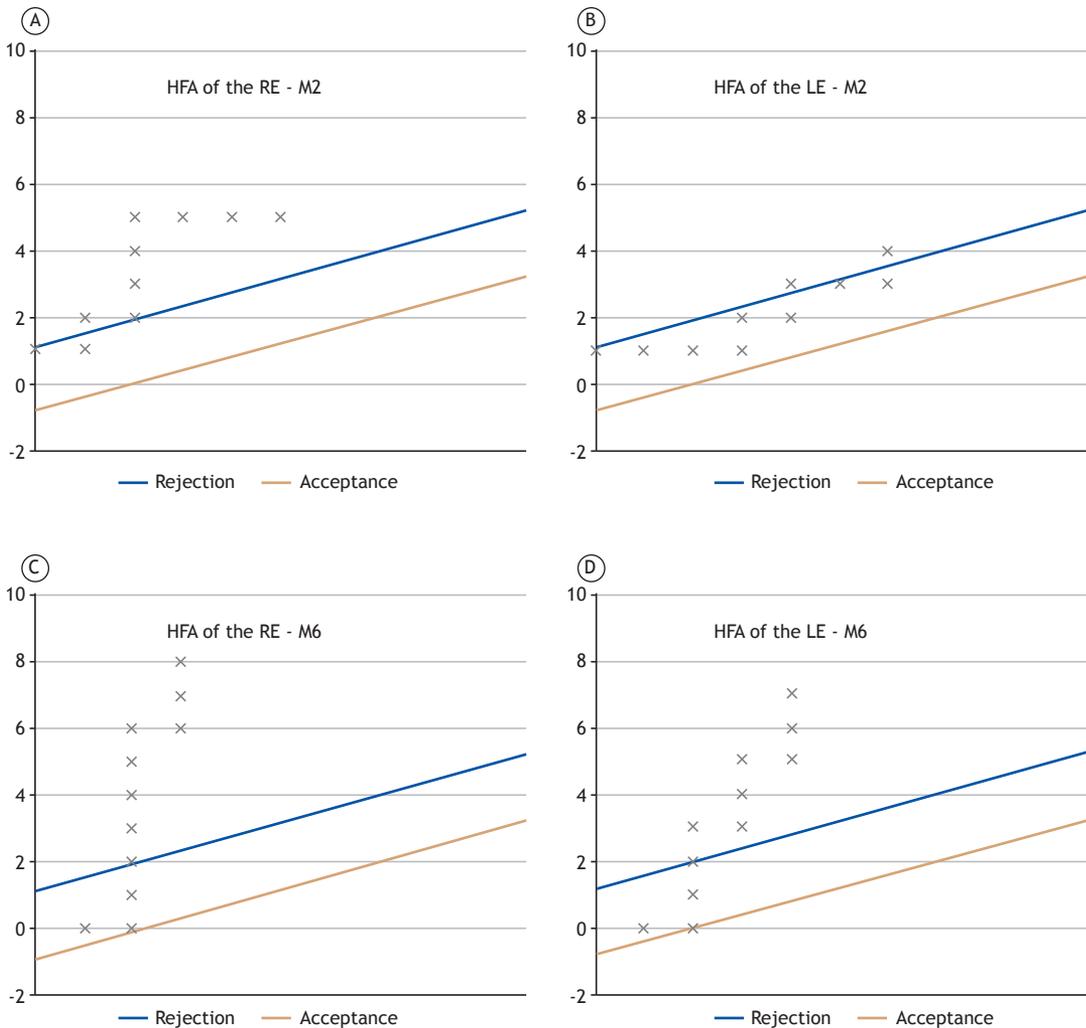


Figure 2. Sequential analysis of high-frequency audiometry (HFA) results for the right ear (RE) and the left ear (LE) at two months of treatment (M2; in A and B) and at six months of treatment (M6; in C and D).

