THE USE OF OTOACOUSTIC EMISSIONS TO PREDICT OTOTOXIC-INDUCED HEARING CHANGES

By

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ABSTRACT

Millions of patients in the US risk experiencing loss in hearing sensitivity from ototoxic medications annually. The impact on older individuals with preexisting hearing impairment may be even greater since additional loss can immediately affect their communication ability. The current gold-standard for monitoring ototoxicity is through repeated measurements of high-frequency pure-tone thresholds. However, patients differ in their ability to respond to behavioral testing, they can be categorized as fully responsive, partially responsive, or fully unresponsive. Different monitoring strategies are required to detect incipient ototoxicity in each of these groups. Distortion-product otoacoustic emissions (DPOAE) have been proposed as one such strategy for monitoring patients who are partially responsive or fully unresponsive. The objective of this study is to determine how well DPOAEs identify audiometric pure-tone threshold shifts resulting from ototoxic medication administration. DPOAEs depend on the physiological status of the outer hair cells which are typically affected first by most ototoxic medications and therefore should be a sensitive and specific measure of hearing change. However, there are no accepted protocols for ototoxicity monitoring using DPOAE, particularly for individuals with preexisting hearing loss. Most investigations into DPOAE applications for detecting ototoxicity have used either statistical tests of difference, or test-retest variability estimates derived from control populations. Though useful, these investigations do not provide overall sensitivity and specificity of DPOAE measures. A prospective, observational study design was undertaken to determine the test accuracy of DPOAEs compared to repeated measures of high-frequency pure-tone thresholds. DPOAE test performance (sensitivity and specificity) was evaluated through the

construction of receiver operating characteristic curves for different metrics derived from DPOAE input/output functions. DPOAE metrics including the sum of input stimulus levels, sum of output emission amplitudes and sum of signal-to-noise ratios were modeled alone and in combination to discriminate patients with and without hearing loss. The result was a discriminate function which included measures of pre-exposure hearing, drug dose and DPOAEs that accurately predicted ASHA significant hearing change in patients exposed to cisplatin. Once the test characteristics of DPOAE are determined, objective ototoxicity monitoring strategies can be developed. DPOAE are quick and unaffected by patient responsiveness. This model of ototoxicity has the potential to increase the number of patients monitored and thus limit the incidence of ototoxicity and its associated side-effects.

INTRODUCTION

Ototoxicity is defined as damage to the cochlea and/or vestibular end organs of the inner ear by medications or chemicals resulting in functional impairment and cellular degeneration. Symptoms include hearing loss, tinnitus, and vertigo. Certain medications preferentially damage either the cochlea and/or the vestibular system; therefore, hearing loss, tinnitus and vestibular dysfunction do not always co-occur. Additionally, damage can present unilaterally or bilaterally of varying degrees or as an aggravation of an existing underlying problem. Hearing impairment can result in significant communicative, emotional and social problems and tinnitus can be psychologically distressing (Shulman, 1999). Vestibular dysfunction can result in gait imbalance, vertigo, oscillopsia, nausea and vomiting. There are at least 130 known ototoxic medications. The medications falling under the classification of platinum-based chemotherapy and aminoglycoside antibiotics are known to have the highest ototoxic potential (Seligmann, Podoshin, Ben-David, Fradis, & Goldsher, 1996). For the duration of this document, the term “ototoxicity” will imply damage to the cochlear end organ.

The anticancer drugs containing platinum are the basis for chemotherapy for a wide range of tumor types including ovarian, testicular, colorectal, head and neck, and lung cancer. The first generation, cisplatin, is widely used in both children and adults and is unrivaled in effectiveness against many cancers; however it is also considered to be the most ototoxic compound in clinical use (Anniko & Sobin, 1986; Hartmann & Lipp, 2003). Cisplatin causes ototoxicity in a large percentage of patients treated. Schweitzer (1993) calculated that the incidence of cisplatin-induced hearing loss averaged across a large number of studies was 62% (the range was 11 to 97%). The second generation

platinum agent is carboplatin and is found to be much less toxic in comparison to cisplatin and has fewer ear-related side effects. The incidence of carboplatin-induced hearing loss is comparatively lower than cisplatin, ranging from 19% (Kennedy, Fitzharris, Colls, & Atkinson, 1990) to 82% (Parsons et al., 1998). In addition to being used in the treatment of cancer, high-dose carboplatin therapy is also used with stem cell/bone marrow transplants. Oxaliplatin is the third generation platinum-based agent and is frequently used in treating colon cancer. It has been suggested that oxaliplatin does not cause any toxicity in the ear (Cavaletti et al., 2001); however this drug has yet to be fully evaluated for ototoxicity.

Results from experimental animal models of ototoxicity and human temporal bone studies suggest that ototoxic agents primarily damage the outer hair cells within the organ of Corti and the stria vascularis, which provides the electrical drive to the outer hair cells. Initially, the first row of outer hair cells is affected, followed by the second and third rows of outer hair cells, inner hair cells and finally supporting cells (Estrem, Babin, Ryu, & Moore, 1981; Marco-Algarra, Basterra, & Marco, 1985; Nakai et al., 1982; Tsukasaki, Whitworth, & Rybak, 2000). Damage typically begins near the high-frequency coding cochlear base and progresses apically, sequentially affecting the mid to low frequencies. (Brummett, 1980; Komune, Asakuma, & Snow, 1981; Konishi, Gupta, & Prazma, 1983; Nakai et al., 1982; Schweitzer et al., 1984). Direct damage to spiral ganglion cells also occurs as a result of cisplatin and carboplatin toxicity (*cisplatin:* Hoistad et al., 1998; van Ruijven, de Groot, Klis, & Smoorenburg, 2005; *carboplatin*: Husain, Scott, Whitworth, Somani, & Rybak, 2001).

Predicting which patients will experience ototoxic hearing loss is a clinical

challenge. The risk for developing hearing loss from ototoxic drugs is generally related to the dose, duration, frequency, and method of medication administration (Rademaker-Lakhai et al., 2006, Vermorken, Kapteijn, Hart, & Pinedo, 1983). For cisplatin, it has been suggested the critical cumulative dose associated with ototoxicity is 3-4 mg/kg body weight (Moroso & Blair, 1983) while others have suggested a cumulative dose of 400 mg is related to an increased risk of ototoxicity (Bokemeyer et al., 1998; de Jongh et al., 2003; Li, Womer, & Silber, 2004; Park, 1996; Waters, Ahmad, Katsarkas, Stanimir, & McKay, 1991). Similarly, high blood concentrations of carboplatin are associated with increased risk of ototoxicity compared to low blood concentrations (Obermair et al., 1998). However, concomitant toxins such as noise, chemicals, and other ototoxic medications can produce a synergistic effect leading to increased rates of ototoxicity and at lower administration levels. Furthermore, in patients who are treated with cisplatin followed by carboplatin (not concurrent regimens but rather as a second line of treatment), the association between increasing carboplatin administration dose and hearing loss is strengthened (Dubs, Jacky, Stahel, Taverna, & Honegger, 2004). Patient factors such as age, biochemical, physiologic, and genetic factors will further impact incidence rates (Fischel-Ghodsian, Prezant, Bu, & Oztas, 1993; Forge & Schacht, 2000). Once the dose sufficient to cause hearing impairment is reached, both older adults (>45 years old) and younger children (<6 years old) demonstrate increased susceptibility. Thus, the risk for developing and monitoring ototoxic induced hearing changes can be variable, difficult to predict, and presents as a clinical challenge (Blakley, Gupta, Myers, & Schwan, 1994; de Jongh et al., 2003; Moore, Smith & Lietman, 1984; Waters et al., 1991).

The only way to identify a patient experiencing ototoxicity related hearing change is by actively and directly monitoring their auditory status. The impact of ototoxicity on adults with pre-exposure hearing loss may be great since additional impairment can immediately affect their communication ability. Early signs of ototoxicity are often missed if hearing is not directly monitored. Patients tend not to complain of hearing difficulties until a communication problem becomes significant, an indication that the hearing changes have progressed from the cochlear base toward the apex and into the frequency range important for speech understanding (mid-to-low frequencies). Thus, waiting for a patient to complain increases the likelihood of having greater amounts of ototoxic hearing change and decreases the likelihood of those hearing changes recovering back to pre-exposure hearing levels. The purpose of ototoxicity monitoring is to prevent, or limit, hearing loss so as to preserve quality of life following treatment. Early detection of ototoxicity provides physicians with the necessary information to prevent progression of hearing loss into frequencies critical for speech communication and the audiologist an opportunity to provide aural rehabilitation when indicated.

The foundation of ototoxicity monitoring is the serial application and collection of pure-tone behavioral thresholds. The goal of serial monitoring for detection of ototoxic hearing loss is typically to categorize patients into two groups: those exhibiting hearing change and those who do not, based on hearing change criteria. For serial audiograms, American Speech-Hearing Association (ASHA, 1994) developed criteria for a clinically significant hearing change based primarily on results of large clinical research studies. The ASHA standards advocate for extended high-frequency testing when possible. Extended high-frequency pure-tone testing maintains a high degree of sensitivity with

minimal false positives rates and is necessary for the detection of incipient ototoxicity. Behavioral audiometry, including extended high-frequency testing when possible, has been labeled the gold standard for the early detection of ototoxic hearing loss (ASHA, 1994; Fausti et al., 1999; 1993; 1992; 1994).

Unfortunately many patients receiving ototoxic drugs are too ill to provide reliable behavioral responses and thus receive no ototoxicity monitoring. Objective measures that do not require physical cooperation or mental concentration are needed to monitor and detect ototoxicity in these patients. Otoacoustic emission (OAE) testing has been proposed as a non-behavioral, or objective, measure of cochlear function because OAE generation depends upon the physiological status of the outer hair cells, which is the initial site of lesion (Hodges & Lonsbury-Martin, 1999). Changes in the outer hair cell mechanism alter OAE responses (reviewed in Campbell & Durrant, 1993 and Whitehead, Lonsbury-Martin, Martin, & McCoy, 1996).

Distortion-product (DP) OAE testing provides a non-invasive, objective measure of cochlear function. A DPOAE is an acoustic response generated by the outer hair cells within the cochlea and reverse-transmitted through the middle ear into the ear canal (Kemp, 1979). The response is initiated in the overlapping region of the basilar membrane's response to two stimulating tones, f_1 and f_2 (where $f_1 < f_2$), somewhat nearer to the f_2 tonotopic place. A second component arises near the basilar membrane place that codes the distortion-product frequency ($2f_1 - f_2$) (Kim, 1980; Shera & Guinan, 1999). Clinical DPOAEs are comprised of these two sources combined within the ear canal. The presence of otoacoustic emissions are generally associated with normal hearing and are reduced in individuals with mild to moderate hearing losses up to approximately 50 - 60

dB SPL. Thresholds greater than 60 dB SPL are rarely associated with otoacoustic emissions (Gorga et al., 1997; Gorga, Stover, Neely, & Montoya, 1996). This observed association has lead several authors to investigate the ability of DPOAEs to estimate audiometric pure-tone thresholds, attempt predictions of auditory status (normal versus impaired), and detect changes in auditory status over time.

