Selective Inner Hair Cell Loss in Premature Infants and Cochlea Pathological Patterns From Neonatal Intensive Care Unit Autopsies

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**Background:** Deafness and handicapping sensorineural hearing impairment occur frequently in neonatal intensive care unit survivors for unknown reasons.

**Patients and Methods:** Hearing was tested early and repeatedly in neonatal intensive care unit patients with an auditory brainstem response (ABR) screener. The temporal bones of 15 nonsurvivors (30 ears) were fixed promptly (average, 5 hours) after death for histological evaluation.

**Results:** Among these patients, 12 failed the ABR screen bilaterally, 1 passed unilaterally, and 2 passed bilaterally. Cochlear histopathologic conditions that could contribute to hearing loss included bilateral selective outer hair cell loss in 2 patients, bilateral selective inner hair cell loss in 3 (all premature), and a combination of both outer and inner hair cell loss in 2. Other hair cell abnormalities were noted; the 2 infants who had passed the ABR screen demonstrated normal histological features. Neuronal counts were normal.

**Conclusions:** Auditory brainstem response failure among these neonatal intensive care unit infants who died was extremely common in part owing to an unexpected histological alteration, selective inner hair cell loss among premature newborns, that should be detectable uniquely by the ABR testing method. Additional histological patterns suggest more than one cause for neonatal intensive care unit hearing loss. Hair cell loss patterns seem frequently compatible with in utero damage.


**HANDICAPPING** sensorineural hearing impairment and deafness occur for unknown reasons in at least 2% to 4% of neonatal intensive care unit (NICU) survivors, an incidence approximately 50 times greater than in normal newborns.1 This heightened vulnerability of critically ill neonates is underscored further by the fact that older critically ill pediatric and adult patients, even those who suffer cardiopulmonary arrest, are unlikely to develop hearing loss. Retrospective studies have suggested several possible causes for neonatal hearing loss2-7 but also have shown that it is impossible to predict which patients will be affected.8 These neonates depend on early auditory function to develop speech and language skills. Insight into the peculiar auditory susceptibility of NICU patients is necessary to optimize appropriate prenatal and postnatal clinical care intervention and to measure and maintain hearing function.

Historically, our understanding of the mechanisms of neonatal hearing loss has been limited by (1) the lack of audiometric data for infants still in the NICU since for technical reasons hearing tests are typically performed only on surviving patients near discharge and (2) the lack of temporal bone tissue in NICU patients from whom correlative audiometric data were available prior to autopsy. In a previous study, we accomplished a unique prospective hearing screening on patients while they were still in critical condition in an NICU, using an automated system designed to measure auditory brainstem responses (ABRs) in the noisy NICU environment.8 This article summarizes the ear histopathologic findings in 15 of these NICU patients. The results are relevant not only to the pathophysiology of the hearing loss but also to possible limitations and interpretations of current techniques used to test hearing.
HEARING TESTS

In the NICU, Hospital Nacional de Niños, San Jose, Costa Rica, 92 infants underwent repeated ABR testing before discharge from the hospital or death. Details of the testing technique have been published previously. This study had received institutional review board approval from both that hospital and the Massachusetts Eye and Ear Infirmary, Boston. The Hospital Nacional de Niños is a modern 350-bed facility. The neonatologists received their training in the United States, and the NICU care was contemporary.

An automated evoked response infant hearing screener (ALGO-1; Natus Medical, San Jose, Calif) was used for all tests. This portable, battery-operated device weighs 3.2 kg and provides a click stimulus with a bandwidth of 750 to 3000 Hz that was modified to increase the output from a 35- to 40-dB hearing loss for this study. Recording electrodes (3M, Minneapolis, Minn) were placed on the vertex of the neonate’s head and nape of the neck, and a separate ground electrode was placed on the chest. Disposable ear couplers were used. These devices surrounded the auricle and gently adhered to the side of the head to prevent collapsing ear canals, avoid differences in earphone placement, and attenuate ambient noise.