PTA is the hearing test that is most widely used in clinical practice because it analyzes the frequency range responsible for discrimination of sounds familiar to human beings, including speech.⁽¹¹⁾ Ototoxicity was found in 20% of our sample, which is in agreement with findings in the literature.⁽¹³⁾ PTA proved to be an appropriate test for monitoring and detecting amikacin ototoxic effect over the six-month period. H_0 was rejected at M_6 for both ears, confirming the association between amikacin use and hearing loss. However, early detection should be routine in order to prevent damage to this region.^(5,13-15)

When assessing the HFA results, we found an association between amikacin use and hearing threshold shifts already at M_2 , and this association persisted and was more accurately observed at M_6 . The proportions found in the present study are, once again, in agreement with data reported in the literature^(5,13-15) and can be explained by the frequency range assessed.⁽¹⁴⁾ HFA is increasingly being included in further hearing assessment,^(5,9-11,13,22,24) but it is far from being considered a routine test, even in cases of auditory function monitoring,⁽¹⁰⁾ because

the equipment used in HFA is costly and the usefulness of HFA is limited by the lack of reference values. The variability of responses, even in individuals without a history of otologic complaints or otologic disease, makes it difficult to establish reference values for this test.⁽¹⁰⁾ Thus, in cases of auditory function monitoring by HFA, the test responses should always be compared with the responses from an assessment performed before the exposure that may carry a risk of auditory function impairment.^(5,10)

DPOAE testing is described as being able to detect ototoxicity as early as possible because it assesses outer hair cells.^(13,14,22,23,26) It is considered a rapid, painless, objective, and reliable test. In the present study, DPOAE testing detected hearing impairment in 20% of the population only in the right ear at M_2 , that is, 2 patients showed DPOAE amplitude reductions, and this finding did not persist at M_6 . DPOAE testing is recommended by the American Speech-Language-Hearing Association⁽²³⁾ for auditory function monitoring. However, given that DPOAE testing results vary greatly, even in normal-hearing listeners, it is suggested that,