Frequently studied populations for the assessment of DPOAEs to detect in ototoxic-induced hearing changes are children and young adults with cystic fibrosis who receive the aminoglycoside antibiotics gentamicin and/or tobramycin on a routine basis. In cross-sectional studies investigating aminoglycoside exposure and DPOAEs, several authors have noted DPOAE input/output growth function differences between those exposed to aminoglycoside antibiotics and healthy matched controls. These differences were noted in the absence of pure-tone audiometric differences (Mulheran & Degg, 1997) including extended high-frequency audiometric differences (Katbamna, Homnick, & Marks, 1999). Stavroulaki et al. (2002) prospectively investigated DPOAE and pure-tone thresholds in children with cystic fibrosis receiving gentamicin, children with cystic fibrosis receiving non-ototoxic drugs and in healthy children of a similar age. The authors noted a statistically significant decrease in DPOAE amplitudes from pre-treatment to post-treatment in the children receiving gentamicin when compared to their age-matched controls. DPOAE changes were noted in the absence of pure-tone threshold changes, which strongly support previous cross-sectional study findings. Stavroulaki and colleagues concluded that DPOAEs were more sensitive to the early effects of aminoglycoside induced ototoxicity when compared to audiometry.

Additionally, in DPOAE investigations related to ~platinum exposures in young children and older adults (cisplatin or carboplatin), authors have noted input/output growth function and amplitudes changes in addition to audiometric changes at corresponding frequencies and at disparate frequencies located apically to the hearing change (Knight, Kraemer, Winter, & Neuwelt, 2007; Ress et al., 1999; Stavroulaki, Apostolopoulos, Segas, Tsakanikos, & Adamopoulos, 2001). Ress and colleagues (1999) performed a prospective study comparing the relative sensitivity of DPOAEs (.8 - 8 kHz), conventional audiometry (.25 - 8 kHz), and extended high-frequency audiometry (>8 kHz) to ototoxic damage in adults receiving cisplatin chemotherapy. Mean age was 62 years (range 42 - 80 years) and some subjects presented with pre-exposure hearing loss. Compared to baseline measures, post-treatment thresholds changed in the frequency range from 2 - 14 kHz, while DPOAE amplitudes were reduced for frequencies >2 kHz and absent at all frequencies >5 kHz. The proportion of ears showing ototoxic change was similar for DPOAE (75%) and behavioral threshold testing in the extended high-frequency range (74%), and both methods were better than conventional audiometry (65%).

Comparably, Knight et al. (2007) found DPOAEs and extended high-frequency testing to be more sensitive to ototoxicity than conventional frequency testing in a cohort of 32 infants, children and young adults receiving cisplatin and/or carboplatin. Additionally, Knight and colleagues noted a temporal effect with extended high-frequency thresholds changing prior to DPOAE responses, suggesting the ototoxic insult is detected first among behavioral pure-tone thresholds >8kHz, then in DPOAE $\leq$8kHz, and finally, after repeated doses, is detected by behavioral pure-tone thresholds $\leq$8kHz.

However, the temporal aspects of DPOAE changes have not been consistently observed. Reavis et al. (2008) reported a roughly equivalent proportion of ears experienced initial DPOAE changes before, during and after documented behavioral hearing changes. The study, in Veterans with some pre-exposure hearing loss, was conducted to determine factors associated with DPOAE sensitivity. The authors concluded that ears successfully monitored for ototoxicity with DPOAEs are those with better pre-exposure hearing, greater post-exposure hearing changes, and baseline DPOAEs near the behavioral region of change. However, these variables could not predict the relative timing of behavioral and DPOAE changes.

Results of these numerous studies in human subjects suggest that DPOAEs are sensitive to pre-clinical and clinical hearing changes (Katbamna et al., 1999; Mulheran & Degg, 1997; Stavroulaki et al., 2001; 2002) and sensitivity may be affected by baseline subject factors (Reavis et al., 2008). However, it remains unclear if extended high-frequency monitoring and DPOAE measurements are equally as sensitive to ototoxic-induced cochlear damage (Ress et al., 1999) or perhaps if extended-high-frequency monitoring is more sensitive than DPOAE (Knight et al., 2007).

It is likely that pre-exposure hearing affects DPOAE sensitivity in an adult population in which normal pre-exposure hearing cannot be assumed. In particular, successful monitoring of ototoxicity with DPOAEs might depend on the ability to record DPOAEs somewhat near to the behavioral change. Few studies have examined these potential relationships (Reavis et al., 2008). Additionally, previous studies have used statistical methods to determine significant DPOAE changes as well as clinical methods, which make statements regarding overall DPOAE test performance (sensitivity and

specificity) challenging. Consequently, the clinical significance of DPOAE changes (or of the lack of DPOAE changes) observed following ototoxic drug exposure is unknown.

An approach to evaluate DPOAE test performance (sensitivity and specificity) is the construction of receiver operating characteristic (ROC) curves. A ROC curve is a plot of the sensitivity as a function of 1-specificity and has been utilized in assessing DPOAE utility to discriminate normal hearing from impaired hearing based on audiometric results (Gorga et al., 1993a; 1993b; 1997; Gorga, Stover, Neely, & Montoya, 1996; Kim, Paparello, Jung, Smurzynski, & Sun, 1996; Stover, Gorga, Neely, & Montoya, 1996). Tests with continuous results, such as DPOAEs, typically have two features 1) a measurable test variable (i.e. DPOAE measurement that relates to the status of the outer hair cell) and 2) a criterion which can be considered a cut point that discriminates two groups of patients (i.e. normal hearing versus hearing impaired). DPOAE measurements will show a substantial spread in values for individuals with and without hearing impairment and these values will likely overlap, making absolute discriminations between two groups improbable. While no single DPOAE measurement (amplitude and signal-to-noise ratio) has been capable of completely separating those with normal hearing from impaired hearings, multivariate DPOAE models have shown increasing test accuracy (Dorn, Piskorski, Gorga, Neely, & Keefe, 1999).

Distinguishing patients with and without ototoxic hearing change is useful for the purpose of subsequent decision making because most medical actions are dichotomous. There are advantages to applying ROC curve analysis to determine DPOAE test performance for detecting changes in cochlear function due to ototoxic medication administration. Namely, it can provide an estimate of test performance for all values of

the DPOAE measurement. However, this approach has not been utilized for DPOAEs in the ototoxicity literature.

The present study involves a subset of DPOAE and pure-tone threshold data obtained as part of a large, prospective study investigating methods of ototoxicity monitoring. DPOAE test performance was compared to the behavioral gold standard method applied to the frequency range most sensitive to early onset ototoxicity. Objectives of this study were:

Specific Aim 1. To establish a discriminate function that incorporates DPOAE measures as well as other factors to classify subjects with and without hearing change as identified by a gold standard test.

Specific Aim 2. To evaluate the accuracy of the discriminate function established in Specific Aim 1 in subjects receiving cisplatin.

Specific Aim 3. To evaluate the accuracy of the discriminate function established in Specific Aim 1 in subjects receiving other ototoxic agents and in subjects not receiving any ototoxic agents.

METHODS

Subjects

Subjects were recruited from the Portland Veteran Affairs Medical Center. Potential subjects were identified from a hospital pharmacy list. Specific Aims 1 and 2 included subjects drawn from patients receiving the anti-neoplastic chemotherapeutic agent cisplatin for the treatment of cancer. Specific Aim 3 included subjects receiving treatment with ototoxic chemotherapeutic agents carboplatin or oxaliplatin and hospitalized patients receiving non-ototoxic antibiotics or non-ototoxic chemotherapeutic agents.

Subjects inclusion criteria included: (a) ability to provide reliable behavioral responses; (b) measureable DPOAEs in at least one ear; (c) no active or recent history of middle-ear disorder, Meniere's disease, or retrocochlear disorder; (d) no concurrently prescribed ototoxin; and (e) a complete baseline test and at least one complete post-treatment evaluation (behavioral audiometry and DPOAEs). All subjects were counseled to reduce their noise exposure and protect their hearing when exposed to loud noise during and following cisplatin administration. All subjects were consented to participate in the study following the guidelines of the medical center's Institutional Review Board and were compensated for their time.

Measurements

Primary audiometric measures included: (1) behavioral pure-tone testing, and (2) DPOAE testing; and additional measures: (a) case history, (b) otoscopy, (c) immittance screening, (d) a tinnitus questionnaire, and (e) a noise exposure questionnaire. All testing

was completed within 2 hours. The measurement schedule and descriptions of the outcome variable, predictor variables, and other additional variables are presented below.

Measurement Schedule: Patients completed a battery of tests and interviews at baseline and during follow-up visits. Baseline was performed within the week prior to or within 24 hours after initial treatment with a platinum based chemotherapeutic agent cisplatin, carboplatin, or oxaliplatin. Baseline testing was conducted within 72 hours after initial drug administration for patients receiving a non-ototoxic agent.

Subsequent monitoring visits were ideally completed within 24 hours of each chemotherapy treatment. The chemotherapy regimen depended on the drug, dose, presence of concomitant radiation, and overall health of the subject; therefore, the schedule was variable across subjects. If a behavioral hearing change was noted, all frequencies were retested and the physician was notified. We attempted to conduct a test every week until thresholds stabilized. Additionally, when possible, testing was performed immediately after treatment had been discontinued but prior to hospital discharge and at one, three and six months after treatment.

Outcome Variable: The outcome variable was the presence or absence of hearing change determined by serial audiometric high-frequency monitoring (gold standard). Behavioral pure-tone thresholds were obtained using the modified Hughson-Westlake technique (Carhart & Jerger, 1959). Pure-tone thresholds were measured from 0.5 - 20 kHz using a Virtual Corporation, Model 320 (V320) audiometer. TDH-50P earphones in MX-41/AR cushions were used for testing 0.5 and 1 kHz thresholds. Koss Pro/4X Plus earphones, modified to improve signal-to-noise ratio for high frequency testing as described in Fausti, Frey, Henry, Knutsen, & Olson (1990), were used for testing

frequencies from 2 - 20 kHz. Reliability, validity, and equipment limits (115 dB SPL) for frequencies 2 - 20 kHz for threshold responses using the Virtual V320 audiometer paired with modified Koss Pro/4X Plus earphones have been documented previously (Fausti et al., 1990).

Calibration of the Virtual V320 audiometer was conducted twice each month. TDH-50P earphones were calibrated according to ANSI S3.6-1989 and IEC 318 specifications. The earphone was coupled to a Bruel & Kjaer (B&K) 4153 artificial ear and the acoustic output was measured by a B&K 4134 ½" condenser microphone and read on a B&K 2231 sound level meter. KOSS Pro/4X Plus earphones were calibrated on a 6cc flat-plate coupler with a B&K 4134 ½" condenser microphone in the center of the cavity (as described in Fausti et al., 1979).

Research results have demonstrated approximately 90% of initial ototoxic threshold changes occur within a small range of frequencies at or near the highest frequencies heard by each individual (Fausti et al., 1999). This range has been designated the sensitive range for ototoxicity or SRO. A behavioral SRO was identified for each ear from the baseline pure-tone thresholds (.5 – 20 kHz). The upper bound of the SRO was defined as the highest frequency the subject responded to a pure-tone signal of 100 dB SPL or less. The pure-tone thresholds of the six lower adjacent frequencies in $1/6^{th}$ octave steps were then obtained. These seven frequencies constituted the behavioral SRO, which was the target range monitored at all visits. Monitoring the SRO is time efficient and maintains a high-degree of sensitivity and reliability, regardless of pre-exposure hearing status (Fausti et al, 1999; Vaughan et al., 2002).

Behavioral hearing change was assessed relative to baseline measurements within the SRO only. Criteria utilized in this investigation are consistent with the published clinical standards for ototoxic behavioral hearing change criteria (ASHA, 1994), which include: (a) ≥20 dB change at any one test frequency; (b) ≥10 dB change at any two consecutive test frequencies; or (c) loss of response at three consecutive test frequencies where responses were previously obtained. Results of the DPOAE measure were not analyzed during the study and thus did not influence audiometric results.

Predictor Variables: The main predictor variables of interest were derived from DPOAE measures. DPOAEs were collected using the Otoacoustic Emission Averager (EMAV; Neely & Liu, 1993) which utilizes a Card Deluxe digital signal processing board to generate stimuli. An optimized probe system consisting of two ER-2 transducers delivered the stimuli coupled to an ER-10B+ microphone to record the responses. The system was electrically calibrated annually according to the manual. System distortion was estimated in a standard cavity (B&K 4153 Coupler). Estimates of system distortion were less than -20 dB SPL for the frequencies and intensity levels used in the present study.

DPOAE stimuli were two continuous tones (f_1 and f_2, where $f_1 < f_2$) presented through separate ER-2 transducers at a fixed primary frequency ratio f_1/f_2=1.22. DPOAE responses were obtained for a primary frequency sweep (DP-gram) from 1 – 8 kHz in $1/6^{th}$ octave increments at stimulus frequency levels of L_1=L_2=65 dB SPL to identify the highest frequency which produced a valid response, which constituted the upper bound of the DPOAE range. The highest f_2 able to generate a valid measure was marked and response growth (input/output) functions were obtained for that frequency and the three

lower adjacent frequencies using 1/3rd octave frequency steps. DPOAE levels were optimized based on a covaried paradigm ($L_1 = .4L_2 + 39$; Kummer, Janssen, & Arnold, 1998) to obtain input/output frequency responses at six intensity levels: L_1/L_2 in dB SPL = 63/60, 61/55, 59/50, 57/45, 55/40 and 53/35. This level paradigm yields DPOAEs characteristic of functional outer hair cells that are likely to be altered by ototoxicity (Kummer et al., 1998; Whitehead, Lonsbury-Martin, Martin, & McCoy, 1996).

Measurement-based stopping rules were used, such that at any frequency, averaging stopped when the noise floor was <-20 dB SPL or after 32 seconds of artifact-free averaging, whichever occurred first. In situ ear canal volume measurements obtained during baseline recordings were employed as a target volume in order to ensure consistent probe placement across follow-up visits and thus improve test-retest reliability. DPOAE level was estimated as the level in the $2f_1$-f_2 frequency bin. Noise level was estimated as the average level in the three closest frequency bins above and below $2f_1$-f_2.

DPOAE responses were considered valid if they met all of the following criteria:

1. DPOAE amplitude was greater than -20 dB SPL, a conservative estimate of the system distortion.
2. DPOAE amplitude was at least 6 dB or greater than the measured noise floor (biologic and system noise).
3. Primary levels L_1 and L_2 measured with the ER-10B+ probe microphone were within 3 dB of the targeted stimulus level.

The DPOAE predictor variables were derived from input/output functions obtained at four frequencies. All DPOAE associated values recorded in dB SPL were

converted to pressure and reported in micropascals (µPa) for all calculations. There were six input levels (L_2) ranging from 35 to 60 dB SPL (.00112 to .02 µPa) resulting in 6 output amplitudes ranging from -20 to 20 dB SPL (.000002 to .0002 µPa) for each function. Three DPOAE metrics were calculated from each input/output function: 1) sum of the stimulus level input, 2) sum of the emission output, and 3) sum of the signal-to-noise ratio. The sum of the stimulus level input (I) is defined as the sum of the L_2 values (in µPa) which were associated with a valid DPOAE response. Sum of the emission output (O) is defined as the sum of the valid DPOAE amplitudes (in µPa) generated within an input/output function. The sum of the signal-to-noise ratio (SNR) is the amplitude of the DPOAE (dB SPL) minus the noise floor (dB SPL) and the difference converted to µPa and summed over the range of valid responses within the input/output function. Any input/output function metric with valid no responses (i.e. lack of response not attributable to a high noise floor masking the DPOAE) was arbitrarily set to 0 µPa. The difference between DPOAE metrics measured at baseline and DPOAE metrics measured during subsequent follow-up visits were calculated. DPOAE metric differences are on a continuous, interval scale and were used for all analyses.

Additional Variables: Many variables were collected as a part of the large, prospective study. However, only certain variables were of interest to this study and mentioned below.

Information was obtained for each subject, either directly from the patient or from the patient's physicians or medical records. Information obtained included patient factors such as gender, age, pre-exposure hearing loss, disease, and drug treatment variables such as type, dose regimen and concomitant radiation therapy. At each session, the presence or

absence of tinnitus was recorded. If the patient reported tinnitus, a questionnaire was administered verbally to characterize the tinnitus. Additionally, the patient was asked about noise exposure during treatment including type of noise and duration, and if hearing protection devices were used. All patient interviews were conducted based on a standard questionnaire. Otoscopy and acoustic immittance testing were performed at every visit.

Statistical Analysis

Specific Aim 1: To establish a discriminate function that incorporates DPOAE measures as well as other factors to classify subjects with and without hearing change as identified by a gold standard test.

The main predictor variables of interest were the DPOAE input/output functions collected at 4 frequencies in each ear and summarized as three metrics per frequency: the sum of the level inputs, the sum of the emission outputs, and the sum of signal-to-noise ratios; this yielded 12 DPOAE metric-frequency combinations per ear. Other non-DPOAE variables considered included age, gender, measures of baseline hearing, baseline tinnitus status, concurrent radiation exposure, and measures of cisplatin exposure. The importance of each measure for predicting hearing change (present/absent) was individually assessed with logistic regression. Statistical significance of each regression coefficient was determined using the likelihood ratio statistic. Variables with p-values $<.10$ were considered for the multivariate discriminate function. The standard errors of the regression coefficients were estimated using Generalized Estimating Equations (GEE) to account for correlation among the repeated hearing measures taken

on each ear. The ear, follow-up visit, rather than the subject, served as the independent unit of analysis.

The optimal discriminate function to predict ototoxicity evolved in 3 steps. First, only baseline characteristics were assessed, yielding a baseline risk factor model. Second, cisplatin exposure variables were introduced to the baseline risk factor model. Third, DPOAE metrics were introduced. All potential 2-way interactions were assessed. Each discriminate function was reduced in a backward fashion and the model fit was assessed at each step. The optimal discriminate function was comprised of significant variables at the .05 level and significant interactions at the .15 level.

Specific Aim 2: To evaluate the accuracy of the discriminate function established in Specific Aim 1 in subjects receiving cisplatin.

Receiver operating characteristic curves and estimates of the area under the ROC curves (AUC) were utilized to determine the accuracy with which the discriminate function established in Specific Aim 1 detected hearing changes using high-frequency audiometry as the gold standard for comparison.

The purpose of building a discriminate function is to optimize the separation between subjects with no hearing change and from subjects with ototoxic-induced hearing changes utilizing the most important variables. The result is the ability to estimate the probability of hearing change based on a host of variables. The estimated probability of hearing change resulting from the discriminate function is a continuous variable that ranges from 0% to 100% and thus a ROC curve can be constructed to evaluate test performance.

The ROC curve evaluates overall test performance independently of the ultimately chosen decision criteria for DPOAE change. ROC curves are plots of sensitivity (y-axis) as a function of 1-specificity (x-axis). Both sensitivity and specificity can range from 0 to 1. Shifting the cut point down will increase the number of patients correctly identified as having behavioral hearing changes (i.e. increase the true positive rate or sensitivity). At the same time, it will also increase the number of patients without hearing changes who are incorrectly labeled by the discriminate function as having hearing changes (i.e. increase the false positive rate). Increasing the cut point will increase the number of patients correctly identified by the discriminate function as having no behavioral hearing changes (i.e. increase the true negative rate or specificity). At the same time, it will also increase the number of patients with behavioral hearing changes incorrectly labeled by the discriminate function as having no change (i.e. increase the false negative rate). Moving the cut point entails a trade-off between sensitivity and false positive rate which can be demonstrated graphically by a ROC curve.

An indication of the overall accuracy of the test is expressed as the AUC and was estimated by the concordance statistic, a nonparametric estimate, similar to the Wilcoxon statistic. Curves close to the diagonal line (AUC = 0.5) are indicative of a discriminate function that has little ability to separate ears with hearing change from ears without hearing change. The closer the curve is to the upper left corner of the plot, the better the diagnostic test. A discriminate function with high predictive accuracy is reflected in a ROC curve that rises quickly and is associated with a large AUC.

Specific Aim 3: To evaluate the accuracy of the discriminate function established in Specific Aim 1 in subjects receiving other ototoxic agents and in subjects not receiving any ototoxic agents.

To determine the clinical utility of the discriminate function established in subjects receiving cisplatin, it will be applied to subjects who were administered other known ototoxic agents (the anti-neoplastic chemotherapies carboplatin and oxaliplatin) and a control group of hospitalized subjects not receiving ototoxic medications. Test performance (sensitivity and specificity) in each group will be evaluated using the same methodology outlined in Specific Aim 2. Discriminate functions with high predictive accuracy will be reflected in a ROC curve that rises quickly and is associated with a large AUC.

RESULTS

Forty subjects receiving the anti-cancer drug cisplatin were consented to participate in the study and underwent baseline testing. Of the 40 subjects, 3 withdrew after the baseline test, 6 had incomplete data at baseline, and 7 others did not meet the inclusion criteria (3=poor thresholds precluded OAE measurements; 2=active middle ear pathologies; 2=unreliable). Of the remaining 24 subjects, 12 contribute one ear and 12 contribute two ears to the analysis. Of the 12 ears excluded, 3 had incomplete data at baseline, 4 had incomplete follow-up data, and 5 ears did not meet the inclusion criteria (2=poor thresholds precluded OAE measurements; 3=active middle ear pathologies). The ear-visit served as the primary unit of analysis rather than the subject. Therefore, 36 ears from 24 subjects were included in the analysis.

