Published in final edited form as:

Otol Neurotol. 2018 October ; 39(9): 1210–1214. doi:10.1097/MAO.0000000000001942.

Otopathology in the United States: History, Current Situation and Future Perspectives:

"The Importance of Otopathology Laboratories"

Rafael da Costa Monsanto, M.D., PhD student[1,2], **Henrique Furlan Pauna, M.D., PhD Student**[1,3], **Michael M. Paparella, M.D.**[1,4], and **Sebahattin Cureoglu, M.D.**[1]

[1]University of Minnesota, Department of Otolaryngology–Head and Neck Surgery, Minneapolis, Minnesota, USA

[2]Department of Otorhinolaryngology Head and Neck Surgery, Universidade Federal de São Paulo / Escola Paulista de Medicina (UNIFESP/EPM), São Paulo, São Paulo, Brazil

[3]Department of Otorhinolaryngology, Head and Neck Surgery, University of Campinas – UNICAMP, Campinas, São Paulo, Brazil

[4]Paparella Ear Head & Neck Institute, Minneapolis, Minnesota, USA

Abstract

Human temporal bone studies have documented the pathophysiologic basis of many pathologic conditions and diseases affecting the ear, contributing to the development of specific clinical knowledge and pathology-oriented treatments. Researchers dedicated to the study of anatomy and histology of the temporal bone emanated from Europe to the United States during the first part of the 20th Century. The first otopathology laboratory was founded in the United States in 1924, at Johns Hopkins University; over time, the otopathology laboratories—considered by some authors as "gold mines" for studying ear diseases—became numerous and very prolific. However, today, only 3 of the temporal bone laboratories are still running and producing scientific knowledge to the Otology/Neurotology field: the ones at Harvard Medical School, University of Minnesota, and University of California. Molecular biologic assay techniques and new microscopy and computer equipment broadened the possibilities for temporal bone studies; however, the current funding for those laboratories are insufficient to cover the costs for processing and studying human temporal bones. The main objective of this study is to briefly describe the history, current situation, and future perspectives of the otopathology laboratories in the United States.

Keywords

Pathology; Temporal bone; Otolaryngology; Laboratories; History; Genomics; Proteomics; Otopathology

Corresponding Author: Rafael Monsanto, MD, Ph.D. Student, Otopathology Laboratory, Department of Otolaryngology, University of Minnesota, Minneapolis, Minnesota, 2001 6th St. SE, Lions Research Building, