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Chirp-Evoked Otoacoustic Emissions and Middle Ear Absorbance for Monitoring Ototoxicity in Cystic Fibrosis Patients

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Abstract

Objectives—The goal of this study was to investigate the use of transient-evoked otoacoustic emissions (TEOAEs) and middle ear absorbance measurements to monitor auditory function in patients with cystic fibrosis (CF) receiving ototoxic medications. TEOAEs were elicited with a chirp stimulus using an extended bandwidth (0.71 to 8 kHz) to measure cochlear function at higher frequencies than traditional TEOAEs. Absorbance over a wide bandwidth (0.25 to 8 kHz) provides information on middle ear function. The combination of these time-efficient measurements has the potential to identify early signs of ototoxic hearing loss.

Design—A longitudinal study design was used to monitor the hearing of 91 patients with CF (median age = 25 years; age range= 15 to 63 years), who received known ototoxic medications (e.g., tobramycin) to prevent or treat bacterial lung infections. Results were compared to 37 normally hearing young adults (median age= 32.5 years; age range= 18 to 65 years) without a history of CF or similar treatments. Clinical testing included 226-Hz tympanometry, pure-tone air-conduction threshold testing from 0.25 to 16 kHz and bone conduction from 0.25 to 4 kHz. Experimental testing included wideband absorbance at ambient and tympanometric peak pressure (TPP), and TEOAEs in three stimulus conditions: at ambient pressure and at TPP using a chirp stimulus with constant incident pressure level across frequency, and at ambient pressure using a chirp stimulus with constant absorbed sound power across frequency.

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D. H. Keefe is involved in commercializing new technology for screening and diagnosis of middle ear and cochlear disorders.

Results—At the initial visit, behavioral audiometric results indicated that 76 of the 157 ears (48%) from patients with CF had normal hearing, whereas 81 of these ears (52%) had sensorineural hearing loss (SNHL) for at least one frequency. Seven ears from four patients had a confirmed behavioral change in hearing threshold over three or more visits during study participation. Receiver operating characteristic (ROC) curve analyses demonstrated that all three TEOAE conditions were useful for distinguishing CF ears with normal hearing from ears with SNHL, with area under the ROC curve values ranging from 0.78 to 0.92 across methods for frequency bands from 2.8 to 8 kHz. Case studies are presented to illustrate the relationship between changes in audiometric thresholds, TEOAEs, and absorbance across study visits. Absorbance measures permitted identification of potential middle ear dysfunction in an ear that exhibited a temporary hearing loss.

Conclusions—The joint use of TEOAEs and absorbance has the potential to explain fluctuations in audiometric thresholds due to changes in cochlear function, middle ear function, or both. These findings are encouraging for the joint use of TEOAE and wideband absorbance objective tests for monitoring ototoxicity, particularly in patients who may be too ill for behavioral hearing tests. Additional longitudinal studies are needed in a larger number of CF patients receiving ototoxic drugs to further evaluate the clinical utility of these measures in an ototoxic monitoring program.

B. Introduction

Cystic fibrosis (CF) is a life-threatening genetic disorder that affects approximately 30,000 people in the United States and 70,000 worldwide (CFF 2014). Aggressive treatment using inhaled or intravenous (IV) aminoglycoside (AG) antibiotics, such as tobramycin, has become an accepted standard of care for the management of CF-related pulmonary bacterial infections (e.g., *Pseudomonas aeruginosa*) (Veenstra et al. 2007). Ototoxicity is a potential side effect of AG exposure, particularly in adult CF patients who have received multiple IV treatments over their lifetime (Mulheran et al. 2001). AG-induced ototoxicity is typically characterized by damage to the cochlear hair cells resulting in permanent sensorineural hearing loss (SNHL). This insult follows a base-to-apex gradient, with initial damage occurring to hair cells in the basal region of the cochlea that are important for the detection of high frequency sounds (Brummett 1980; Komune et al. 1981; Nakai et al. 1982; Konishi et al. 1983; Schweitzer et al. 1984). The literature suggests the incidence of AG-induced ototoxic hearing loss ranges from 0 to 47% in adults and from 0 to 24% in children (Fausti et al. 1992; Mulheran et al. 2001; Cheng et al. 2009; Martins et al. 2010; Al-Malky et al. 2011; Al-Malky et al. 2015). This wide variation in incidence is likely due to dissimilar testing methodologies, different study populations, genetic predispositions, variable regimens of drug administration, and duration of treatment.

Routine audiologic monitoring for ototoxicity is not yet commonplace in CF centers worldwide. Recent studies have reported that 26% of centers in the United States, 13% of centers in the United Kingdom, and 15% of centers in Australia regularly monitor CF patients for hearing loss (Phillips and Bell 2001; Tan et al. 2003; Van Meter et al. 2009), often using behavioral audiometry in the conventional frequency range of 0.25 to 8 kHz as the sole monitoring tool. The conventional range is important for speech understanding, and

hearing loss detected in this frequency range indicates permanent damage has already occurred in the cochlea that is likely affecting the patient's ability to understand speech.

According to the American Speech Language and Hearing Association guidelines (ASHA 1994), a significant increase in hearing thresholds ("ASHA shift") in hearing may include:

20 dB HL air-conduction threshold shift at one frequency, 10 dB HL shift at two adjacent frequencies, or thresholds shifting from a response to no response at three consecutive frequencies. The ASHA guidelines also specify that a shift must be confirmed at a follow-up test visit in order to be considered a true change in hearing (i.e., confirmed ASHA shift). ASHA and the American Academy of Audiology (AAA) have established guidelines for developing an effective ototoxic monitoring program. These guidelines, consistent with recommendations found in the literature, (e.g., Fausti et al. 1993; Ress et al. 1999; Fausti et al. 1999; 2003; Knight et al. 2007; Konrad-Martin et al. 2014), recommend serial monitoring using extended high-frequency (EHF) (>8 kHz) behavioral audiometry combined with a physiologic (objective) measure of auditory function, such as otoacoustic emissions (OAEs), at baseline and during routine follow-up visits (ASHA 2013; AAA 2009). If an ASHA shift in hearing or a significant physiologic change (e.g., OAE level shift) is evident during monitoring, then the patient and their physician should be notified immediately to discuss treatment and care options.

Unfortunately, completing this comprehensive test paradigm can be lengthy and unrealistic for some patients in an ototoxic monitoring program, particularly if they are too ill to provide reliable behavioral responses. For example, studies of patients receiving cisplatin for cancer treatment, another class of ototoxic drug, report 30 to 41% of study participants became untestable or had unreliable behavioral responses due to side effects associated with illness and/or medication regimens (Fausti et al. 1991; Fausti et al. 1992; Dille et al. 2010; Konrad-Martin et al. 2014). A similar situation occurs with CF patients receiving ototoxic drugs for lung infections. A physiologic assessment using OAEs is preferred in these instances because a behavioral response is not required from the patient, and valuable information about cochlear function may be obtained (Konrad-Martin et al., 2014).

A double-evoked, transient OAE elicited using acoustic chirps (TEOAEs) or clicks (CEOAEs) presented in the ear canal has shown promise for distinguishing normal hearing (NH) from SNHL (Keefe et al. 2011; Putterman et al. in press). This procedure measures a nonlinear residual TEOAE that arises due to the compressive nonlinearity of cochlear mechanics to frequencies higher than are available in clinical TEOAE systems (Goodman et al. 2009). TEOAEs and CEOAEs have similar properties across stimulus conditions, as long as the stimuli have the same energy spectrum and that the effects of any stimulus phase differences are removed from the TEOAE response (Keefe et al. 2016a). Both measures are thought to be generated by the linear coherent reflection mechanism within the cochlear tonotopic region (Shera and Zweig 1993; Zweig and Shera 1995; Shera and Guinan 1998; Kalluri and Shera 2013; Keefe et al. 2016a). High-frequency TEOAE measurements may be particularly useful when monitoring for changes in high-frequency cochlear function, such as in ototoxic monitoring.

Middle ear conditions, such as negative middle ear pressure or otitis media, may affect the presence of evoked OAEs by reducing the stimulus level as well as the emission amplitude (e.g., Kemp et al. 1990; Lonsbury-Martin and Martin 1993). Sun (2012) showed that negative middle ear pressure of -40 to -425 daPa resulted in the largest reduction in distortion-product OAE (DPOAE) levels at frequencies below 1 kHz, but DPOAE levels were also reduced at frequencies from 2 to 6 kHz. Marshall et al. (1996) found that fluctuations in negative middle ear pressure ranging from -105 to -165 dekaPascals (daPa) affected CEOAE spectra, and that compensation for negative middle ear pressure by pressurizing the ear canal corrected this reduced amplitude effect for frequencies between 1.5 to 2 kHz consistent with other reports (Naeve et al. 1992; Trine et al. 1993; Sun 2012).

The effect of middle ear pressure may be particularly important when testing patients with CF because the build-up of mucous in the lungs can cause frequent clearing of the throat, coughing, and wheezing that may alter middle ear pressure and impedance across tests. A physiologic test battery that includes wideband absorbance and OAE tests at ambient and tympanometric peak pressure may provide a longitudinal assessment of cochlear function that is corrected for middle ear fluctuations over time in the frequency range of the OAE response.

The present study was part of a multi-year investigation evaluating a wideband acoustic immittance and TEOAE test battery for the assessment of auditory function across the age range. The current investigation describes the application of TEOAE tests to monitor auditory function in CF patients receiving ototoxic IV-AG treatments, while partially controlling for differences in middle ear function across tests using data obtained from absorbance testing.

C. Materials and Methods

1. Participants

Ninety-one participants (43 females and 48 males) with CF were recruited from the Oregon Health & Science University (OHSU) pediatric and adult CF centers. Participants were enrolled into the study if they had a history of or were currently treated with inhaled or IV-AGs. The median age was 25 yrs., with a range of 15 to 63 yrs. CF participants were excluded from data analyses if they had abnormal 226-Hz tympanometry consisting of peak-compensated static acoustic admittance (Y_{tm}) <0.3 mmho or >1.7 mmho, or air-bone gaps (ABGs) >10 dB from 0.25 to 2 kHz, or >15 dB at 4 kHz. This initial inclusion criterion for middle ear function in the normal range placed all participants in the longitudinal study at a common baseline. There was no ear canal equivalent volume or TPP criterion for CF patients.

Seventeen test visits from CF ears were excluded from group analyses because they did not meet the 226-Hz tympanometric inclusion criteria, and eight different ear visits were excluded due to equipment issues (Total= 25 ears excluded). These criteria resulted in the complete exclusion of data from six CF participants (12 ears), and excluded tests in 13 ears from 13 different participants. Final data analyses used two ears from 72 participants (144 ears) and one ear from 13 participants, resulting in 85 participants and 157 ears contributing

to the final dataset. These ears were evaluated for an ASHA shift at each follow-up test visit, or a confirmed ASHA shift for ears with 3 visits (see Introduction for a description of ASHA guidelines).

Control participants without CF were recruited from OHSU campus and clinics to establish normal test-retest variability for wideband absorbance and TEOAEs. These participants consisted of 37 adults (25 females and 12 males), with NH (<25 dB HL for 0.25 to 8 kHz) and a negative history of AG treatments. The median age was 34 yrs. with a range of 18 to 65 yrs. Data from control participant ears were excluded from analyses if there was abnormal 226-Hz tympanometry consisting of $Y_{tm} < 0.3$ or > 1.7 mmho, and TPP $> +100$ or < -100 daPa. Control data were also excluded if the ear had ABGs > 10 dB from 0.25 to 2 kHz, or > 15 dB at 4 kHz. Following the exclusion criteria, 18 ears did not meet the middle ear or ABG criteria for at least one visit, therefore 56 of 74 ears were used for data analyses.

2. Equipment

2.1 Clinical Systems—A Grason Stadler Instrument (GSI) model 61 audiometer calibrated to ANSI standards (S3.6-1996) was used to measure pure-tone air conduction and bone conduction thresholds. A GSI Tymstar V2 calibrated to ANSI standards (S3.39-R2007) was used to measure clinical immittance at 226 Hz.

2.2 Wideband Research System—An Interacoustics Wideband Research System was used with custom software to collect experimental data on ear-canal acoustic absorbance and admittance at the probe tip using procedures described in Keefe et al. (2015), and TEOAE data using procedures described in Keefe et al. (2016a, 2017). This system included a Windows computer with a two-channel sound card (CardDeluxe) and bi-directional serial port (RS-232), which was coupled to a modified AT235 tympanometer (Liu et al. 2008). The sound card had two channels for digital-to-analog conversion (DAC) to drive the pair of receivers, and a channel for analog-to-digital conversion to record the microphone output with a sample rate of 22.05 kHz. The tympanometer consisted of an air pump, controller, and a custom ear-level probe containing two receiver ports and one microphone port. The tympanometry pump and controller allowed computer control of air pressure generated by the pump via a serial port.

3. Procedures

All study materials and test protocols were approved by the OHSU Institutional Review Board. Informed written consent was obtained at the participant's clinic visit or at the beginning of the first scheduled test session. Parental consent was obtained for CF participants between 15 to 17 years of age. Participants within this age group co-signed the consent form to provide assent to the test procedures and protocol. Control participants completed two visits approximately one-month apart. CF participants returned for follow-up visits, when possible, at ~3 to 6 month intervals for up to 2.2 years. Clinical testing was completed first during each study session. Participants who met the clinical test inclusion criteria (see Participant's section) were then tested with the experimental wideband test battery. The same tests were completed in the same order for both the CF and control participants. All clinical and experimental testing was completed at OHSU facilities in a

sound-treated booth located in either the Otolaryngology Clinic or the Oregon Hearing Research Center.

3.1 Clinical Tests—The following clinical tests of auditory function were conducted using the standard clinical equipment described above: 1) tympanometry with a 226-Hz probe tone; 2) pure-tone air conduction audiometry in the conventional frequency range (0.25, 0.5, 1, 2, 3, 4, 6, 8 kHz) using Etymotic Research ER-3A insert earphones; 3) pure-tone air conduction audiometry in an extended HF range (9, 10, 11.2, 12.5, 14, 16 kHz) using Sennheiser HDA-200 circumaural headphones; and 4) pure-tone bone-conduction audiometry (0.25, 0.5, 1, 2, 4 kHz) using a model B71 bone oscillator.

3.2 Wideband Research System

3.2.1 Wideband Absorbance Tests: Following a wideband calibration procedure over a frequency range from 0.25 to 8 kHz (Liu et al. 2008), the probe was inserted securely in the participant's ear canal. Using procedures described in Keefe et al. (2015), wideband pressure reflectance and admittance at the probe tip were obtained in the ear canal from the response to a series of clicks presented over seconds using time averaging. These measurements were made at ambient pressure and as a wideband tympanogram with pressure varying from +200 daPa to −200 daPa.

For ambient absorbance (Aa) measurements, the air pressure in the ear canal was adjusted to ambient pressure by the pump circuit after the probe was inserted into the ear canal and prior to any measurements. Aa was calculated as one minus the squared magnitude of the pressure reflectance of the ear at the probe tip. Because ear-canal wall losses are small, this Aa at the probe tip is assumed identical to the Aa of the middle ear at the tympanic membrane. This absorbance varied from a value of “0” with no power absorbed by the ear to a value of “1” when the ear absorbed all the sound power from the incident click. The ambient acoustic admittance was also calculated at the probe tip, and the acoustic conductance was obtained as the real part of this admittance. This information was used to calculate power weighting for chirp stimuli as described below. The absorbed sound power of the ear was mainly due to power absorption by the middle ear at the tympanic membrane, such that only negligible amounts of sound power were absorbed at the ear-canal walls in the TEOAE bandwidth of interest (above 0.71 kHz) (Keefe et al. 2017).

Pressurized measurements of reflectance were interpreted in the present study in terms of an absorbance tympanogram. The pressure in the ear canal was varied from maximum to minimum between ± 200 daPa in a downswept absorbance tympanogram (AtD), or from ± 200 daPa in an upswept absorbance tympanogram (AtU). A nominal sweep rate of 100 daPa/s was used, for which the pressure in the ear canal changed by 3.2 daPa between clicks. This was brief enough to characterize the air pressure as quasi-stationary over the inter-click interval, which was desirable for interpreting wideband tympanograms. Detailed procedures to measure ambient and tympanometric absorbance and admittance are described in Keefe et al. (2015). The AtD tympanogram was the initial test in the battery. It was effective in confirming that the probe insertion was leak-free based on the ability to initially pressurize the ear canal up to +200 daPa. The ambient and up-swept wideband tympanometric tests

followed. All TEOAE tests were performed immediately after these tests without further adjustment of the probe (unless the test was paused or some problem, such as a leaky seal in the ear canal, was observed).

3.2.2 TEOAE tests: Measures of cochlear function consisted of double-evoked TEOAEs that were measured at 1/6th-octave frequency bands from 0.71 to 8 kHz at ambient pressure (Keefe et al. 2016a) and at TPP (Putterman et al. in press), and at ambient pressure with a power-weighted chirp (Keefe et al. 2017). In approximately 20% of the initially tested control ears and CF ears, the chirp stimulus bandwidth was extended from 8 to 10 kHz with a similar chirp sweep rate and the same stimulus peak-to-peak equivalent sound pressure level (peSPL). The peSPL is a relevant stimulus level to normalize across TEOAE measurement types and was determined in a short-duration measurement prior to the main TEOAE measurement. Data from these ears with the 10 kHz stimulus bandwidth were included in the study, but analyzed only over the reference bandwidth from 0.71 to 8 kHz.

Preliminary findings showed that the sound sources produced insufficient sound energy above 8 kHz. The test protocol was modified early in the data collection process so that all subsequent TEOAE tests used the chirp stimuli with a maximum frequency of 8 kHz. Because the peSPL was adjusted to the same value for both chirp stimuli, the spectral density magnitudes generated by the 10 kHz bandwidth stimulus were slightly smaller (by 1 dB) than those generated by the 8 kHz bandwidth stimulus. This complication arose in testing both control and CF ears, and it is assumed that any TEOAE level differences due to this effect, which were on the order of 0.4 dB due to the compressive nonlinearity of cochlear mechanics, were sufficiently small to neglect compared to the TEOAE level differences in ears with NH versus ears with SNHL.

The TEOAE stimulus sequence included repeated presentations of a stimulus set comprised of three equal-duration chirp stimuli, each having a duration of 1536 samples. The duration of the stimulus set was 209 ms. Each stimulus set consisted of a first chirp generated by DAC1 at a reference output voltage level, a second chirp generated by DAC2, and the simultaneous presentation of both chirps by DAC1 and DAC2. The stimulus levels in each DAC were the same within the stimulus sequence. The chirp in DAC2 was the same waveform as the chirp in DAC1 except that the DAC2 chirp was presented at an output voltage level 12 dB higher than the reference output voltage level of DAC1. Based on in-the-ear measurements, the reference output voltage level produced an average sound pressure level (SPL) and peSPL for each stimulus set with values listed in Table 4. The mean stimulus SPL and peSPL in CF ears in this study were within 0.8 dB of the mean SPL and peSPL in NH adult ears in the TEOAE study of Putterman et al. (in press), which used the same stimuli at the same reference output voltage level applied using the same measurement system and probe.

A TEOAE residual was calculated for the response to each stimulus set by subtracting the response to the third stimulus from the sum of the responses to the first and second stimuli. This residual differed from zero due to the presence of a compressive nonlinearity in the cochlear mechanics in an ear with normal function. It also included measurement noise from the system and any sources of biological and environmental noise. Each chirp application

used 288 buffers split into 16 time-averaged blocks (18 buffers for each block) over a one-minute duration of measurement. Each block was analyzed in the $1/6^{\text{th}}$ octave frequency domain for 0.71 to 8 kHz. The so-called positive chirp waveform swept linearly with increasing time from low to high frequencies (Keefe et al. 2016a).

TEOAE (residual) responses were evaluated by examining the signal-to-noise ratio (SNR) and coherence synchrony measure (CSM) in $1/2$ octave frequency bands for comparison to audiometric data. CSM is a phase relationship detection criterion quantifying the degree of synchrony between the TEOAEs and chirp stimuli. The degree of synchrony varies between “0” (asynchronous relationship) and “1” (completely synchronous relationship) (Keefe 2012; Valdes et al. 1997). The CSM provided a phase-sensitive measure of TEOAE strength, while the SNR provided a magnitude-sensitive measure. Putterman et al. (in press) provides chirp-evoked TEOAE normative data for both SNR and CSM for the same TEOAE conditions reported in the current study.

3.2.2.1 Ambient TEOAE: The ambient pressure TEOAE was generated using a flat stimulus chirp spectrum, where “flat” means that the incident pressure spectrum level measured in a reflection-less tube was approximately constant across frequency. The ambient TEOAE was the first test completed in the TEOAE test sequence for each study participant.

3.2.2.2 TPP TEOAE: TEOAEs were recorded at the TPP measured for the absorbance tympanograms for each test ear. The stimulus eliciting the TPP TEOAE was the same flat chirp stimulus used in the ambient TEOAE test. Following Keefe et al. (2015), the TPP was derived from the low-frequency (0.376 to 2 kHz) absorbance averaged across measurements from the downswept and upswept absorbance tympanograms to compensate for any hysteresis due to sweep direction (see Liu et al., 2008; Feeney et al. (in press) for additional information). Air pressure during testing was monitored by a sensor located within the tympanometry cable between the pump and the probe. If air pressure varied more than 10 daPa, then the test was automatically paused and the pressure was re-calibrated to its target value. TEOAE testing at TPP was then restarted at the beginning of each 6-s recording interval until all data were acquired. The operator also had the option of stopping and re-starting the entire test if determined necessary.

3.2.2.3 Power-Weighted TEOAE: TEOAEs were next recorded using a power-weighted chirp technique at ambient pressure in the ear canal. This test method used a sound stimulus with an equal level of sound power absorbed by the ear across frequency to generate a TEOAE response (Keefe et al. 2017). The TEOAE stimulus was calculated in terms of the measured ambient conductance $G(f)$ at each frequency f at the probe tip (conductance is the real part of the admittance at the probe tip). Group results for conductance level, which is calculated as ten times the common logarithm of the conductance $G(f)$, are shown for normal-hearing adults (and infants) in Keefe et al. (1993), and replicated for adults in Keefe et al. (2017).

For a mean-squared magnitude of ear-canal pressure $|p(f)|^2$ measured in response to the flat chirp, the corresponding sound power $W(f)$ absorbed by the ear was $W(f) = G(f) \times |p(f)|^2$. Because the chirp waveform had a local frequency that varied linearly with time during the

waveform, it was possible to assign to each time a local frequency, and to assign the conductance that was measured during the earlier measurement of wideband admittance to that local frequency. The effect of the frequency variation in conductance was cancelled out by multiplying the DAC voltage waveform that produced the flat chirp sound in the ear canal by the inverse square root of the conductance at the local frequency corresponding to that sample in time. The resulting squared pressure in the ear canal from this new chirp would then have a factor of conductance in the denominator. This produced a constant absorbed sound power across frequency in the ear. The new power-weighted chirp was scaled in amplitude to generate the same in-the-ear peSPL as was measured in the ear for the flat chirp in an initial 7.5 s calibration, prior to the main TEOAE test of one-minute duration. The data in the initial 7.5 s calibration were discarded. The resulting power-weighted chirp had constant absorbed sound power in the TEOAE test. To reduce the effect of a potential amplitude modulation (or distortion), conductance smoothed over each one-sixth octave frequency band was used to calculate the stimulus rather than the conductance at each frequency of the spectrum (calculated using a 1536 sample discrete Fourier transform). Any conductance value smaller than 0.5 mmho was set equal to 0.5 mmho to avoid any numerical problems in calculating the new chirp (although this was not a concern in practice). Keefe et al. (2017) provides a detailed review of the power-weighted chirp stimulus design, the accompanying TEOAE test procedures, and normative TEOAE results in adult ears.