The majority of subjects in the analysis were Caucasian males with a mean age of 58.5 years. On average, each subject had 3.4 follow-up visits. Of the 24 subjects receiving cisplatin chemotherapy, half met the criteria for a hearing change according to the ASHA definition of ototoxicity in at least one ear, during at least 1 follow-up visit. On average patients received approximately 350 mg of cisplatin over 42 days. Table 1 summarizes subject characteristics.

Baseline mean behavioral hearing thresholds for the entire sample (solid gray line) are plotted in Figure 1 as a function of audiometric test frequency from 0.5 to 20 kHz. Pure-tone threshold responses unable to be obtained at equipment limits (115 dB SPL) were arbitrarily set to 120 dB SPL for inclusion into the average. Ninety-seven percent (35/36) of ears in this sample had thresholds above 8 kHz at baseline. The percentage of ears with pure-tone thresholds which could be measured within the

intensity limits of the audiometric equipment (115 dB SPL from 2 - 20 kHz) declined as frequency increased. At baseline, approximately 83% of ears (30/36) had measurable hearing thresholds at 12.5 kHz and above; this rapidly declined to 28% (10/36) at 16 kHz, and 0% (0/36) at 20 kHz. In Figure 1, mean thresholds at frequencies beginning around 16 kHz were near 120 dB SPL, indicating that many subjects had no responses at these higher frequencies.

Baseline testing involved the determination of an individualized, sensitive range for ototoxicity (SRO) defined as the uppermost frequency at which threshold is 100 dB SPL or less and the six lower adjacent frequencies in 1/6-octave steps (Fausti et al., 1999). Therefore, depending on the patient's hearing thresholds, testing in 1/6-octave intervals could extend below 8 kHz to as low as 2 kHz. Since hearing changes typically occur within the SRO (Fausti et al., 1999), the baseline audiometric frequencies were normalized to each subject's highest audible frequency, termed F_B. For subsequent test sessions, pure-tone thresholds were obtained only within the subject's SRO, as defined at baseline. If a hearing change was noted within the SRO, then full frequency testing resumed. In this study, the median upper bound of the SRO at baseline was 12.5 kHz (range: 5 – 16 kHz) and baseline SRO pure-tone thresholds averaged 74.3 dB SPL (range: 48.6 – 97.5 dB SPL).

Specific Aim 1: To establish a discriminate function that incorporates DPOAE measures as well as other factors to classify subjects with and without hearing change as identified by a gold standard test.

Outcome Variable

The outcome variable in this analysis, hearing change (present/absent) was defined according to ASHA 1994 guidelines (outlined in Methods) applied to the SRO region. This approach to monitoring hearing for ototoxicity was considered to be the gold standard for the purpose of this analysis. Post-exposure hearing changes for normalized behavioral SRO frequencies are plotted in Figure 2. The plot includes magnitude of hearing change (dB) at the final test by the seven SRO frequencies in rank order of the lowest frequency, F_B*-6*, to highest frequency, F_B (F_B-1 is 1/6-octave below F_B, F_B-2 is 1/6-octave below F_B-1, and so on). The ears with hearing change (white bars) experienced at least 10 dB threshold shifts across all frequencies (F_B*-6* through F_B) and at least 15 dB threshold shifts across the four highest frequencies (F_B*-4* through F_B*)*.

Predictor Variables

Non-DPOAE Measures as Predictors of Hearing Change: In addition to baseline hearing thresholds for the entire group, the baseline hearing thresholds (dB SPL) for the no hearing change group and the hearing change group are plotted in Figure 1. Both groups follow a similar audiometric pattern with normal to near-normal hearing in the low frequencies followed by a sloping hearing loss. However, on average, the ears that went on to experience hearing change had better pre-exposure hearing across the majority of frequencies tested compared to the ears that did not have hearing change.

Tables 2 and 3 depict the predictor variables (continuous variables and categorical variables, respectively) by hearing change group. Table 2 includes age, high-frequency pure-tone average (PTA) at 2, 4, 6 kHz at baseline, SRO average pure-tone threshold at

baseline, upper-frequency bound of the SRO at baseline, cumulative cisplatin dose, total number of days exposed to cisplatin, and the total number of cisplatin doses by hearing change group. The degree of hearing loss reported by high-frequency PTA at 2, 4, and 6 kHz is restricted to the lower conventional frequencies, whereas the SRO average threshold reflects the degree of hearing loss in the range most sensitive to ototoxicity. Therefore, though high-frequency PTA and SRO average threshold measures are highly correlated (r = 0.78), they do represent hearing in different regions of the cochlea. If a patient had a severe hearing loss (e.g. baseline behavioral hf-limit was 8 kHz) these two measurements would overlap; however most often these two measurements were different. Similarly, upper-frequency bound of the SRO is correlated with both SRO average threshold (r = -0.62) and high-frequency PTA (r = -0.66). However, it was of interest to determine which measure of baseline hearing was the best predictor of hearing change, so all three measures were evaluated individually as well as together.

Each baseline hearing measure (high-frequency PTA, SRO average threshold, and upper-frequency bound of the SRO) reflected significant differences at baseline between the ears that went on to experience hearing change and no hearing change. Ears with subsequent hearing changes had better hearing at baseline, roughly 14 dB better, and this difference was statistically significant (both high-frequency PTA and SRO average threshold *p*-value <.01) and they could hear more extended-high-frequencies (upper-frequency bound of the SRO *p*-value <.01).

Furthermore, ears with hearing changes also received significantly greater cumulative drug doses (*p*-value = <.01). The mean cumulative cisplatin dosage among ears with hearing change was 425 mg, consistent with previous findings that ototoxicity

is associated with cumulative doses of approximately 400 mg (Schaefer, Post, Close, & Wright, 1985). However, other cisplatin exposure descriptors such as total number of days exposed and total number of doses were not different between groups.

Age was also significantly different among groups, with the hearing change group being slightly younger than the no hearing change group, 60 years of age versus 56 years of age, respectively (*p*-value = .03). Gender, tinnitus at baseline, and presence of concurrent radiation did not differ between groups (presented in Table 3).

DPOAE Measures as Predictors of Hearing Change: The overall goal of the analysis was to establish a discriminate function that incorporates DPOAE measures as well as other subject characteristics to classify subejcts with and without hearing change as identified by a gold standard test. Therefore, the main predictor variables of interest are the DPOAE input/output function summary metrics. The predictive utility of all DPOAE metrics were individually assessed through logistic regression analysis adjusted for repeated measures.

Input/output functions were summarized using three separate, but related metrics including the sum of the stimulus level input (I), the sum of the emission output (O), and the sum of the signal-to-noise ratio (SNR). Each valid emission, corresponding input level, or corresponding signal-to-noise ratio was first converted to pressure (micro pascals) before summing and the sum multiplied by 10,000 for scaling purposes. Therefore, each frequency had three DPOAE metrics associated with it: I, O, and SNR. By converting the measurements from sound pressure level to pascals, zero reflects of the absence of sound and thus the absence of emissions, a potential scenario during follow-up visits.

DPOAE input/output function summary metrics, I, O, and SNR, were evaluated at four frequencies in each ear, thus yielding 12 metric-frequency combinations for analysis. For each ear, DPOAE input/output functions were collected at the highest frequencies with emissions. Similar to the behavioral SRO, DPOAE frequencies were normalized to each subject's highest frequency with an emission and termed F_D, which was defined during the baseline test. DPOAE input/output functions were then collected at 4 frequencies, 1/3 octave apart. Thus, F_D -1 is 1/3-octave below F_D, F_D -2 is 1/3-octave below F_D -1, and so on. The median high-frequency with a valid OAE was 4 kHz and ranged from 1.5 to 8 kHz.

The difference in DPOAE metrics was calculated by subtracting the follow-up visit from the baseline visit. Table 4 shows the average difference in the DPOAE metrics by normalized test frequency between the two groups, ears without hearing change and ears with hearing change. Positive differences indicate a decrease in the DPOAE metric from baseline to follow-up and a negative difference indicates an increase in the DPOAE metric from baseline to follow-up. It is apparent the largest positive differences (i.e. an indication of decreasing emissions) occurred at the highest frequencies measured, normalized to F_D. Statistically significant differences were observed for the I:F_D, O:F_D, and SNR:F_D (all p-values <.05). The predictive utility (area under the curve estimates; y-axis) of each DPOAE metric is plotted as a function of normalized frequency (x-axis) in Figure 3. Overall, the difference in the O:F_D (solid black line, open squares) was the best predictor of ototoxicity, however all three metrics at F_D exhibited test accuracy better than chance (AUC = .5). Generally, the usefulness of DPOAE metrics as predictors of ototoxicity declined as test frequency declined.

Initial Discriminate Function

Model 1: Any variable whose univariable test above had a *p*-value <.10 was a candidate for the initial discriminate function. Therefore, the initial main effects model included the baseline variables that were significantly different between groups: age, SRO average threshold at baseline, high-frequency PTA at baseline, and the upper-frequency bound of the SRO at baseline. This model was then reduced through backward elimination to include only the best predictor(s) of hearing change. The resulting model included only the SRO average threshold at baseline. Age was no longer a significant variable after controlling for hearing ability at baseline. SRO average threshold was inverted such that larger values would reflect better hearing. However, the scaling of the reciprocal of the SRO average threshold variable was problematic. To correct for the scaling problem, the reciprocal of the SRO average threshold variable was standardized to a mean of 0 and a standard deviation of 1 and used for all model estimates.

Model 2: Added to the model at this time was cumulative dose, the only significant cisplatin exposure variable. Both the SRO average threshold and cumulative dose remained significant when included in the model together. Additionally, there was a significant interaction between SRO average threshold and cumulative dose; as such the discussion of dose toxicity cannot be fully explained without discussing pre-exposure hearing.

The predicted probability of hearing loss was determined using Model 2 for a range of cumulative drug doses and a limited range of pre-exposure hearing (using the standardized, reciprocal of the SRO average threshold) and plotted in Figure 4. The predicted probability of ototoxicity (y-axis) was plotted by cumulative drug dose (x-axis)

ranging from 100 mg to 600 mg and stratified by pre-exposure hearing for the mean (dashed line with open squares), one standard deviation below the mean (poorer hearing indicated by the dotted line with open circles) and one standard deviation above the mean (better hearing indicated by solid line with open triangles). At drug doses of 300mg or lower, the predicted probability of ototoxicity differs very little by pre-exposure hearing status. At cisplatin doses of 400 mg and greater, those with better hearing have a higher probability of experiencing ototoxicity. At 400 mg cumulative drug dose, the predicted probability of ototoxicity increases from 28% to 64% to 90% with increasingly better pre-exposure hearing status. At a cumulative cisplatin dose of 600 mg, a dose typically associated with ototoxicity, the predicted probability of ototoxicity is near 100% for ears with better hearing. However, for ears with poorer hearing (one standard deviation below the mean), the predicted probability of ototoxicity does not reach 50%. At high cisplatin doses, those with better hearing are 2 times more likely to experience ototoxicity.

