

Seminar Summary Report:

“Perilymph Sampling for Understanding Sudden Sensorineural Hearing Loss (SSNHL)”

Date: Saturday, 10th May 2025

Time: 09:00–11:00 GMT-07:00

Speakers:

- Hinrich Staecker, MD, PhD, University of Kansas
- Adele Moatti, PhD, University of Pittsburgh
- Konstantina Stankovic, MD, PhD, Stanford University
- Andrea Vambutas, MD, Northwell Health
- William Slattery, MD, House Ear Clinic, House Institute Foundation

Attendees:

- Ali Andalibi
- Seilesh Babu
- John Butman
- Jane Cabison
- Sujana Chandrasekhar
- Nathan Creber
- John Galvin
- John House
- Benson Jung
- Hiroki Kagoshima
- Jeffery Lichtenhan
- Sean Lang
- Sudhir Manickavel
- Shemms Najjar
- Norma Penido
- Stefan Plontke
- Madelynn Poe
- Teres (*full name not provided*)
- Seth Schwartz
- Nobuyoshi Tsuzuki
- Brian Westerberg
- Mary Ying

Introduction: HSA and SSNHL

The House Institute Foundation Hearing Science Accelerator (HSA) is a strategic initiative designed to advance biomedical research in hearing disorders that have historically lacked effective treatment options. By bringing together leading experts in hearing science and thought leaders from other disciplines to an in-person workshop, the HSA fosters innovative, cross-disciplinary collaboration. For each targeted disorder, the HSA identifies critical research questions with the aim of shifting from progress to transformative innovation.

One such focus area is Sudden Sensorineural Hearing Loss (SSNHL), a condition characterized by the rapid onset of hearing loss, typically in one ear and often without a clearly identifiable cause. Following the HSA workshop, several research questions were developed. Two of those research questions we addressed in this seminar focused on the development of perilymph sampling (Slattery et al., 2024). Perilymph sampling has emerged as a promising technique to better understand the underlying mechanisms of SSNHL. This involves collecting fluid from the inner ear's perilymphatic space to analyze and identify potential biomarkers associated with hearing loss.

Q4: Can a biobank and patient registry be developed in order to determine the etiology of SSNHL? Possible specimens would include genetic, serum, and/or perilymph testing.

- a. a. Can Genetic testing determine susceptibility or predict prognosis of SSNHL?
- b. b. Are there biomarkers in the serum predictive of etiology, diagnosis or treatment?
- c. c. What test of perilymph would aid in the treatment of SSNHL?

Q5: Can perilymph obtained during cochlear implantation or during the acute presentation aid in diagnosis and/or treatment of SSNHL?

- a. a. Would biologic markers elucidate perilymph antigens helpful in etiology or diagnosis?
- b. b. Could the etiology of hearing loss be determined by analysis of perilymph content in the future?
- c. c. Would variation in perilymph composition be predictive of different SSNHL etiologies?
- d. d. A standard procedure for perilymph collection should be developed for maximum yield and minimal risk to hearing and balance.

Perilymph Sampling Seminar

The “Perilymph Sampling for Understanding Sudden Sensorineural Hearing Loss” seminar brought together over 30 leading clinicians and researchers from institutions worldwide to advance collaborative research on Sudden Sensorineural Hearing Loss (SSNHL).

The objective of this seminar was to evaluate the current state of perilymph sampling as it relates to SSNHL and to develop a robust protocol aimed at improving early diagnosis and informing treatment strategies.

Key goals of the seminar included determining standardized protocols for both human and animal perilymph sampling, identifying the most pressing unanswered questions surrounding this methodology, and establishing a framework for multi-institutional collaboration.

The meeting was hosted by the HSA chair, William Slattery, MD, and featured focused presentations, followed by open discussions on future research directions and ethical considerations.

Speaker Presentations

Hinrich Staecker, MD, PhD: “Perilymph Sampling to Develop Biomarkers for SSNHL”

Dr. Hinrich highlighted that the inner ear is unique among human organs in that it has traditionally been inaccessible for biopsies, unlike most other organs. However, advances in genetic testing for hearing loss are well underway, with whole exome sequencing already established and whole genome sequencing approaching practical application. Furthermore, recent research suggests that analyzing the perilymph metabolome—a fluid in the inner ear—can distinguish between individuals with long-term hearing loss and those with more recent onset, offering a potential diagnostic pathway.