3.4 Data Analyses—Descriptive statistics were used to characterize the data from visit 1 in terms of participant, age, gender, and presence or absence of SNHL. An independent t-test was used to compare mean differences between CF and control ears for clinical 226-Hz tympanometric data. A repeated-measures analysis of variance (RM-ANOVA) was conducted to determine differences for the factors group (CF ears with NH or CF ear with SNHL), absorbance method (ambient or pressurized) and frequency (half-octave frequencies from 1 to 8 kHz). A separate RM-ANOVA was conducted to identify group and method (ambient, TPP and power-weighted) differences for mean TEOAE responses in SNR. Receiver operating characteristic (ROC) curve analyses were used to determine the performance of each TEOAE method in SNR and CSM to identify SNHL in CF ears at each $\frac{1}{2}$ octave frequency band of the TEOAE closest to their audiometric frequency ranging from 1 to 8 kHz.

TEOAE SNRs for CF ears with NH and SNHL were compared statistically using a multivariate-T testing adjustment to estimate p-values for each individual contrast. Ninety-percent test-retest reference limits were estimated using the control ears with NH to determine if a shift (i.e., a decrease) in TEOAE SNR or absorbance for CF ears exceeded the normal range of variability. These reference limits are used in the Results to interpret responses in the four case studies of CF ears tested over multiple visits. If a particular absorbance test in a CF ear was outside this range, it was interpreted as a high risk for conductive hearing loss. If a particular TEOAE test in a CF ear was outside this range, it was interpreted as a risk for hearing loss (SNHL or CHL). If both TEOAE and absorbance responses were outside the normal range, then it was interpreted as a high risk for significant increases in hearing threshold (i.e., ASHA shift) according to non-invasive physiological tests of cochlear function and middle ear function.

D. Results

1. Clinical Test Results

1.1 Audiometric Test Results—Ninety-one CF participants (157 ears) with useable data were evaluated in this study. Table 1 classifies the number of CF ears with NH or SNHL for each pure-tone air conduction frequency from 0.25 to 16 kHz at baseline (visit 1). Seventy-six of 157 ears (48%) had NH (≤ 20 dB HL) across the entire frequency range of 0.25 to 16 kHz. Eighty-one of 157 ears (52%) had SNHL for at least one frequency (>20 dB HL) in this same frequency range. One hundred and twenty nine of 157 ears (82%) had at least one follow up visit, with up to seven total visits. Table 2 provides a summary of the number of ears with useable data across test visits for which the inclusion criteria were met and no equipment issues arose. Thirty-seven control participants (56 ears) met the inclusion criteria for NH in the conventional range of hearing (0.25 to 8 kHz), in whom test-retest data were acquired.

ASHA shifts in hearing for CF ears were evaluated using the 1994 ASHA guidelines, described in the Introduction. For the present study, a confirmed ASHA shift required that the participant had at least one baseline visit and two follow-up visits. Figure 1 provides a flow chart of CF ears tested in this study, as well as a breakdown of the number of ears that provided useable data across visits. Visit 1 is the baseline visit, and each visit afterwards was considered a follow-up or monitoring visit. Figure 1, Box A shows that 91 participants (182 ears) were initially tested, of which 157 ears (Figure 1, Box D) had baseline data and 129 ears had ≥ 2 visits (Figure 1, Box F). Given the ASHA criteria described above, 87 ears did not have ASHA shifts (Figure 1, Box G) and 42 ears had ASHA shifts (Figure 1, Box H). Of these 42 ears, 18 ears had tests for ≥ 3 visits (Figure 1, Box J). Of those 18 ears, seven ears had a confirmed ASHA shift at all follow up visits (Figure 1, Box L). One ear had a shift in the extended HF region only, four ears had a shift in the conventional region only, and two ears had a shift in both frequency regions (Figure 1, Box K). The remaining 11 of 42 ears did not have confirmed ASHA shifts, because their hearing thresholds ‘recovered’ or returned to baseline hearing levels at their following visit (Figure 1, Box M).

1.2 Clinical tympanometric results—All participants included in the analyses met the 226-Hz inclusion criteria described in the Methods. Table 3 provides descriptive information about the mean estimates for ear canal volume, middle ear pressure, and static acoustic admittance magnitude for the CF group at visit 1. Middle ear pressure ranged from -125 to $+85$ daPa with a mean of $+7.2$ daPa (standard deviation, SD = $+20$ daPa).

2. Experimental Test Results

2.1 Wideband Absorbance Results—A secondary goal of this study was to contrast the information that an *Aa* measurement reveals about middle ear function relative to that in a tympanometric absorbance (*AtD* or *AtU*) measurement. Some aspects of middle ear function are described by changes in an ambient response across frequency, whereas the wideband tympanometric measurement captures changes across frequency as well as changes related to an air pressure difference across the tympanic membrane (Feeney et al., in press). Comparing the potential middle ear differences between CF ears and control ears is

relevant, partly due to the high incidence of congestion in the respiratory tract, i.e., the wheezing breathing issues that CF patients experience. We combined all CF ears with NH and SNHL and compared them to control ears for this comparison. This information is particularly valuable when considering a TEOAE test that may compensate for middle ear pressure fluctuations during recording. Evidence suggests that negative middle ear pressure reduces TEOAE response levels (dB SPL) (Marshall et al., 1997).

Absorbance results showed that *AtD* had similar results to the *AtU* condition; therefore, only *AtD* is contrasted with *Aa* results. A three-way repeated measures ANOVA model was used to contrast CF and control groups across absorbance methods (*Aa* and *AtD*) and frequencies. There was a statistically significant three-way interaction [$F(10, 213) = 202.0, p=0.04$], which indicates that the mean absorbance differences between CF and control subjects depended on method (*Aa* or *AtD*) and frequency. Specifically, CF and control ears had lower mean absorbance at frequencies below 1.5 kHz in the *Aa* condition, compared to the *AtD* condition. For *Aa*, CF ears exhibited lower mean absorbance compared to control ears from 0.35 (CF= 0.17; Control= 0.19) to 0.71 kHz (CF= 0.39; Control= 0.44), and again at 8 kHz (CF= 0.37; Control= 0.41). For *AtD*, responses were similar across all frequencies for both groups, except the CF ears had slightly lower mean absorbance at 8 kHz.

2.2 TEOAE Results—Results are described below for three different types of TEOAE measurements under a common condition in which the peSPL was maintained as approximately constant calculated over the response to the three equal-duration chirp stimuli in a TEOAE stimulus set. Results were obtained to check how similar the peSPLs were across the three conditions in the final measurements, and to assess the stimulus SPL across the three conditions. Table 4 shows the mean and SD of the TEOAE stimulus SPL and peSPL for all CF ears at the first visit across the three TEOAE methods: flat-weighted at ambient pressure, flat-weighted at TPP, and power-weighted at ambient pressure. This stimulus SPL was calculated over the response to the three equal-duration chirp stimuli comprising a single TEOAE stimulus set, which was repetitively output by the two DACs. The mean SPL for the power-weighted chirp stimulus (67.1 dB with standard deviation (SD) of 1.2 dB) was approximately 2 dB greater than both the mean SPLs of the ambient (65.1 dB with SD of 1.3 dB) and TPP condition (65.2 dB, SD=1.4 dB). The mean peSPL was similar for the three stimulus conditions at around 77.0 dB peSPL (Table 4).

Figure 2 displays box and whisker plots for SNR (upper panels) and CSM (lower panels) for each TEOAE method in both NH and SNHL groups of CF ears at the first visit with useable data. This visit was one in which the clinical and experimental data inclusion criteria were met (see Participants section). Boxplots for each ½-octave frequency band are shown for CF ears with NH (white boxes) or SNHL (gray boxes) at TEOAE frequency bands closest to their audiometric frequency from 1.0 to 8 kHz (see Table 1 for the counts in CF ears of the numbers of NH and SNHL ears within each audiometric frequency band). In these CF ears, the TEOAE SNR and CSM showed clear differences between ears with SNHL and ears with NH at each frequency. CSM had slightly larger differences between groups at higher frequencies (>2 kHz) in the power-weighted condition compared to SNR.