Optimal Discriminate Function

Model 3: The three significant DPOAE metrics were introduced to the model: difference in I:F_D, difference in O:F_D, and the difference in SNR:F_D. The model was again reduced in a backward fashion. All DPOAE metrics fell out of the model with the exception of the difference in I:F_D. Two-way interactions between the SRO average threshold and cumulative dose and the remaining DPOAE metric were introduced (i.e. SRO average threshold x difference in I:F_D and cumulative dose x difference in I:F_D) and were found not to be significant. The only significant interaction remaining in the model was between the SRO average threshold at baseline and cumulative dose. The final model

included: SRO average threshold at baseline, cumulative cisplatin dose, difference in I:F_D, and the interaction between SRO average threshold at baseline and cumulative dose. The optimal discriminate function was assessed by cumulative residuals for each variable in the model. All cumulative residuals suggest the model fit to the data was adequate.

Each model derived from the GEE approach yields an estimate of the relative risk of hearing change in an ear at a visit given that a hearing change occurred at another visit in that ear. The estimated relative risk was 2.14 (95% CI: 0.67 – 6.86), indicating that the risk of hearing change in an ear more than doubles if hearing change occurs at any other time. However, the relative risk 95% confidence interval includes 1 which suggests there is no increase in the risk of a hearing change at a visit, in an ear, given hearing changed occurred at another visit.

Table 5 reflects the order and results of building the optimal discriminate function. It is sorted by predictor variables (baseline variables, other subject variables, and DPOAE variables) and organized by the built model. Model 1 included only baseline variables, yielding a baseline risk factor model. Model 2 included adding other subject variables to the initial baseline variables established in Model 1. Model 3 includes the addition of DPOAE variables to Model 2. Information includes the regression coefficient, standard error of the coefficient, and type 3 *p*-value. Within each model (1, 2, and 3), the initial parameter estimates and *p*-values are shown for all variables. The models were then systematically reduced (backward elimination) to leave only the significant variables, which are highlighted within each model in bold italics.

Based on the estimated regression coefficient of the optimal discriminate function, the relative risk of ototoxicity can be determined based on a variety of

differences in I:F_D. At a difference of 0, the relative risk is 1 indicating no increased risk. For every 100 Pa drop from baseline in I:F_D, there is a 2.3 fold increase (95% CI: 1.53 – 3.36) in the predicted risk of hearing change, after controlling for baseline hearing and cumulative cisplatin dose. The relative risk of ototoxicity increases as the difference in the I:F_D increases.

Specific Aim 2: To evaluate the accuracy of the discriminate function established in Specific Aim 1 in subjects receiving cisplatin.

A ROC curve and it's corresponding AUC was estimated to determine the accuracy with which the discriminate function established above detected hearing changes using behavioral audiometry (within the SRO) as the gold standard for comparison. Figure 5 is a plot of sensitivity (y-axis) as a function of 1-specificity (x-axis), a ROC curve. Plotted in Figure 5 are the ROC curves for the optimal discriminate function (thick solid black line) and single predictor variables: cumulative drug dose (thin solid black line), SRO average threshold at baseline (dashed black line) and difference in the I:F_D (dotted black line). The ROC curve for the optimal discriminate function rises quickly suggesting high predictive accuracy. Furthermore, the calculated AUC is .94, an indication of the overall accuracy of the discriminate function. The calculated AUC for the individual variables cumulative dose, SRO average threshold at baseline, and difference in the I:F_D were .84, .76, and .67 respectively. The optimal discriminate function predicts hearing change better than any single variable. However, the AUC estimates may be overly optimistic because the estimation of test accuracy was based on the data for which the model was derived.

Specific Aim 3: To evaluate the accuracy of the discriminate function established in Specific Aim 1 in subjects receiving other ototoxic agents and in subjects not receiving any ototoxic agents.

The goal of Specific Aim 3 was to evaluate the optimal discriminate function on a separate validation group, a random subset of the data not included in the establishment of the optimal discriminate function. For this purpose, subjects who received carboplatin, oxaliplatin, or non-ototoxic agent were held out so they could serve as a validation group which would lead to inferences regarding the generalizability and validity of the discriminate function. It was my intention to develop a model that did not include cumulative drug dose, but rather a proxy of drug dose through time exposed. However, number of total days exposed was not a significant predictor and cumulative drug dose was too strong of a predictor to not be included. Omitting this strong predictor could have resulted in a miss-estimation of the regression coefficients yielding a biased discriminate function. Once cisplatin drug dose was in the discriminate function, the validation groups were no longer a viable option. Though there exists dose equivalency measures between cisplatin and other anti-neoplastic agents, there is no dose-toxicity equivalency measure. This rendered Specific Aim 3 unachievable as it was originally described.

DISCUSSION

The rationale for this study is that once the test characteristics of DPOAE are determined, objective ototoxicity monitoring strategies can be developed. DPOAE are quick and unaffected by patient responsiveness. This measure of ototoxicity has the potential to increase the number of patients monitored and thus limit the incidence of ototoxicity.

Changes in the DPOAE response have previously have been associated with ototoxicity in both children and older adults (Katbamna et al., 1999; Knight et al., 2007; Ress et al., 1999; Stavroulaki et al., 2001; 2002). These previous findings suggested DPOAEs would provide a useful means of monitoring hearing changes in individuals with limited behavioral responses. Most investigations into DPOAE applications for the detection of ototoxicity have utilized either a) statistical tests of difference, or b) clinical tests of difference derived from control populations. Though useful, these investigations add little to the literature with respect to overall sensitivity and specificity of DPOAE measures for predicting hearing changes.

In this study DPOAE test performance was evaluated for detecting changes in cochlear function due to ototoxic medication administration through ROC curves, which allowed for an estimate of test performance for all values of the DPOAE measurement. This appears to be the first study to apply ROC curve analyses to the investigation of DPOAE test accuracy in detecting ototoxicity. Distortion-product otoacoustic emissions were first evaluated as a univariable predictor of ototoxicity and the significant predictors were modeled with other subject information to achieve an optimal discriminate function.

DPOAE Test Performance

There were 12 DPOAE metric–frequency combinations evaluated for predictive accuracy. Of the four normalized frequencies (F_D through F_D-3), the most predictive frequency was located at normalized F_D, the highest frequency with a valid emission, for each metric based on estimates of the AUC. Predictive accuracy decreased as the frequency decreased. These results suggest the most DPOAE sensitive region to ototoxic insult is similar to the behavioral SRO. Additionally, the functional changes in the DPOAEs located at the highest frequencies support the animal and human temporal bone studies suggesting the lesion progresses in a base to apex order. Previous reports in adults have also noted the higher DPOAE frequencies changed first with lesser or no changes observed in DPOAEs located more apically (Ress et al., 1999; Reavis et al., 2008). However, this study appears to be the first to demonstrate that higher DPOAE test frequencies are statistically more predictive of incipient ototoxicity than lower DPOAE test frequencies.

Recall that the DPOAEs being monitored for ototoxicity were most often located apically to the behavioral region monitored by extended high-frequency pure-tone thresholds. At baseline, the median DPOAE upper-frequency bound was 4 kHz and the median upper bound of the behavioral SRO was 12.5 kHz. Previous studies have demonstrated that DPOAE amplitude measures highly correlate with pure-tone thresholds obtained at the same test frequency. It has also been suggested that extended high-frequency hearing may be associated with DPOAEs measured at lower frequencies (Arnold, Lonsbury-Martin, & Martin, 1999). To this effect, several authors have noted DPOAE changes in the absence of corresponding, parallel pure-tone threshold changes in

patients receiving ototoxic medications (Katbamna et al., 1999; Mulheran & Degg, 1997, Stavroulaki et al., 2001; 2002). However, extended high-frequency pure-tone thresholds were rarely measured, thus the relationship between DPOAEs obtained at frequencies lower than the extended high-frequency hearing threshold change could not be explored. Similar to previous studies, this study found DPOAE changes in the absence of parallel pure-tone threshold changes and in the presence of extended high-frequency pure-tone threshold changes. This pattern of change could potentially be reflecting a) the hearing threshold shifts noted remotely at the base of the cochlea, b) pre-clinical degradation of the outer hair cells within that frequency region, or c) a combination of both the influence of degrading high-frequency hearing on remotely located DPOAEs and parallel outer hair cell destruction at the DPOAE test frequency. It is beyond the scope of this study to address the complex pathophysiology of ototoxicity; however, regardless of the mechanism, it is clear that DPOAEs generated apically predicted behavioral hearing threshold shifts occurring basally.

To maximize the difference between ears with hearing change and ears without hearing change a multivariate model was explored allowing for the established predictors of cumulative dose and pre-exposure hearing status to be included and controlled for. The addition of DPOAEs to the model increased test accuracy and test performance over what was achieved with cumulative dose, baseline hearing, or the DPOAE metric alone. This model may have implications for the use of DPOAEs clinically in assessing pure-tone threshold shifts resulting from cisplatin administration in terms of both clinical improvements in test performance and by providing a means for interpreting the DPOAE test results.

When interpreting DPOAE test results it is important to remember that DPOAEs never achieved perfect test performance (AUC=1.0) when compared to the gold standard. Most clinical tests are not perfect and diagnostic errors will occur. Several cross-sectional studies have demonstrated DPOAE differences in the absence of behavioral hearing differences among those exposed to ototoxic agents. Those authors concluded DPOAEs may be useful as a pre-clinical (i.e. pre- pure-tone threshold shift) screening tool in patients receiving ototoxic medications. If DPOAEs measures were, in truth, functionally reduced from baseline prior to hearing changes detected behaviorally, this would render the discriminate function less accurate because DPOAEs changes in the absence of pure-tone threshold changes would be considered a false-positive (a diagnostic error). However, many of the studies which have suggested the pre-clinical utility of DPOAEs did not evaluate hearing thresholds >8 kHz. As suggested by Knight and colleagues (2007), behavioral thresholds at extended high-frequencies (>8 kHz) may initially be more sensitive to ototoxic change compared to DPOAE amplitudes in the conventional frequency range. In this situation, changes in behavioral thresholds would precede DPOAE changes in the conventional range, and would be considered a miss. Until more information regarding the relative timing of changes noted among DPOAEs measured in the conventional range and pure-tone thresholds measured the extended high frequencies, DPOAE interpretations will include some diagnostic errors. It is important to recognize these potential diagnostic errors when applying and interpreting DPOAE measurements in the clinic. Knowing more about the timing will help reduce clinical errors but is outside the scope of this study.

Risk Factors for Ototoxicity

Prior to assessing the ability of DPOAE measures to discriminate between those with hearing changes from those without hearing changes, it was important to first control by study design and if need be, statistically adjust for other potential risk factors found to be important predictors in the multivariate model. The data presented here were from a homogenous group of older, adult, male Veterans who received only the ototoxin, cisplatin. Furthermore, no subject reported noise exposure during treatment without the use of hearing protection devices. Therefore concurrent exposure to other ototoxins such as noise and other medications were controlled by the study design and not evaluated. Other baseline variables that were not controlled by the study design and were investigated included age, gender, pre-exposure hearing status, pre-exposure tinnitus status as a proxy for cochlear degradation, concurrent radiation and the number, dose, and duration of cisplatin exposure. Evidence suggests that other patient factors such as biochemical, physiologic, and genetic factors also may impact incidence rates though these were not evaluated in this study.