MicroRNA Research in Hearing Loss:

Initial studies in microRNA research included 10 patients undergoing procedures such as cochlear implantation, stapedectomy, and labyrinthectomy. These studies identified approximately 300 microRNAs that were common across all participants. Using machine learning, researchers successfully differentiated between microRNA profiles in patients with different conditions including Meniere's disease, otosclerosis, and sensorineural hearing loss, pointing to the diagnostic potential of microRNA patterns. MicroRNAs are released into the body either bound to proteins or enclosed in extracellular vesicles—both of which play key roles in cellular communication and may be important biomarkers. These extracellular vesicles are already implicated in conditions like glaucoma and autism. A technique developed by a collaborator, originally intended for ovarian cancer research, now enables the capture of these vesicles from perilymph samples. This breakthrough allows researchers to isolate microRNA and study cell-specific activity within the cochlea, opening new avenues in hearing research and diagnostics.

Adele Moatti, PhD: “Pig Perilymph Sampling Protocol”

Dr. Moatti discussed how the identification of biomarkers for sensorineural hearing loss faces significant challenges, primarily due to the rarity of suitable human perilymph samples. Most available samples come from patients with severe inner ear conditions or those undergoing

cochlear implant surgery, making it difficult to establish a baseline for normal perilymph composition. Even rarer are samples from healthy individuals, which are essential for comparative analysis and accurate biomarker identification.

Another major hurdle in hearing loss research is the lack of a controlled model for sensorineural hearing loss. Animal models present difficulties due to significant biological differences between species, with proteomic overlap being only about 10%. However, pigs have emerged as a promising model because their genome and amino acid sequences of deafness-related genes more closely resemble those of humans compared to mice. This genetic similarity could improve the translational relevance of research findings.

There are substantial anatomical and physiological differences across species that complicate inner ear research. Human perilymph samples typically range from one to five microliters, while mice yield only about 0.3 microliters, limiting the volume available for study. Sample contamination with cerebrospinal fluid (CSF) or endolymph is a concern, particularly in smaller animals like mice. To address these issues, researchers plan to analyze perilymph from pigs and compare it with human samples, including those from patients with meningiomas. However, anatomical differences in pig ear structures, such as the longer and narrower ear canal and the mastoid bone's location, make surgical access challenging.

Sampling techniques also raise concerns regarding potential hearing damage. The use of needles that apply suction and create negative pressure may harm sensory cells and introduce contaminants. To mitigate this, the team developed a surgical approach involving drilling through the temporal bone and opening the bulla, which they published in a recent study. Moving forward, they aim to assess the impact of perilymph sampling on hearing thresholds post-procedure and to continue proteomic and microRNA profiling to better understand the inner ear's molecular environment.

Konstantina Stankovic, MD, PhD: “Perilymph Sampling in Humans: Insights from Cochlear implantation and Labyrinthectomy Procedures”

Dr. Stankovic discussed a study she investigated involving nearly 500 patients with vestibular schwannoma and found no correlation between tumor size and the severity of hearing loss. This suggests that factors beyond tumor volume contribute to auditory damage. Recent research over the past decade has shown that these tumors may secrete ototoxic and neurotoxic substances that impair hearing function. Furthermore, scientists have demonstrated the ability to detect molecular biomarkers of hearing loss using as little as half a microliter of inner ear fluid, paving the way for more precise diagnostic methods. The next phase of the study focused on analyzing blood samples from affected patients to identify potential biomarkers for non-invasive diagnosis.

TWEAK (TNF-related weak inducer of apoptosis) and TNF have emerged as significant contributors to hearing loss in vestibular schwannoma patients. Higher levels of TNF in tumor-conditioned media correlate with poorer hearing performance, including both word recognition

and pure tone hearing thresholds. It has been observed that patients with vestibular schwannoma have elevated concentrations of both TWEAK and TNF in their perilymph compared to their blood, and those with the highest levels tend to experience the most severe hearing impairment. The combined presence of TWEAK and TNF has been shown to greatly increase ototoxicity, indicating that their synergistic effect may be a key mechanism in tumor-related auditory damage.