TEOAE SNRs for CF ears with NH and SNHL were compared statistically. There were significant differences in TEOAE SNR between NH and SNHL ears for CF participants at each frequency under each method ($p < 0.01$), except at 2 kHz for TPP ($p = 0.092$). The CF ears with SNHL exhibited significantly smaller SNRs across TEOAE methods and frequencies compared to NH CF ears (see Figure 2, top row). For the ambient condition (left panel), the mean SNRs for NH ears were smallest at 2 kHz (9.4 dB, SD= 5.2 dB) and largest at 4 kHz (15.1 dB, SD= 7.2 dB). In contrast, mean SNRs for ears with SNHL were smallest at 1 kHz (1.4 dB, SD= 1.2 dB) and largest at 5.7 kHz (6.6 dB, SD= 5.9 dB). Results for the TPP TEOAE condition (middle panel) were similar to the ambient condition for both NH and SNHL ears. The power-weighted TEOAE condition (right panel) had larger *mean* responses than the other two conditions for both NH and SNHL ears. For example, the mean SNRs for the power-weighted condition in NH ears ranged between 11.4 to 18.6 dB across frequency bands. This was in contrast to the range of mean SNRs in the ambient unweighted (9.4 to 15.1 dB) and TPP conditions (8.7 to 14.6 dB). A similar pattern was evident for SNHL ears across TEOAE methods.

2.3 ROC analyses—ROC analyses were used to determine the performance of each TEOAE method to identify SNHL in CF ears at the $\frac{1}{2}$ octave frequency band of the TEOAE closest to each audiometric frequency between 1 to 8 kHz. Figure 3 shows the non-parametric area under the ROC curve (AUC). The AUC is an indicator of diagnostic performance representing the average value of sensitivity for all the possible values of specificity. An ANOVA was used to compare AUC results for TEOAE SNR and CSM responses across frequency that were measured in the ambient, TPP and power-weighted conditions. AUCs were compared to determine if they were statistically different across methods (DeLong et al. 1988). The frequency bands 4 kHz had 10 or fewer SNHL ears (Table 1), so the AUC results at these frequencies were not considered to avoid over-interpretation of data. AUCs for SNRs were 0.80 for all three TEOAE methods at 5.7 kHz, and were 0.85 or larger for TEOAE SNRs at 8 kHz. A similar trend was shown for the CSM data. There were no significant differences at the $p < 0.05$ level when contrasting TEOAE methods for TEOAE SNR or CSM at 5.7 or 8 kHz.

The limited scope of these AUC analyses is a consequence of the fact that few CF ears developed a SNHL at frequencies up to 4 kHz, and there were only 16 to 17 ears with SNHL at 6 and 8 kHz (see Table 1). For this reason, the examination of individual-ear case studies across their longitudinal visits was used to reveal relationships between elevated hearing threshold and the TEOAE and absorbance data.

2.4 Case Studies—Four case studies are presented to demonstrate the relationship between audiometric hearing thresholds, TEOAEs and absorbance in CF participants. These were four of the 18 ears tested in the box labeled “visit 3” in Figure 1, Box J. Figures 4–7 display individual data for each case study. Panel A of Figures 4a–7a shows audiometric thresholds across visits. Panels B, C and D display TEOAEs measure for all three methods. Panels E and F show absorbance data for the ambient and TPP tests, respectively. Figures 4b–7b show individual data overlaying the 90th percentile test-retest reference limits (gray shaded area) of the change in TEOAE SNR on later visits relative to SNR in the first visit

(SNR in top panel) and relative change in CSM (CSM in bottom panel). These 90th percentile test-retest limits were based on results in control participants with NH. All CF participants in these case studies had a history of, or were currently on, a twice-daily inhaled antibiotic therapy (e.g., colistin, tobramycin or azithromycin). In addition, participants may have been admitted to the hospital during a CF-related exacerbation typically marked by poor pulmonary function test outcomes, abnormal lab results or reported symptoms. As inpatients, they typically received a single daily dose of IV-AG tobramycin based on body mass (10 mg/kg) and/or amikacin (20–25 mg/kg) drug therapy administered for up to 14 days. Table 5 provides information about cumulative IV-AG doses taken of mg/kg/day that each of the four patients received prior to and during study participation (relative to the body mass of each patient).

2.4.1 An ASHA shift in hearing is associated with a change in the TEOAE

Participant #1: This participant was a 27-year-old female with confirmed bilateral ASHA shifts in audiometric thresholds. She reported no history of familial hearing loss, but she did have a history of IV-AG treatments (288 mg/kg/day doses over her lifetime) prior to visit 1 (Table 5). Figure 4a (panel A) displays right ear air-conduction threshold data. Her hearing test at visit 1 revealed a slight hearing loss at 0.25 kHz, NH from 0.5 to 4 kHz and a moderate to severe SNHL at higher frequencies. The participant had 2.25 mg/kg/day doses of IV-aminoglycosides (amikacin) in the four months between visits 1 and 2. At visit 2, she exhibited a large ASHA shift in hearing at frequencies >0.5 kHz. The patient reported having constant bilateral tinnitus after her last IV treatment of amikacin, and noticed her hearing began to decline (e.g., sounds were “muffled”). She had no IV-AG treatments between visits 2 and 3 (~ 3 months between visits) and visits 3 and 4 (~ 5 months between visits). However, her thresholds continued to increase at frequencies at and above 1 kHz. She had a CF health exacerbation between visit 4 and 5 that resulted in 8.5 mg/kg/day doses of IV-tobramycin treatments. A continued decline in hearing was evident through the participant’s last visit (five total visits). Her hearing at her last visit showed NH thresholds at 0.25 kHz, and a sharply sloping loss to a profound SNHL with absent responses at and above 2 kHz.

Figure 4a (panels B–D) shows TEOAE SNRs for all three methods: ambient (panel B), TPP (panel C), and power-weighted (panel D). At her first visit, this participant had present and robust TEOAEs for each method (6 to 23 dB SNR) for frequency bands between 1.0 to 5.7 kHz, and absent TEOAEs at 8 kHz. The SNRs at frequencies from 1.0 to 5.7 kHz were reduced on all subsequent visits. The A_2 and AtD increased at frequencies above 4 kHz compared to baseline on visits 2–5 (see Figure 4a, panels E and F, respectively).

Figure 4b displays the 90th percentile test-retest reference limits (gray shaded area) of TEOAE SNR (top panel) and CSM (bottom panel) based on control participants with NH for TEOAEs using each of the three methods. Also shown are the changes in TEOAEs for participant #1 from visits 2–5 referenced to visit 1 for SNR (top row) and CSM (bottom row) for each ½ octave frequency band. The y-axes display changes in TEOAE SNR and CSM re: visit 1 and the x-axes show each half-octave frequency band. Thus, the line at 0 dB represents no change in the corresponding TEOAE measure. This participant’s TEOAE

shifts were outside SNR and CSM reference limits from 1.0 to 5.7 kHz in the ambient and TPP methods. Although the shifts in TEOAE were not outside the test-retest range for 6 to 8 kHz, TEOAEs were not measurable at baseline for these frequencies. Similar shifts in SNR and CSM were observed for the three TEOAE methods. Findings for the left ear were similar to the right ear, and are not displayed.

2.4.2 A confirmed ASHA shift is detected by a change in TEOAEs in the traditional audiometric range

Participant #2: This participant was a 29-year-old female with confirmed bilateral ASHA shifts in hearing. This participant reported no history of familial hearing loss, but she did have an extensive history of IV-AG treatments (52 mg/kg/day doses of tobramycin) during her inpatient hospital stays prior to visit 1 (Table 5). Her hearing test at visit 1 revealed NH across the entire test frequency range (Figure 5a, panel A). She subsequently received 1.75 mg/kg/day doses of IV-tobramycin in the eight months between visits 1 and 2 that resulted in constant bilateral tinnitus. At visit 2, the participant's hearing declined to a mild to moderate range of hearing loss from 10 to 16 kHz. She had continued IV-AG treatments between visits, when she started to notice difficulties listening in noise. Hearing continued to decline over subsequent visits from higher to lower frequencies, and finally resulted in a mild to moderate SNHL from 6 to 16 kHz.