Stimuli were provided to the patient at a rate of 37 per second, and the machine computed a binary averaged response from the electroencephalogram. At each increment of 300 sweeps, the screener compared the cumulative response with a normal response template. If the fit was fewer than 4 SDs from chance, another 500 sweeps were added. If the fit exceeded 4 SDs, it scored a “pass.” The test was terminated and scored as a “refer (fail)” when 15000 sweeps had been accumulated without a significant (4 SDs) fit to the template. The statistical evaluation of the fit was a maximum likelihood test using a Nieman-Pearson criterion, giving exact control over false-negative error. False-positive errors were controlled by an artifact rejection algorithm matched to the 15000-sweep limit. Because of the myogenic and ambient noise criteria for data acquisition, testing could be suspended for indeterminate periods. This could lead to an inability to complete a test within permissible time limits, and the result was scored as “cannot test.” Objective screening results were displayed as pass, refer (fail), or cannot test based on machine scoring. Waveforms were not displayed for subjective interpretation. Patients were tested from 1 to 4 times (Table).

HISTOLOGICAL STUDY

Of the 20 infants who died after being tested, 15 underwent autopsy for histological evaluation, providing a total of 30 temporal bones for analysis (Table). The bones were promptly fixed, 1 to 15 hours (average, 5 hours) after death, in neutral-buffered (10%) formalin solution, decalcified with 9% trichloroacetic acid, and embedded in celloidin over a period of 12 weeks. The blocks were cut at a thickness of 20 µm in the horizontal plane, and every 10th section was stained with hematoxylin-eosin and mounted on slides.

The inner ear could be evaluated in all 30 bones. A 2-dimensional projection technique was used for reconstructing the cochlear spiral and computing the percentage of distance from the base of each section through the cochlear duct. Cochlear location was converted to frequency via a mathematical fit to the cochlear map data provided by Schuknecht and Greenwood. The number of inner hair cells (IHCs) and outer hair cells (OHCs) missing in each row was estimated within each section, and the percentage of hair cell loss was calculated for each millimeter interval of the organ of Corti. The hair cell counts were performed with high-powered (×100) phase-contrast objectives. The tissue preservation was generally excellent and, thus, the cuticular plates and stereociliary tufts of hair cells were usually clearly visible, adding a valuable histological marker for hair cell presence that was often used in cases that might have been otherwise ambiguous. The condition of the supporting cells, stria vascularis, spiral ligament, and the Reissner membrane were qualitatively evaluated in each section. The presence of blood and/or proteinaceous precipitate in the cochlear scalae was noted.

A separate graphic reconstruction was used to evaluate the spiral ganglion. Cochlear nerve cells (nuclei) were counted in each section with the aid of an ocular grid. To maintain a close relationship to the sense organ, the spiral ganglion was divided into 4 contiguous segments from base to apex. The ganglion cell populations were determined for each segment and multiplied by 10 to account for the unmounted sections and by a factor of 0.61 to compensate for double counting.

RESULTS

CLINICAL PROFILE

The population studied consisted of 10 male and 5 female patients, ranging in age from 1 day to 8 months and in birth weight from 0.9 to 4 kg. Seven patients were premature (<37 weeks’ gestation). The medical conditions reflected that of a standard NICU patient population (Table); the name of each condition reflected the terminology used in the clinical record.

HISTOLOGICAL FINDINGS

Middle Ear

As summarized in the Table, the middle ear cavity contained purulent exudate or eosinophilic precipitate in 8 patients. In 2 patients, there was a middle ear inflammatory reaction. In 2 patients, the middle ear cavity appeared to be normal. Because of the unintentional opening of some middle ears at autopsy, these spaces could not be evaluated confidently in the remaining 3 patients.
Hair Cell Loss or Damage

As summarized in the Table, significant cochlear hair cell loss or damage was seen in 7 of the 15 patients; the damage was bilateral and was usually symmetrical between ears. In 2 patients (patients 1 and 2) selective OHC loss was seen. A representative photomicrograph of a normal organ of Corti in Figure 1 can be compared...
with OHC loss as shown in Figure 2. The cytocochleograms from patient 1 demonstrate that the OHCs were missing preponderantly in the basal turn. In patient 2 the hair cells were missing preponderantly in the apical half of the cochlea. In both cases the loss was symmetrical (Figure 3).

Selective IHC loss is an extremely rare finding in the temporal bones of humans or other mammals. Nevertheless, selective and widespread loss of IHCs was seen in 3 (patients 3-5) of the 15 patients in this study. Representative photomicrographs from 2 of these infants are shown in Figure 4; the cytocochleograms are shown in Figure 5. As illustrated by the photomicrographs, tissue preservation was generally excellent, the patterns of hair cell loss, and particularly this unique pattern of selective IHC loss, were unambiguous when viewed with high-powered, phase-contrast objectives. In patient 4 the pattern of hair cell loss was symmetrical between both ears. In patients 3 and 5 the loss was more extensive in the left ear than in the right ear (Figure 5). In these infants, concurrent OHC loss was minimal except for a restricted area in the lower basal turn in patient 5. All 3 patients with this rare pattern of selective IHC loss were premature (Table).