Pre-exposure hearing status/cochlear degradation: Evidence from experimental animals and human temporal bone studies have identified the base of the cochlea as physiologically more vulnerable to ototoxic damage than the apex. This might suggest that for a given cisplatin dose, patients that hear at higher absolute frequencies may be more susceptible to ototoxic hearing changes than patients who hear poorly or not at all at high frequencies. However, pre-exposure hearing loss as a risk factor for ototoxicity has not been well studied.

The data presented within this study examined hearing using a monitoring strategy that tests the entire range of human hearing (frequencies from 0.25 to 20 kHz) in order to determine the highest audible frequencies, considered the most vulnerable frequency range, for each patient. The result of this SRO monitoring strategy yielded results which suggested individuals with better than average hearing at baseline are at greater risk for experiencing pure-tone threshold shifts compared to individuals with poorer than average hearing at baseline.

On the contrary, several clinical studies (Aguilar-Markulis et al., 1981; Fleming et al., 1985; Bokemeyer et al., 1998; van der Hulst et al., 1988) have suggested that patients with pre-exposure sensorineural hearing loss may develop greater hearing loss from cisplatin than those with normal hearing sensitivity, however associations have been inconsistently observed in both animal and human studies (Boheim & Bichler, 1985; Laurell & Borg, 1985; 1988; Laurell & Jungnelius, 1990). In reports demonstrating an association between poor pre-exposure hearing and ototoxicity, hearing threshold shifts were monitored up to 8 kHz. Fausti and colleagues (1994) evaluated incipient ototoxicity in a sample of Veteran subjects receiving cisplatin and found that 64.3% of ears reflected incipient hearing threshold shifts in the extended high-frequency range (>8 kHz) compared to only 11.9% within the conventional frequency range (1-8 kHz), and 23.8% in both ranges (i.e. conventional and extended high-frequency). Furthermore, Fausti and colleagues (1999) noted that initial threshold shifts occurred at the highest audible frequency for each ear (otherwise known as the SRO), which suggests those individuals with hearing threshold shifts within the conventional frequency range likely had much poorer pre-exposure hearing than subjects with hearing threshold shifts detected in the

extended high-frequency range. Therefore, monitoring only up to 8 kHz can be interpreted as monitoring within the SRO only for people with pre-exposure hearing loss, and below the SRO of people with normal to near-normal hearing. Thus, monitoring only the conventional frequency range (i.e. 250 – 8000 Hz) will not be equally as sensitive for all audiometric configurations; in fact, monitoring the conventional frequency range will initially be less sensitive in ears with good high-frequency hearing. This could have erroneously led investigators to conclude that pre-exposure hearing loss is a predictor of ototoxic hearing threshold shifts. Monitoring frequencies that are within the SRO only for people with pre-exposure hearing loss and below the SRO for people with normal hearing may make it appear that people with poorer than average hearing have an increased risk of experiencing ototoxicity.

Cisplatin Exposure: Cisplatin is a known ototoxin and the incidence of cisplatin ototoxicity is related to dose. Studies have shown the risk for hearing threshold shifts from ototoxic medications is related to the individual dose and cumulative dose, the duration of exposure, and number of doses. Several prospective human studies have noted increasing incidence of ototoxicity with increasing cumulative dose noting a dramatic increase in the rate of ototoxicity as the cumulative dose exceeds 400 mg (Bokemeyer et al., 1998; de Jongh et al., 2003; Li et al., 2004; Park, 1996; Waters et al., 1991; Vermorken, 1983). The incidence of ototoxicity increases from approximately 20% at low doses to 40% at high doses to near 100% at very high doses (Vermorken et al., 1983; Waters et al., 1991). Half of our subjects experienced cisplatin-induced hearing threshold shifts in at least one ear with an average cumulative dose of 425 mg and is in

good agreement with previously published data. No effect was observed between the total number of cisplatin doses and ototoxicity or total duration exposed and ototoxicity.

Pre-exposure hearing and cisplatin dose interaction: Furthermore, we noted that pre-exposure hearing was an effect modifier of the cisplatin dose - hearing change relationship. A logistic regression model predicts ototoxicity twice as frequently in ears with better than average pre-exposure hearing (which average pre-exposure hearing was a mild sloping to a moderate sensori-neural hearing loss) compared to ears with poorer than average pre-exposure hearing. The predicted probability of ototoxicity in ears with average to better-than-average pre-exposure hearing markedly rises as cumulative cisplatin dose exceeds 300 mg and reaches near 100% at 600 mg. The predicted probability of ototoxicity at 600 mg in ears with poorer than average pre-exposure hearing is less than 50%. However, in individuals with poor pre-exposure hearing, the functional risk of hearing threshold shifts may be even greater than for patients with good pre-exposure hearing since additional loss can immediately affect their communication ability. Furthermore, there is no indication that individuals with poorer hearing at baseline experience less (dB) hearing change. Reavis et al., (2008) found no differences in the magnitude of hearing change between a group of cisplatin exposed Veterans with good hearing (high-frequency PTA = 43 dB SPL) and exposed Veterans with poor hearing (high-frequency PTA = 70 dB SPL) at baseline.

Other non-significant risk factors: No effects were found between the variables age, gender, tinnitus at baseline and concurrent radiation, and the outcome variable,

hearing change. After controlling for pre-exposure hearing, age was no longer significantly associated with hearing change. Tinnitus at baseline, a proxy in this study for cochlear degeneration resulting from noise-induced hearing loss, yielded no association with hearing change. Additionally, concurrent radiation was found not be associated with hearing change. However, these findings should be interpreted with caution because concomitant toxins such as noise (Boettcher, Henderson, Gratton, Danielson, & Bryne, 1987; Gratton, Salvi, Kamen, & Saunders, 1990) and radiation therapy to the head and neck (Chen et al., 2006; Pearson, Meyer, Adams, & Ondrey, 2006) have previously been shown to produce a synergistic effect leading to increased rates of ototoxicity. Neither of these two variables was explored in any detail and their potential synergy is beyond the scope of this study.

Limitations

There were several limitations to this study and data analysis. First, the audiologists were not blinded to the drug exposure. This could bias the sample since the audiologist knew, a priori, whether or not the subject was at risk for developing hearing changes based on the ototoxic drug administered. Additionally, each subject was questioned (using a standard questionnaire) regarding new complaints of hearing loss, tinnitus, or dizziness. If a subject was more likely to subjectively report changes in hearing ability or tinnitus, the audiologist may have been more likely to readily accept a hearing change when in truth there was no change. This bias could result in misclassification of the outcome. However, all changes were confirmed either within the test session or within 24 hours, which should limit the effects of any bias.

Furthermore, the discriminate function established in this study can only be applied to a population with a similar demographic profile and collected with the same stimulus parameters and recording conditions used in this study, which limits its generalizability. The Veteran study participants were mostly older Caucasian males with some pre-exposure hearing loss, which is fairly representative of the Portland VA Medical Center (PVAMC) population. The Veteran population is different from non-Veterans in their overall health status. Veterans traditionally present to the hospital with more advanced stages of cancer and multiple, more significant co-morbidities (Agha, Lofgren, VanRuiswyk, & Layde, 2000). The inherent homogeneity among the PVAMC population makes external validity and generalizability of this study to other populations challenging even among other VA medical centers especially those with greater ethnic diversity. Future ototoxicity hearing loss investigations would benefit from being conducted in more racially and ethnically diverse VA medical centers. It is known there are innate genetic factors that contribute to the risk of ototoxic hearing loss. However, it is not readily known which, if any, group of individuals share this genetic pre-disposition. The inclusion of minorities and women by which an analysis could be stratified would help to determine if the observed associations were confounded by a cultural trait, either social and/or genetic.

A potential limitation in this study is the statistical methodology employed to determine test accuracy. Though widely popular in psychology literature, ROC curve analysis is relatively new to medical literature with the most popular being the investigation of the sensitivity of radiographic images. ROC curve analysis (sometimes referred to as clinical detection theory) recently has been found in the OAE literature

related to predicting hearing status, normal versus impaired (Gorga et al., 1993b; 1996), but has never been applied to evaluate DPOAE's ability to predict ototoxicity. Though novel in its application, it does present with noted limitations. As mentioned previously, DPOAE changes may reflect pre-clinical cochlear degradation; as such a DPOAE change would inaccurately be categorized as a diagnostic error. The effect of this misclassification is unknown and would have greater impact on test accuracy in the univariable model. The misclassification, if apparent, appears to have a lesser effect on the multivariate model considering discriminate function accuracy was estimated to be 94%. However, there are other variations to ROC curve analysis which can be applied in the absence of a gold standard which may better assess DPOAE's pre-clinical detection rates.

ROC curve analyses limit the outcome variable to a dichotomous measure. There are some inherent limitations to dichotomizing ototoxic hearing change as present or absent. Quantitative relationships (magnitude) between the ototoxic induced hearing threshold shifts and DPOAE changes remain to be found and warrant further investigation. A DPOAE model predicting magnitude of hearing change would afford the prescribing clinician more detailed information for determining continued treatment.

Additionally, as depicted in Figure 5, the ROC-curves for individual predictors are somewhat disjointed and do not reflect a smooth curve more typical in ROC-curve analyses. It is unknown whether this slight deformity is related to the small sample size, or is inherent to the DPOAE data itself. A larger sample size is preferable for a ROC-curve analysis in addition to model validation.

The major limitation to this study is the lack of model validation. It is possible the optimal discriminate function established is entirely idiosyncratic to the data set from which it was derived. The best way to access the predictive accuracy of a discriminate function is to apply it to an independent data set that was not used to fit the discriminate function. This can be done by holding out a portion of the sample, often times referred to as a validation group. However, the small sample size in the study prohibited the use of a validation group. Assessing the accuracy of the model using the same data set as was used to determine the model results in overly optimistic estimates of sensitivity and specificity. One method of addressing this limitation is to evaluate the optimal discriminate function on a separate group of cisplatin subjects. Other methods of model validation include computer intensive statistical approaches such as jack-knife and bootstrap which are both methods for estimating generalization error based on "resampling." Both approaches should be considered and the model validated.

SUMMARY

The investigation of DPOAE changes as an indicator of hearing changes attributed to ototoxic medication administration is fundamentally about disability control. Platinum-based chemotherapeutic agents are widely used in both children and adults and are unrivaled in their effectiveness against cancers. As long as the best, evidence-based practice for the treatment of certain cancers includes treatment with cisplatin, patients will experience ototoxic hearing loss. Screening with DPOAEs for hearing threshold shifts will allow for early intervention, aural rehabilitation, and appropriate disability control measures. The aims of this study were to develop an optimal discriminate function that predicts the probability of hearing change at each follow-up visit for each subject's ear utilizing DPOAE measures and other subject characteristics. The optimal discriminate function was compared to the behavioral gold standard method of repeated measures pure-tone threshold testing, which was applied to a frequency range sensitive to early onset ototoxicity. The accuracy of all predictor variables and the multivariate discriminate function were evaluated through ROC curves. Though ROC curve analyses have been used previously to determine DPOAE test performance in predicting normal versus impaired hearing, it has never been utilized to determine DPOAE test performance to predict ototoxicity.