At the participant's first visit, she had present and robust TEOAEs across methods from 1 to 8 kHz, evidenced by SNRs ranging from 6 to 32 dB (Figure 5a, panels B–D). TEOAE SNRs continued to decline over subsequent visits (2–4) at 5.7 and 8 kHz for all three methods. This subject had a marked reduction in TEOAE responses by visit 4, when her hearing loss progressed to frequencies below 8 kHz. The TEOAE SNR at 8 kHz was approximately 0 dB for all methods on visit 4. These changes did not appear to be related to changes in middle ear absorbance, which was relatively stable in the 5.7 to 8 kHz range (Figure 5a, Panels E and F). Figure 5b shows that this participant's change in TEOAEs was slightly outside the control reference at 8 kHz for visit 3 for the ambient SNR condition, but not for the ambient CSM condition. The power-weighted TEOAE SNR was also slightly below the normal test-retest range at 8 kHz on visit 3, but not for CSM. Figure 5b also shows that the change in TEOAEs was outside the control reference limits at 8 kHz on visit 4 compared to baseline for all three conditions for both SNR and CSM. Results were similar for the right ear, but not displayed.

2.4.3 TEOAEs were not sensitive to small increases in hearing thresholds in the traditional audiometric range

Participant #3: This participant is a 21-year-old male with a confirmed ASHA shift in his left ear at 6 kHz (15 dB increase in hearing threshold) and 8 kHz (10 dB increase in hearing threshold) at visits 2 and 3 (Figure 6a, panel A). This participant reported no history of familial hearing loss, and no difficulties with hearing. He had 14 mg/kg/day doses of IV-tobramycin prior to his enrollment in the study (Table 5). This participant's hearing test at visit 1 revealed NH for 0.25 to 12.5 kHz and hearing in the mild hearing loss range for 14 to 16 kHz (Figure 6a, panel A). He subsequently had 3.5 mg/kg/day doses of IV-tobramycin in the five months between visit 1 and 2, and 7 mg/kg/day doses in the six months between

visits 2 and 3. Although his hearing remained in the normal range, this participant exhibited a confirmed ASHA shift in threshold from visit 1 to visits 2 and 3 for 6 kHz (a 15 dB increase in hearing threshold) and 8 kHz (a 10 dB increase in hearing threshold). The 1 kHz threshold was also elevated by 15 dB at visit 2, however this recovered to within 5dB of baseline at visit 3.

There were no persistent changes in any TEOAE measures (Figure 6a, panels B, C, D), or in *Aa* and *AtD* absorbance (panels E & F), across the three visits in the frequency range of the hearing shift (6 to 8 kHz). This indicates that neither TEOAE or absorbance measures up to 8 kHz appeared to be related to this participant's confirmed ASHA shift in hearing. Figure 6b showed that the ambient TEOAE condition was the only one to exhibit a change, albeit borderline, in visit 3 at 1 kHz, which was outside of the TEOAE reference limits in control ears. Figure 6b also showed that this participant's change in TEOAEs from baseline were primarily within control reference limits for both visits 2 and 3. The TEOAE in the TPP condition for visit 2 was slightly outside limits at 4 kHz, but this was only for CSM and not SNR.

2.4.4 Changes in TEOAEs are associated with a temporary change in absorbance

Participant #4: This participant was a 41-year-old female with a history of 9 mg/kg/day cumulative doses of IV-tobramycin before visit 1 (Table 5). Although this participant had a negative history of familial hearing loss, she reported a diagnosis of SNHL in her left ear associated with previous ototoxic IV-AG treatments. No IV-treatments were documented between her visits 1 and 2 (~4 months) and visits 2 and 3 (~3 months). Her hearing test at visit 1 revealed a primarily mild SNHL for 0.25 to 16 kHz. She experienced an ASHA shift in her right ear at 16 kHz (20 dB decline in hearing) at visit 2, which recovered to baseline by visit 3 (Figure 7a, panel A). This participant also had a reduced absorbance and reduced TEOAE SNR in the right ear at 5.7 kHz at visit 2 that each recovered to baseline by visit 3.

Figure 7a (Panels B, C, D) shows this participant had primarily absent TEOAE responses for all methods from 1 to 4 kHz, but TEOAEs were present at 5.7 and 8 kHz. There was a decline of about 9 dB in TEOAE SNR at 5.7 kHz for visit 2 for all three conditions that largely recovered by visit 3. Figure 7a (Panels E and F) shows that the ambient and TPP absorbance were also lower than baseline at visit 2 between 1.4 and 8 kHz. Interestingly, audiometric thresholds were slightly lower overall on visit 2 compared to visit 1, particularly at 6 and 16 kHz. Although absorbance measurements had an upper frequency limit of 8 kHz, it is possible that the overall decline in absorbance at visit 2 produced the observed TEOAE and threshold changes. Figure 7b shows that this participant's TEOAE data were outside reference limits at 4 to 6 kHz when comparing visits 1 to 2, and within the TEOAE reference limits when comparing visits 1 and 3. Figure 7c displays this participant's change in absorbance across visits. The grey shaded region indicates the 90%-tile test-retest reference limits for absorbance in control ears with NH. The absorbance at ambient pressure and at TPP decreased outside the normal control reference limits at 2.8 kHz for visit 2 relative to visit 1, but recovered to show essentially no decrease compared to reference limits on visit 3.

This evidence for transient middle ear dysfunction at visit 2 that resolved by visit 3, parallels the ASHA hearing shift at visit 2, which recovered at visit 3 when the absorbance returned to baseline. Nevertheless, the absorbance changed in visit 2 in the 1.4 to 8 kHz frequency range (depending on its measurement condition), whereas the ASHA shift was measured at 16 kHz.

Previous research on the conductive hearing loss associated with otitis media in children has shown that the specific frequencies at which absorbance is shifted relative to that in an ear with NH do not necessarily predict the frequency of the associated conductive hearing loss (Keefe et al. 2012). Notwithstanding that fact, the presence of a shift in absorbance in that study accurately classified an ear as having NH or a conductive hearing loss with AUCs in the range of 0.95 to 0.99. One possible explanation for a lack of frequency alignment between an absorbance shift and hearing change may be that the frequency profile of the absorbance measured in the ear canal may vary from the power transferred through the middle ear to the cochlea, which cannot be directly assessed by an acoustic ear-canal measurement.

It is proposed that the frequency-dependent effects of middle ear dysfunction in the test ear of participant #4 in visit 2 at 1.4 to 8 kHz were associated with a reduced conductive forward and/or reverse transmission at frequencies near 16 kHz. The purpose of a longitudinal monitoring program for ototoxicity is to identify a risk of SNHL. While the audiogram in this individual showed a transient hearing loss at 16 kHz in visit 2, the lack of persistence of this loss suggests that the hearing loss was conductive rather than sensorineural. That may be relevant information in a monitoring program to estimate separate risks of whether a hearing change may be sensory or conductive in nature. It also suggests that it might be beneficial to develop absorbance measures at higher frequency bands to understand the effects of middle ear dysfunction on hearing for frequencies above 8 kHz.

E. Discussion

The AAA 2009 guidelines recommend that ototoxic monitoring protocols should include both subjective and objective measures. Testing should include conventional (up to 8 kHz) and extended HF (up to 16 kHz) audiometric testing and OAEs, which are performed at a baseline initial visit (pre-treatment) and in follow-up evaluations. This study provides data in 91 CF patients on the clinical utility of TEOAEs measured using three methods to detect SNHL in patients receiving potentially ototoxic IV-AG treatments. Four cases were also presented to illustrate the relationship between TEOAEs, absorbance and hearing thresholds.