In patients 6 and 7 there was total loss of both IHCs and OHCs in the basal cochlear regions. Patient 6 was a newborn with trisomy 13 syndrome.

In patients 8 through 11 there were significant abnormalities in many of the hair cells remaining in the cochlea. These abnormalities consisted of (1) swollen OHCs, common in all of these patients or (2) herniation of the IHC contents into the scala media, which was common in patients 8 and 10. It is difficult to determine whether these abnormalities represent incomplete premortem damage or postmortem autolysis; however, these ears had acceptably short postmortem fixation times ranging from 2 to 11 hours (Table), making autolysis less likely.

In all of the 4 remaining patients (patients 12-15), the cochleae were well preserved, as illustrated by the photomicrograph in Figure 1; there was no significant hair cell abnormality. Visual inspection of the spiral ganglion in these 4 patients showed a high density of neurons throughout the Rosenthal canal, and neuronal counts in these 8 ears were among the highest of the 30 ears in the study (see “Neuronal Loss” subsection of the “Results” section).

Neuronal Status

Qualitative analysis of the spiral ganglion region in all patients suggested there was no neuronal loss. The Rosenthal canal, where the cell bodies are found, appeared full of neurons, without the empty spaces seen in cases of clear-cut neuronal loss.

For more quantitative analysis, the number of spiral ganglion cells was counted and total cell counts were estimated (see “Subjects, Materials, and Methods” section). As seen in Figure 6, when these counts are compared with those obtained, using identical techniques, from older human temporal bones (aged, 1-10 years), these data suggest that (1) neonatal ears have fewer spiral ganglion cells than in the adult ears and (2) the number of ganglion cells increases systematically during this immediately postnatal period. The same conclusion was reached when data were plotted separately for each of the 4 longitudinal segments of the ganglion (data not shown).

Other Abnormalities of the Cochlear Duct

Congenital middle and inner ear malformations were seen only in 1 patient (patient 6) with trisomy 13 syndrome who demonstrated the shortest cochlear duct (24 mm); most ducts ranged from 30 to 32 mm. In patients 3 and 4 the Reissner membrane was contacting the organ of Corti. It may be significant that both of these infants also
showed hydrocephaly; an association has been suggested between the increased intracranial pressure of hydrocephaly and the collapse of the Reissner membrane.14

Patients 2, 9, and 12 showed basophilic deposits in the stria vascularis bilaterally. In infants 6 through 8, there were cells extruding from the surface of the stria vascularis and also swollen OHCs. Blood or proteinaceous precipitate was frequently noted but did not correlate with the condition of the organ of Corti (data not shown).

COMPARISON OF HEARING TESTS AND HISTOLOGICAL FINDINGS

Patients 14 and 15 passed the hearing tests bilaterally. Neither infant had significant hair cell loss, and both sets of cochlea were extremely well preserved. Patient 15 had blood in the perilymphatic spaces bilaterally, and both patients 14 and 15 had a fluid-filled middle ear bilaterally.

Patient 5 passed the test unilaterally. This patient also showed selective IHC loss that was extensive on both sides (Figure 5). The right ear, however, passed the screening test, and the right ear also had less IHC loss than the left ear.

Of the 12 patients who failed the hearing tests bilaterally, 2 (patients 1 and 2) had selective OHC loss, 2 (patients 3 and 4) had selective IHC loss, and 2 (patients 6 and 7) had total loss of IHCs and OHCs in the basal cochlear regions. The remaining 6 (patients 8-13) had no significant loss of hair cells, although 4 (patients 8-11) did demonstrate hair cell abnormalities. Of these 12 patients, 2 (patients 2 and 9) showed a middle ear space that appeared to be free of fluid at the time of death.

The patients described in this article are from a larger study using prospective ABR screening to test auditory function in critically ill NICU infants.9 The conventional rate of ABR screening failure among study survivors was 6%, an expected result. However, since premortem ABR results had never been collected by anyone, the failure rate of 25 of 30 ears in nonsurvivors was unexpectedly high. To gain anatomic insight into the cause of neonatal hearing loss, systematic au-
tyspy recovery and analysis of temporal bones were necessary.