Andrea Vambutas, MD: “Perilymph Sampling and Autoimmune Inner Ear Disease”

Dr. Vambutas reported that while 70% of patients with autoimmune inner ear disease (AIED) initially respond to corticosteroid treatment, long-term effectiveness is limited, with only 14% maintaining responsiveness after repeated courses. As a result, many patients eventually require hearing aids or cochlear implants. The study examined a small group of patients with varied systemic autoimmune conditions—such as rheumatoid arthritis, type 1 diabetes, and Hashimoto's thyroiditis—highlighting the heterogeneity in immune profiles. During cochlear implantation, researchers used a neonatal spinal catheter to collect perilymph fluid, which was then used to stimulate patients' own peripheral blood immune cells. Microarray analysis identified only 10 genes with notable findings, including the IL-1 type 2 decoy receptor and glutathione peroxidase, the latter currently being studied in clinical trials.

Her team conducted a study involving 20 patients to investigate decoy receptor gene expression using quantitative real-time PCR. It was found that steroid-resistant individuals exhibited higher levels of interleukin-1 beta (IL-1 β) in their plasma compared to steroid-responsive patients, pointing to a potential biomarker for predicting treatment outcomes. These findings led to the initiation of a clinical trial using anakinra, an IL-1 receptor antagonist. In this trial, steroid-resistant patients first received high-dose corticosteroids for 30 days, followed by 84 days of anakinra treatment, and were monitored for a total of 264 days. The trial was stopped early by the Data Safety Monitoring Board after achieving its efficacy goals ahead of schedule, indicating a strong therapeutic effect.

A separate but related clinical trial assessing Anakinra for Meniere's disease is nearing completion. It has successfully passed a futility analysis at 25% enrollment, affirming the study's progress. The trial includes three blinded arms: one group receives anakinra for 84 days, another receives anakinra for 42 days followed by placebo, and the third receives placebo for 42 days followed by anakinra. Interestingly, a 46% response rate was observed in patients who did not meet eligibility criteria, while an 83% response rate was seen in steroid-dependent individuals. These results support the continued exploration of IL-1-targeted therapies. Perilymph sampling during cochlear implantation remains a key aspect of the research, offering a valuable tool for

understanding molecular mechanisms underlying treatment responses. A paper detailing these findings is expected to be published in the journal *Otology & Neurotology*.

Summary:

A recurring challenge raised by multiple participants was the limited availability of normal perilymph samples, which are critical for establishing baseline data and interpreting disease-related changes. The group discussed the importance of developing a centralized biobank or patient registry, as well as coordinating efforts across institutions to collect, process, and analyze samples consistently. Existing resources from Hanover and Vienna were noted as valuable foundations, and there was a strong consensus around improving data sharing and harmonizing protocols.

Ethical considerations were a key part of the conversation. There was agreement that sampling procedures must be justified, particularly in patients with residual or normal hearing. Pre-procedural evaluations, including imaging and blood tests, were recommended to ensure patient safety. A phased research approach was also proposed, starting with safety studies on microneedle sampling in humans without therapeutic delivery, followed by more complex trials. These steps aim to ensure scientific rigor while minimizing patient risk.

Animal models, especially pigs, were discussed as useful platforms for validating sampling techniques and biomarker analysis due to their anatomical and genomic similarity to humans. However, significant differences in proteomic profiles and surgical access between species were noted, underscoring the need for careful cross-species comparison.

Overall, this seminar demonstrated strong engagement and a shared commitment to advancing the science of SSNHL. The productive dialogue, coupled with clearly defined goals and next steps, positions this research initiative for impactful progress in the understanding and treatment of hearing loss.

Next steps:

The seminar concluded with a set of actionable next steps.

1. A standardized protocol needs to be developed for human sampling, including guidelines for multi-institutional collaboration.
2. At this stage, early perilymph sampling in patients with sudden sensorineural hearing loss is not appropriate until additional protocols are established.
3. Animal sampling protocols should also be developed with a focus on maximizing hearing preservation and avoiding damage to the inner ear.
4. Proposed multicenter collaboration to establish perilymph collection/testing of:
 - a. Normal hearing patients undergoing surgical procedure in which perilymph can be obtained.

- b. SSNHL patients undergoing CI surgery with correlation in looking for etiology.
 - c. All patients undergoing CI surgery.
- 5. A standardized testing protocol for proteomics should be developed for all the above.