The TEOAE tests used in the present study had a higher frequency bandwidth (8 kHz) than is typically used in clinical CEOAE tests (up to 4 to 5 kHz). The higher-bandwidth TEOAE tests may be more useful for monitoring ears for ototoxicity given that the initial ototoxic effects occur at the basal end of the cochlea. In a task to classify CF ears as having normal function or a SNHL using TEOAEs recorded with three methods, the resulting frequency-specific AUCs were 0.80 at 5.7 kHz and between 0.85 to 0.88 for 8 kHz. This indicates that all three TEOAE methods adequately distinguished ears in CF patients with SNHL from

those with NH at these frequencies. The question of which of the three stimulus methods might be best in TEOAE testing to monitor for ototoxic SNHL is a topic for future research.

In general, all three TEOAE tests performed similarly based on AUC to performance of DPOAE and traditional CEOAE tests at identifying ears with NH versus HL (Gorga et al. 1993a; Dorn et al. 1999; Harrison and Norton 1999). A difference in the present study was that the SNHL group included only ears from patients with CF. Ototoxic medications in CF patients are known to preferentially affect outer hair cell function, which suggests that ototoxic damage should be detected using an OAE test. The degree to which a more general group of CF ears with SNHL would have a similar type of relative damage to outer hair cell function is unknown.

Ress et al. 1999 showed that DPOAE levels in the 3 to 5 kHz were most sensitive to cisplatin treatments in cancer patients. Specifically, DPOAE levels were reduced between 2 to 6 dB in this frequency range. Ress et al. concluded that OAEs were more sensitive to detecting early indicators of ototoxicity than audiometry between 0.25 to 20 kHz. This is not surprising given that the presence of OAEs are typically associated with NH, and reduced DPOAEs and TEOAEs are thought to be correlated with outer hair cell loss even at low hearing thresholds 25 dB HL (Gorga et al. 1996; 1997). Previous investigations using a large cohort of participants have shown that tests, such as DPOAEs, are more accurate at identifying ears with high-frequency hearing loss compared to traditional click-evoked OAEs that are band limited to frequencies 4 to 5.5 kHz (Gorga et al. 1993a; 1993b; Prieve et al. 1993; Gorga et al. 1994). This is particularly important for ototoxic monitoring protocols given the typical base to apex progression of hearing loss with ototoxic drugs. Keefe et al. (2011) showed that CEOAEs with wider bandwidths accurately classified ears as having either SNHL or NH for frequencies up to 10.1 kHz. The present study demonstrated the clinical utility of monitoring TEOAE changes in auditory function at frequencies up to 8 kHz. The limitation of the maximum analysis frequency to 8 kHz in this study was related to probe performance above 8 kHz. It would be advantageous to measure TEOAEs at frequencies above 8 kHz to detect early ototoxic hearing loss in CF patients.

Some studies suggest that OAE protocols should include the use of normative test-retest limits to determine whether a patient's change in cochlear function is significant (Konrad-Martin et al. 2012; Beattie et al. 2003; Franklin et al., 1992; Roede et al., 1993). The current study used the 90th percentile test-retest reference limits in NH controls to identify changes in wideband TEOAE responses for participants with hearing shifts. This approach showed that in the few subjects with CF who had a confirmed ASHA threshold shift, the wideband TEOAE methods were capable of identifying participants with changes in hearing with, at the minimum, a 20 dB shift for at least one frequency band (e.g. case studies #1 and #2). However, the TEOAE tests were not as sensitive for identifying changes in cochlear function in participants whose hearing remained in the normal range for the OAE frequencies tested or who had only small changes in hearing (i.e., ~10 dB shift at two adjacent frequencies) (e.g., case study #3). This case study illustrates a possible benefit of testing TEOAEs at frequencies above 8 kHz, because the confirmed audiometric shifts occurred only at these higher frequencies.

Of the 157 CF ears tested at visit 1, 81 ears had SNHL (>20 dB HL) based on behavioral audiometric testing for at least one frequency band. The present results show a slightly higher prevalence of SNHL (52%) in CF patients in contrast with other hearing-related studies reporting up to a 44% incidence in patients receiving ototoxic medications (Mulheran et al. 2001; 2006; Cheng et al. 2009; Martins et al. 2010). However, we tended to have an older cohort (median age= 25 yr.) compared to these studies. For example, Mulheran and colleagues (2006) showed that a single course of IV-tobramycin administration in 168 CF patients (mean age= 14.8 yr.; median age not reported), without preexisting hearing loss, did not produce ototoxicity. These authors reported no significant differences in behavioral hearing thresholds (0.25 to 16 kHz) when comparing results at baseline to follow-up testing at 6 to 8 weeks post IV-AG treatment (Mulheran et al. 2006). Similarly, a retrospective study of 19 patients, ages 16 to 38 years old, with 8 courses of tobramycin treatments (one treatment course= twice-daily dosing for up to three weeks) showed no change in hearing after baseline and follow-up behavioral hearing tests for 0.25 to 16 kHz (Scheenstra et al. 2010). In contrast, Cheng et al. (2009) showed that six of 14 (42%) pediatric patients, with a mean age of 11.4 yrs., with > 10 courses of IV-AGs showed signs of ototoxicity compared to one of 36 patients (2%) who received less than 10 treatment courses. Differing results among investigations are likely due to factors such as age group, assessment methods, variability in treatment regimens and disease status, and the definition used for hearing loss.

A practical limitation of this study was the inability to test CF patients prior to any history of their receiving IV-AG treatment, which would have provided a “true” baseline of pre-treatment hearing. This did not interfere with the ability in this study to measure changes in hearing in relation to changes in TEOAEs and absorbance in later participant visits relative to the initial baseline visit. However, we may have identified initial ototoxic cochlear damage or shifts in hearing thresholds in some CF ears prior to study participation. The youngest CF participant in the present study was 15 years old, however many patients receive their first treatment prior to 10 years old. It might be possible in future studies to enroll younger children with CF as participants in a pre-treatment condition, which would improve the definition of the initial baseline visit.

In the present study, 42 CF ears had an ASHA shift in hearing compared to baseline (visit 1). However, not all of these ears had this shift confirmed by a subsequent visit. These ears primarily exhibited a reduction in extended HF hearing rather than in low-frequency hearing changes, consistent with previous research (Tan et al. 2003; Van Meter et al. 2009). Of the 42 ears with an ASHA shift in hearing for the present study, only 18 ears had at least three visits and thus could be evaluated for a confirmed ASHA shift in hearing. Therefore, the hearing loss in the remaining 24 of the 42 ears could not be confirmed, even though they exhibited an ASHA shift at their last visit. It was difficult for many patients to return for follow-up appointments due to distance, transportation constraints, CF-related illnesses, and numerous medical appointments that required priority. This made it difficult to evaluate participants longitudinally as often as desirable. Due to these factors, we were only able to verify that seven ears had a confirmed ASHA shift in hearing.

Accounting for changes in middle ear function during longitudinal OAE testing may help in test interpretation of TEOAE responses, particularly in patients who experience middle ear

pressure fluctuations during testing. Case study #4 is an example of how an overall reduction in absorbance at frequencies ~ 1.4 kHz for visit 2 may have accounted for changes in TEOAE SNR and hearing when compared to baseline (See Figure 7a). However, the change in middle ear function was not explained by a change in middle ear pressure alone, because the decrease in absorbance on visit 2 occurred for both ambient and TPP absorbance (TPP method controlled for middle ear pressure). Changes in absorbance might also occur for other transient middle ear conditions such as the presence of middle ear fluid. Further research is needed to establish the relationship between absorbance, TEOAE and hearing thresholds. This is particularly important in children, for whom middle ear conditions may be more prevalent and likely to impact upon audiometric and OAE measurements.

F. Conclusion

The findings of the present study show promise for utilizing the wideband TEOAE test described in this study as part of an ototoxicity monitoring protocol. Results also suggest the hypothesis that controlling for middle ear function in the OAE test procedures by testing at the TPP or calibrating the TEOAE stimulus in terms of constant absorbed sound power across frequency may be useful for an ototoxicity monitoring program. While more research is needed to evaluate these differing types of TEOAE tests, all TEOAE test methods were sensitive to changes in cochlear or middle ear function. In order to better understand whether a change in TEOAEs is caused by a change in cochlear or middle ear function, it may be clinically advantageous to add a wideband absorbance test to a longitudinal protocol of OAE testing to obtain a combined test assessment of cochlear and middle ear function.