The data reported here are consistent with previous results showing that the risk for hearing threshold shifts from ototoxic medications is generally related to the drug dose. However we find that especially at high cisplatin dose levels and in Veterans with good hearing at baseline, dosage is a particularly important indicator. In contrast to previous reports, we find that good pre-exposure hearing is an important indicator of

threshold shifts following cisplatin exposure, with differences across studies attributable to testing methodology, specifically different test frequency ranges. Further, we find that DPOAEs combined with information about drug dosage and pre-exposure hearing provides a highly accurate multivariate predictor of hearing threshold shifts resulting from cisplatin administration. Compared to the use of DPOAEs alone, this multivariate approach greatly increases the accuracy with which DPOAEs can determine whether or not a significant hearing threshold shift has occurred. This model represents an important first step in providing evidence to allow the rational design of future cisplatin ototoxicity monitoring protocols, with the aim of maximizing efficacy while reducing the long-term consequences, both in responsive and non-responsive patients.

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TABLES

Table 1. Characteristics of cisplatin subjects (n=24)	
GENDER	
MALE	22 (91.7%)
FEMALE	2 (8.3%)
ETHNICITY	
NON-HISPANIC WHITE	15 (62.5%)
AMERICAN-INDIAN/ALASKAN	1 (4.2%)
HISPANIC	0 (0.0%)
AFRICAN-AMERICAN	1 (4.2%)
OTHER	7 (29.2%)
AGE (MEAN)	58.5 (range: 28-75)
# OF FOLLOW-UP TESTS (MEAN)	3.4 (range: 1-9)
FINAL CUMULATIVE DRUG DOSE (MEAN, MG)	347.5 (range: 150-600)
TOTAL DAYS EXPOSED (MEAN)	41.7 (range: 1-160)
TOTAL # OF DOSES (MEAN)	3.3 (range: 1-14)
# OF SUBJECTS WITH NO HEARING CHANGE	12
# OF SUBJECTS WITH UNILATERAL HEARING CHANGE	8
# OF SUBJECTS WITH BILATERAL HEARING CHANGE	4

Table 1. Characteristics of cisplatin subjects.

Table 2: Continuous predictor variables according to hearing change

	No Hearing Change					Hearing Change					$p^{\dagger}$
Variable	**N***	**Mean**	**SD**	**Min**	**Max**	**N***	**Mean**	**SD**	**Min**	**Max**	
Age	79	59.8	6.4	28	75	47	56.4	7.0	28	68	.03
Baseline SRO Average Threshold (dB SPL)	79	76.1	13.2	48.6	97.5	47	62.3	13.6	48.6	93.6	<.01
Baseline High-frequency PTA (2, 4, 6 kHz)	79	57.3	16.2	25.0	83.3	47	43.3	13.0	25.0	71.7	<.01
Baseline SRO Upper Frequency Bound (kHz)	79	11.2	2.3	5.0	14.0	47	12.6	1.8	8.0	16.0	<.01
Cumulative Dose (mg)	79	252.4	98.6	55.0	540.0	47	425.3	121.1	150.0	600.0	<.01
Total number of Days Exposed	79	57.7	43.7	1	160	47	54.2	33.7	1	160	.95
Total Number of Doses	79	3.2	1.7	1	14	47	3.4	2.4	1	14	.55

** N is ear-visits*
† *p-value from GEE logistic regression model coefficients*

Table 2. Continuous variables by hearing change group across all visits. Variables with *p*-values <.10 were considered in the multivariable model.

Table 3. ***Categorical predictor variables according to hearing change***

Variable	No Hearing Change	Hearing Change	Total	*p*[†]
GENDER	--	--	--	.27
MALE	70 (65%)	38 (35%)	108 (100%)	
FEMALE	9 (50%)	9 (50%)	18 (100%)	
TOTAL	79	47	126	
TINNITUS AT BASELINE	--	--	--	.21
NO	28 (84.9%)	5 (15.1%)	33 (100%)	
YES	51 (54.8%)	42 (45.2%)	93 (100%)	
TOTAL	79	47	126	
CONCURRENT RADIATION THERAPY	--	--	--	.96
NO	20 (69.0%)	9 (31.0%)	29 (100%)	
YES	59 (60.8%)	38 (39.2%)	97 (100%)	
TOTAL	79	47	126	

** N is ear-visits*

[†] *p-value from GEE logistic regression model coefficients*

Table 3. Categorical variables by hearing change group across all visits. Variables with *p*-values <.10 were considered in the multivariable model.

TABLE 4: DPOAE predictor variables according to hearing change

		No Hearing Change					Hearing Change					
		N*	**Mean**	**SD**	**Min**	**Max**	**N***	**Mean**	**SD**	**Min**	**Max**	***p*†**
Difference in the Sum of the Inputs	F_D	56	13.6	126.5	-270.1	343.7	42	110.3	170.2	-176.4	438.5	.04
	F_D -1	72	19.7	110.7	-295.1	335.2	44	30.6	101.2	-355.2	302.0	.38
	F_D -2	69	29.8	121.3	-289.1	301.9	39	27.6	86.8	-139.3	317.2	.67
	F_D -3	66	25.5	144.6	-346.8	376.1	32	14.7	97.4	-100.6	335.6	.40
Difference in the Sum of the Outputs	F_D	56	-0.02	0.15	-0.68	0.23	42	0.15	0.13	-0.19	0.39	<.01
	F_D -1	72	0.003	0.17	-0.37	0.49	44	0.09	0.29	-1.63	0.43	.24
	F_D -2	69	0.09	0.28	-0.68	1.19	39	0.09	0.43	-0.89	1.63	.76
	F_D -3	66	0.13	0.44	-0.95	1.31	32	0.13	0.82	-0.58	3.25	.60
Difference in the Sum of the SNRs	F_D	56	-1.81	11.37	-57.87	10.74	42	6.19	5.26	-3.69	14.30	.03
	F_D -1	72	0.02	8.56	-43.53	22.69	44	0.00	6.34	-19.19	13.14	.25
	F_D -2	69	0.43	6.93	-15.42	17.03	39	-0.63	9.51	-23.01	22.26	.35
	F_D -3	66	2.37	13.86	-64.50	37.56	32	-2.83	17.26	-30.60	43.69	.28

** N is ear-visits*

† *p-value from GEE logistic regression model coefficients*

Table 4. DPOAE variables by hearing change group across all visits. Variables with *p*-values <.10 were considered in the multivariable model.

Table 5: Building the optimal discriminate function

	MODEL 1 Initial DF: Baseline Hearing Variables			MODEL 2 Initial DF: Model 1 + Other Subject Variables			MODEL 3 Optimal DF: Model 2 + DPOAE Variables		
VARIABLE	β	SE	$p^{\dagger}$	β	SE	$p^{\dagger}$	β	SE	$p^{\dagger}$
Baseline Hearing Variables									
Age	.014	.031	.66[1]	--	--	--	--	--	--
PTA at 2, 4, and 6 kHz	-.022	.019	.31[3]	--	--	--	--	--	--
SRO Average Threshold[†]	.711 ***1.057***	.340 ***.205***	.17 ***< .01***	***-3.641***	***1.024***	***< .01***	-3.352 ***-3.504***	1.174 ***1.136***	.01 ***.01***
SRO Upper Frequency Bound	.097	.183	.60[2]	--	--	--	--	--	--
Other Subject Variables									
Cumulative Dose				***.016***	***.003***	***< .01***	.017 ***.018***	.004 ***.004***	<.01 ***< .01***
SRO Average Threshold[††] x Dose				***.014***	***.004***	***< .01***	.013 ***.014***	.004 ***.003***	.03 ***.01***
DPOAE Variables									
I:F_D							.006 ***.007***	.002 ***.002***	< .01 ***< .01***
O:F_D							-.606	4.143	.86[1]
SNR:F_D							.078	.104	.42[2]

[†] p-value from GEE logistic regression model coefficients
[††] Reciprocal of 'SRO Average Threshold' and standardized to a mean of 0 and standard deviation of 1
-- Variables evaluated in a prior model and excluded from further modeling
[1,2, or 3] Indicates order in which variables were removed from model

Table 5. Regression table by predictor variables and organized by the built model. Information includes the regression coefficient (β), standard error of the coefficient (SE), and type 3 *p*-value (*p*) for baseline variables, other subject variables, and DPOAE variables as they were entered into the model. Within each model (1, 2, and 3), the initial parameter estimates and *p*-value is shown for all variables and for the remaining significant variables (*p*-value <.05) and their associated parameter estimates in bold italics.

FIGURES

Figure 1.

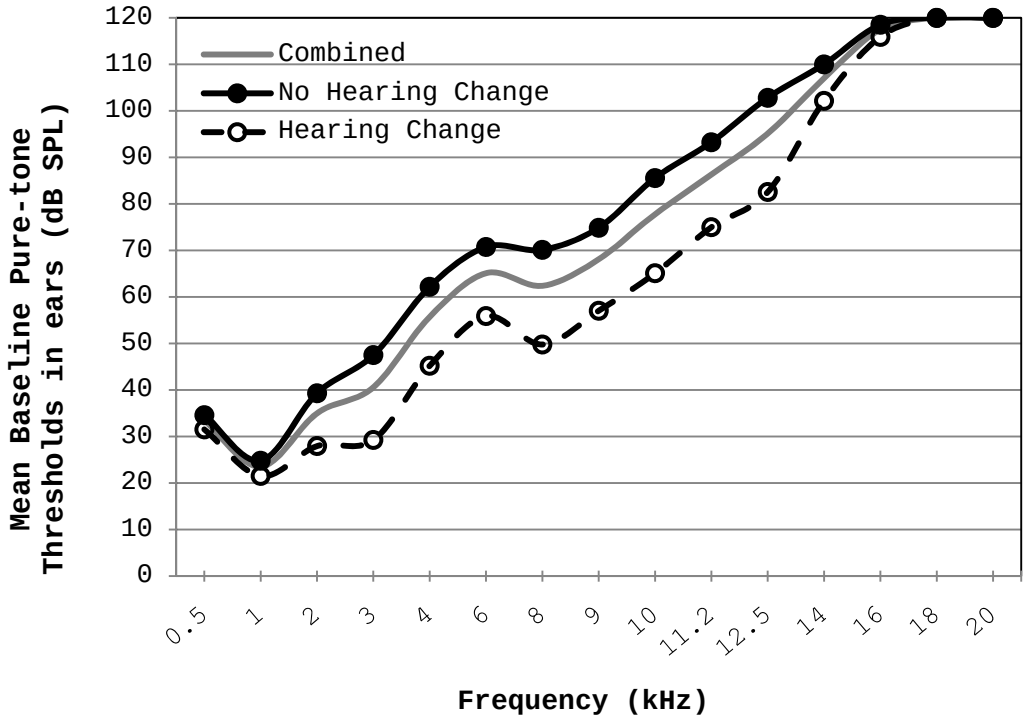

Figure 1. Mean baseline pure-tone thresholds by audiometric frequency. Mean thresholds in dB SPL are given for all ears at baseline (gray solid line), ears that eventually experienced subsequent hearing changes (black dashed line, with open circles), and ears with no hearing changes (black solid line, filled circles). For ease in displaying results, no response was set to 120 dB SPL.