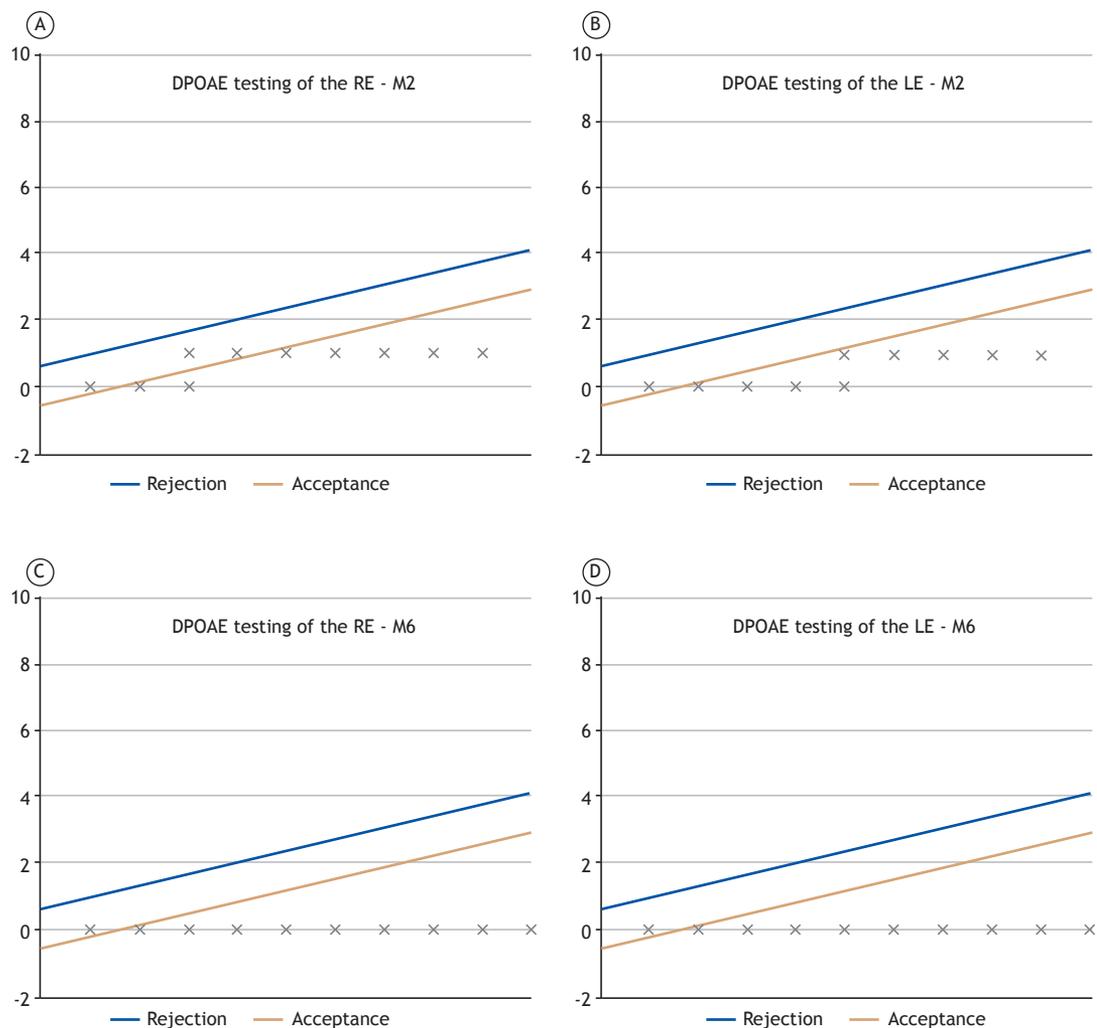


Figure 3. Sequential analysis of distortion-product otoacoustic emission (DPOAE) testing results for the right ear (RE) and the left ear (LE) at two months of treatment (M2; in A and B) and at six months of treatment (M6; in C and D).

when using DPOAE testing, test-retest comparison of responses should always be considered. The American Speech-Language-Hearing Association⁽²³⁾ has associated response variability with the different equipment used, the different parameters defining the presence or absence of OAE, the way probes are placed, or the statistical methods used in the different studies. Since no record has been found in the literature that can explain the increase in and persistence of DPOAEs during aminoglycoside therapy, it is possible that the variation found in the present study occurred because the probe was not properly placed during the test. In addition, the persistence of the amplitudes and even the slight increases recorded may have occurred because of the overall health status of patients with MDR-TB. In this case, the reference values used in the present study (i.e., the values obtained at M_0) could have been influenced by the overall health status of patients who were starting treatment and showed responses that would not correspond to their true hearing status; that is,

the responses that served as reference were, at that point, inadequate. This would be an uncontrollable bias. In DPOAE testing, sounds are generated in the cochlea by healthy hair cells. Physiological changes may interfere with the responses,⁽¹¹⁾ and it is known that, in general, the health status of patients with MDR-TB is precarious before treatment. Another factor that should be considered when monitoring auditory function by DPOAE testing is the frequency range assessed by the test. DPOAE testing does not assess the frequency range in which aminoglycoside-induced hearing impairment begins. In animal model studies, an improvement in DPOAE responses was observed over a period of time after the use of ototoxic agents. This improvement in responses was followed by a recorded decrease in responses. The authors explain that areas adjacent to those that were damaged by the drug may at first respond in an attempt to compensate for the damage to a specific area of the cochlea.^(11,28,32)

The establishment of causality is one of the central components of studies in health care. Determining how causality functions in a representative way in a given population is a challenge for researchers. Establishing the level of statistical significance of a given event has been presented as evidence of a causal relationship; likewise, the absence of a causal relationship leads to rejection of the hypotheses tested.⁽²⁹⁾ Sequential analysis allowed us to find a causal relationship between amikacin use and hearing threshold shifts in the high-frequency range, demonstrating that it is possible to use this method also in health care.

The limitations of the present study include the facts that cognitive function was not systematically assessed in the patients who attended the interviews,

that the strategy of directly observed treatment was not used, and that patient serum levels of amikacin were not determined over the study period.

The statistical method used in this study makes it possible to conclude that, over the six-month period, amikacin-associated hearing threshold shifts were detected by HFA and PTA, and that DPOAE testing was not efficient in detecting such shifts.

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