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Figure 1.

Flow chart of CF study participants and ears evaluated for ASHA hearing shifts.

Figure 2.

Box and whisker plots displaying the TEOAE means (in box, *), interquartile ranges, medians (horizontal line through the IQR) and outliers (outside box, +, o) for both CF ears with NH (white box) and CF ears with SNHL (gray box). Results are displayed for each 1/2-octave frequency band for all three TEOAE methods: Ambient (left), TPP (middle) and Power-Weighted (right). TEOAEs are plotted on the y-axis as SNR (top row) and CSM (bottom row).

Figure 3.

AUC for classifying CF ears as having NH or having SNHL based on TEOAE SNR (left) or CSM (right). Results are shown for the ambient TEOAE method ($\circ$), TPP TEOAE method (+), and Power-Weighted TEOAE method (X).

Figure 4a.

Individual clinical and experimental data for case study #1. Panel A shows audiometric thresholds for 0.25 to 16 kHz across all five visits. Asterisks indicate there was no response for a specific frequency band. Panels B, C and D show SNRs for ambient (panel B), TPP (panel C) and power-weighted (panel D) TEOAEs at $\frac{1}{2}$ octave frequency bands from 0.71 to 8 kHz. Absorbance at ambient (*Aa* in panel E) and absorbance at TPP in a downswept pressure (*AtD* in panel F) are also displayed.

Figure 4b.

Individual change (Δ) in TEOAE response from baseline for ambient, TPP and power-weighted conditions SNR (top row) and CSM (bottom row) from case study #1. The gray shaded area displays the 90th-ile test-retest reference limits based on control ears with NH.

Figure 5a.

Individual clinical and experimental data for case study #2. Panel A shows audiometric thresholds for 0.25 to 16 kHz across all five visits. Panels B, C and D show SNRs for ambient (panel B), TPP (panel C) and power-weighted (panel D). TEOAEs at $\frac{1}{2}$ octave frequency bands from 0.71 to 8 kHz. Absorbance at ambient (A_a in panel E) and absorbance at TPP in a downswamp pressure (A_{tD} in panel F) are also displayed.

Figure 5b.

Individual change () in TEOAE response from baseline for ambient, TPP and power-weighted conditions SNR (top row) and CSM (bottom row) from case study #2. The gray shaded area displays the 90th-ile test-retest reference limits based on control ears with NH.

Figure 6a.

Individual clinical and experimental data for case study #3. Panel A shows audiometric thresholds for 0.25 to 16 kHz across all five visits. Panels B, C and D show SNRs for ambient (panel B), TPP (panel C) and power-weighted (panel D) TEOAEs at $\frac{1}{2}$ octave frequency bands from 0.71 to 8 kHz. Absorbance at ambient (Aa in panel E) and absorbance at TPP in a downswep pressure (AtD in panel F) are also displayed.

Figure 6b.

Individual change (Δ) in TEOAE response from baseline for ambient, TPP and power-weighted conditions SNR (top row) and CSM (bottom row) from case study #3. The gray shaded area displays the 90th-ile test-retest reference limits based on control ears with NH.

Figure 7a.

Individual clinical and experimental data for case study #4. Panel A shows audiometric thresholds for 0.25 to 16 kHz across all five visits. Panels B, C and D show SNRs for ambient (panel B), TPP (panel C) and power-weighted (panel D) TEOAEs at $\frac{1}{2}$ octave frequency bands from 0.71 to 8 kHz. Absorbance at ambient (A_a in panel E) and absorbance at TPP in a downswep pressure (A_{tD} in panel F) are also displayed.

Figure 7b.

Individual change () in TEOAE response from baseline for ambient, TPP and power-weighted conditions in SNR (top row) and CSM (bottom row) from case study #4. The gray shaded area displays the 90th percentile test-retest reference limits based on control ears with NH.

Figure 7c.

Individual change in absorbance from baseline data for Absorbance at ambient (*Aa*, left panel) and absorbance at TPP in a downswept pressure (*AtD*, right panel) for case study #4. The gray shaded area displays the 90th percentile test-retest reference limits based on control ears with NH.

Table 1

This table describes the number of CF ears (total= 157) with normal hearing (≤ 20 dB HL) or SNHL (>20 dB HL) for each pure-tone air conduction frequency from 0.25 to 16.0 kHz at visit 1. Means, SDs and the range of hearing thresholds are described for each frequency band.

AC Frequency (kHz)	CF Group	Hearing Threshold (dB HL)				
		N (Ears)	Mean	SD	Min	Max
0.25	NH	150	9.4	4.8	0	20
	SNHL	7	26.4	2.4	25	30
0.50	NH	154	9.9	4.8	0	20
	SNHL	3	31.7	7.6	25	40
1.0	NH	155	8.4	5.0	-5	20
	SNHL	2	42.5	10.6	35	50
2.0	NH	151	7.5	5.9	-5	20
	SNHL	6	31.7	12.1	25	55
3.0	NH	147	6.0	6.1	-10	20
	SNHL	10	42.0	27.1	25	95
4.0	NH	149	3.2	6.9	-10	20
	SNHL	8	50.0	30.5	25	100
6.0	NH	141	8.7	6.3	-10	20
	SNHL	16	43.4	21.8	25	80
8.0	NH	140	6.6	6.8	-10	20
	SNHL	17	47.9	19.7	25	80
9.0	NH	129	6.9	7.0	-10	20
	SNHL	28	41.0	16.9	25	80
10.0	NH	129	3.2	8.1	-10	20
	SNHL	28	47.7	17.4	25	75
11.2	NH	124	2.4	7.8	-10	20
	SNHL	33	50.3	18.1	25	80
12.5	NH	126	0.4	8.6	-10	20
	SNHL	31	56.9	15.9	25	85
14.0	NH	111	0.0	9.6	-20	20

Hearing Threshold (dB HL)		AC Frequency (kHz)	CF Group	N (Ears)	Mean	SD	Min	Max
			<i>SNHL</i>	46	47.9	16.0	25	75
			<i>NH</i>	93	1.3	11.6	-20	20
		16.0	<i>SNHL</i>	64	40.2	10.8	25	65

Table 2

This table shows the total number of CF ears tested across visits.

Test Visit	Number of CF ears
1	157
2	129
3	74
4	37
5	14
6	5
7	2

Table 3

226-Hz tympanometric results for CF ears. Means, SD and range of values are reported for acoustic admittance (in mmho, left column), tympanometric peak pressure (in daPa, middle column) and ear-canal volume (in cm³, right column).

	Acoustic Admittance (mmho)	Tympanometric Peak Pressure (daPa)	Ear-canal Volume (cm ³)
Mean	0.64	+7.2	1.1
SD	0.32	+20	0.29
Minimum	0.3	−125	0.4
Maximum	1.6	+85	2.4

Table 4

Mean and standard deviation (SD) of the SPL and peSPL of the positive chirp for each TEOAE condition (ambient, TPP, and power-weighted) in all CF ears.

TEOAE Method	Mean SPL (SD)	peSPL (SD)
Ambient	65.1 dB (1.31 dB)	76.8 dB (0.91 dB)
TPP	65.2 dB (1.44 dB)	77.0 dB (1.0 dB)
Power-Weighted	67.1 dB (1.2 dB)	76.9 dB (0.8 dB)

Cumulative IV-AG doses are reported for each case study participant. The total number of IV-AG doses (mg/kg/day) are displayed prior to study visit 1, and between each study visit. The number of months between visits (MBV) are also displayed.

Table 5

Participant	Doses prior to Visit 1	Doses between Visit 1 & 2	Doses between Visit 2 & 3	Doses between Visit 3 & 4	Doses between Visit 4 & 5
1	288	2.25 (4) *	0 (3) *	0 (5) *	8.5 (13) *
2	51.5	1.75 (8) *	6.75 (4) *	8.75 (8) *	
3	14	3.5 (5) *	28 (6) *		
4	9	0 (5) *	0 (3) *		

* Months Between Visits (MBV)