Figure 2.

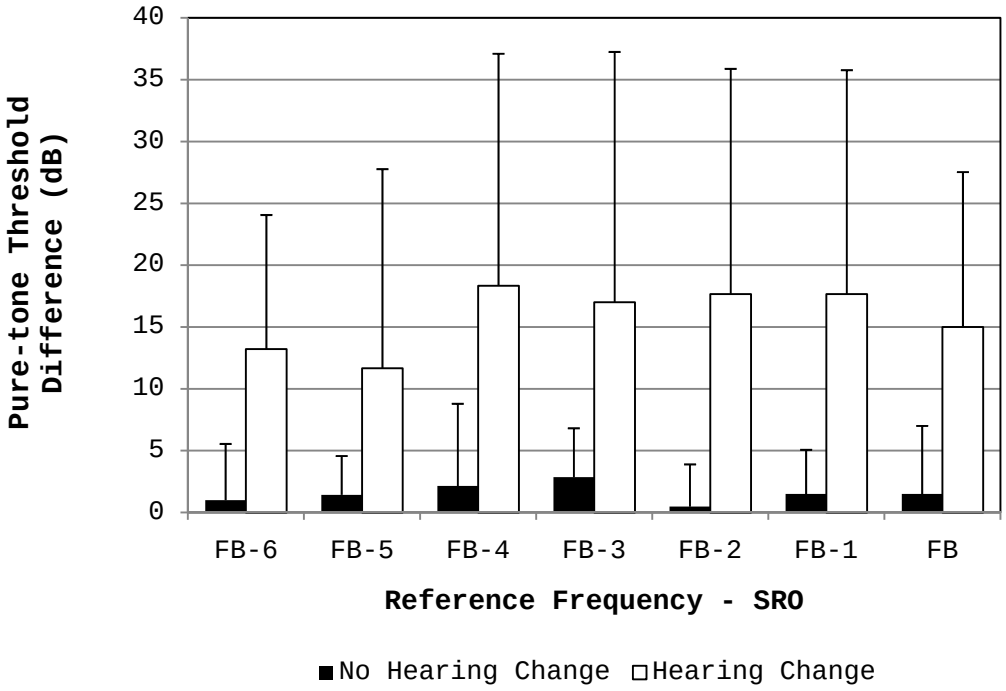

Figure 2. Post-exposure pure-tone threshold shifts by audiometric frequencies from baseline to final test. Amount of hearing change (in dB; y-axis) at the seven SRO normalized frequencies (x-axis) is plotted in Figure 2 by hearing change group in rank order of the lowest frequency, F_B*-6*, to highest frequency, F_B (F_B -1 is 1/6-octave below F_B, F_B -2 is 1/6- octave below F_B -1, and so on). Hearing change was defined according to ASHA (1994). Error bars represent 1 standard-deviation.

Figure 3.

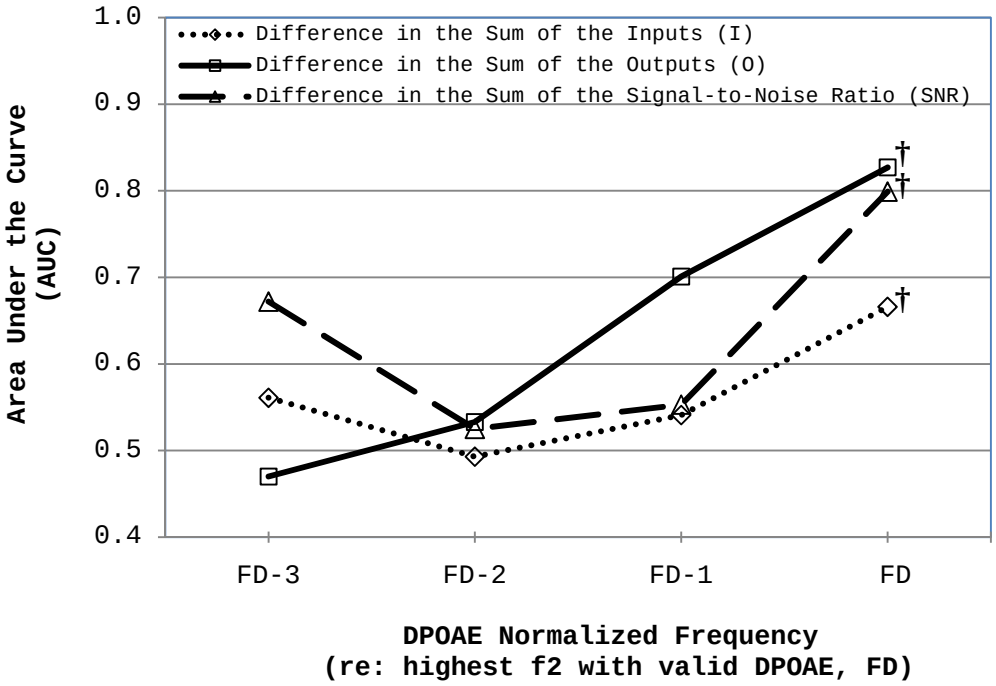

Figure 3. ***Accuracy of OAE metrics as predictors of ototoxicity by DPOAE frequency.*** The area under the ROC curve (AUC; y-axis) is plotted for each DPOAE metric as a function of normalized frequency (x-axis). (†*p-value <.05; derived from GEE logistic regression model coefficients*).

Figure 4.

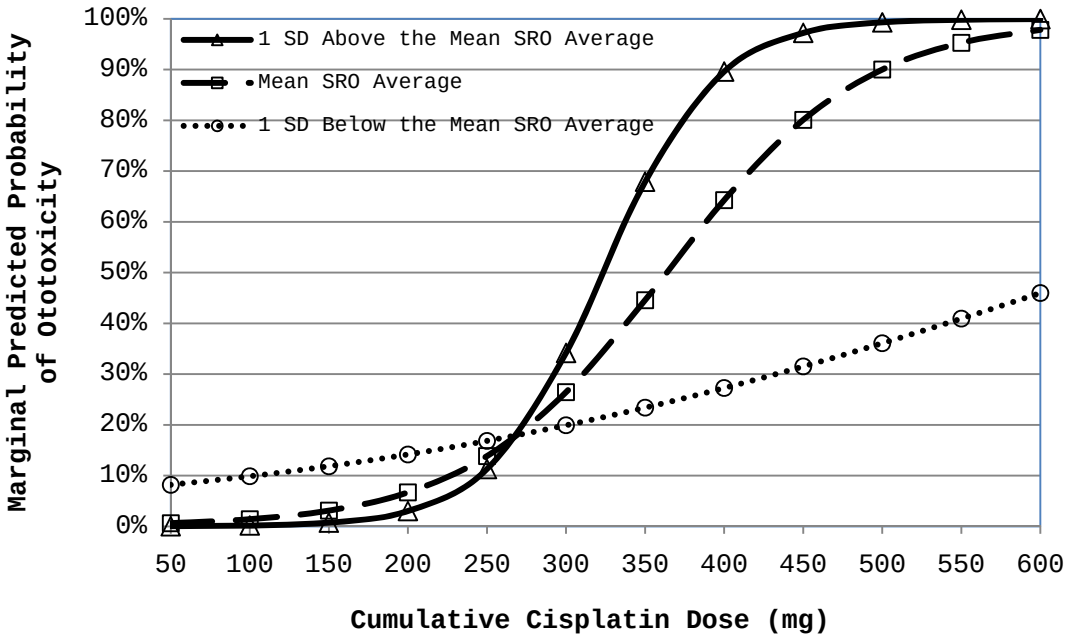

Figure 4. Marginal Predicted probability of ototoxicity by cumulative drug dose and pre-exposure hearing. Figure 4 plots predicted probability of ototoxicity (y-axis) by cumulative drug dose (x-axis), ranging from 100 mg to 600 mg, and stratified by pre-exposure hearing for the mean (dashed line with open squares), one standard deviation below the mean (poorer hearing indicated by the dotted line with open circles) and one standard deviation above the mean (better hearing indicated by solid line with open triangles).

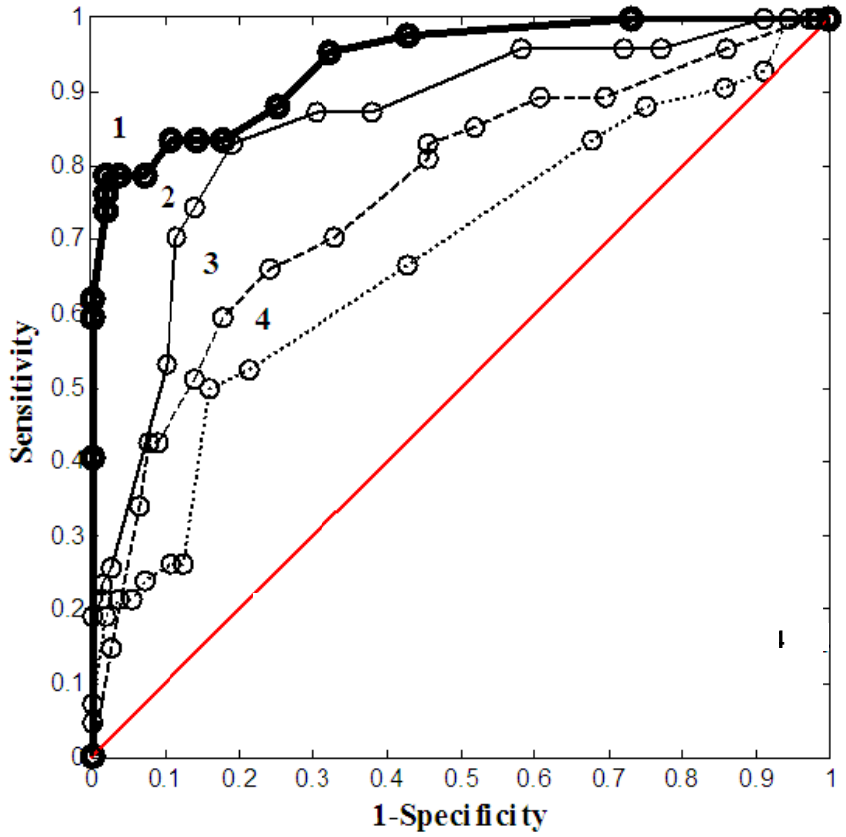

Figure 5. Univariable and multivariable ROC plots. Sensitivity (y-axis) is plotted by 1-Specificity (x-axis) for the independent variables in the discriminate function and for the optimal discriminate function (DF).