Generally, inner ear histopathologic features were heterogeneous among these patients, suggesting more than 1 cause for hearing loss. Therefore, hearing loss is not analogous to neonatal vision loss, in which a single postnatal treatment variable of oxygen toxicity has been able to be modified with great success to diminish the incidence of retinopathy of prematurity. In the cochlea, 2 patients showed loss of both OHCs and IHCs, 2 showed selective loss of OHCs, and 3 showed selective loss of IHCs. Additionally, these hair cell losses usually appeared to reflect fully resolved degeneration, with phalangeal scars resealing the reticular lamina, reinforcing a previous impression that hair cell loss occurred in utero.9 One implication of these findings is that hearing loss might be better considered a coexisting medical condition rather than an adverse effect of necessary NICU treatment. Another implication is that an effort to improve hearing in most NICU survivors via postnatal treatment modification will be fruitless.

Passing the final ABR screen bilaterally was noted for 2 patients who also had normal and well-preserved cochlear tissues. Patient 5 unilaterally passed the final ABR screen, despite a significant selective IHC lesion (right ear), indicating that fewer than half of the normal number of IHCs are sufficient to generate a detectable ABR. Selective IHC loss in this model does not greatly shift the threshold for evoked electrical responses until that loss exceeds 70% theoretically; a 50% loss of neuronal elements can be compensated by a 200% (6-dB) increase in stimulus intensity.

Important and unsuspected, the selective and widespread loss of IHCs seen in 3 infants in this study is not found in the cochleae of older patients. Platinum antineoplastic agents produce widespread and selective IHC loss in some animals16,17; but such medications are not used in the NICU. High doses of some ototoxic antibiotic agents have also been reported to produce a restricted region in the cochlear apex in guinea pigs where only IHCs are destroyed; however, in all such cases there was also a much larger area of the basal, and sometimes middle turns, in which all OHCs were destroyed.18 To our knowledge, there is only one other histological report of selective IHC loss in a human cochlea of any age and that patient was also a premature infant.19 Given that all 3 patients with selective IHC loss in this study were premature, and that 1 of the other 2 premature patients showed widespread herniation of the IHCs, the clear implication is that selective IHC damage is significant and common in the temporal bones of premature infants. This distinctly remarkable finding provides some explanation for the higher incidence of hearing impairment specific to an NICU population.

The loss of OHCs is expected to account for hearing loss; either combined loss of OHCs and IHCs or se-

Figure 5. Cytocochleograms for right and left ears in the 3 infants showing selective inner hair cell (IHC) loss. All other conventions for data display are as described in the legend to Figure 3.
selective OHC loss is a common cause for sensorineural hearing loss in older patients. However, the OHC patterns in the other infants distinct from the premature infants were atypical and did not explain fully the causes of hearing loss in those patients.

The loss of hair cells in this study was not associated with a significant secondary loss of cochlear neurons as a cause for hearing loss. Significant neuronal degeneration is not expected with OHC loss because 90% to 95% of the auditory nerve fibers contact IHCs only.13 Following IHC loss, significant loss of cochlear neurons is demonstrable within a few months,20,21 first visible as a degeneration of the peripheral axon within the osseous spiral lamina, followed later by degeneration of the cell body in the spiral ganglion. Thus, given the young age of these NICU patients, it is expected that even IHC loss was not accompanied by significant neural degeneration.

Excellent correlation (96% agreement) has been demonstrated for detection of sensorineural hearing loss with the ABR screener used in this study compared with complete ABR testing in NICU survivors.22-24 The ABR screener was set to fail those with more than a 40-dB hearing loss. The widespread presence of fluid in the middle ear in the infants of this study could have complicated the interpretation of how much hearing loss was sensorineural (and permanent) and how much was conductive (and reversible). Middle ear status did not influence testing results in this study since the presence or absence of middle ear fluid did not correlate with pass or fail results.

In older populations, a theoretical auditory neuropathy of the eighth cranial nerve has been postulated based on the clinical finding of normal otoacoustic emissions (OAEs), a complementary audiometric measure-

Figure 6. Spiral ganglion cell counts in each ear from 14 of 15 patients in the present study plotted as a function of postconceptual age. (Data from patient 6 with trisomy 13 is not included). The cell counts are expressed as a percentage of the mean neuronal counts found in a study of temporal bones from older patients (adapted from Otte et al12).


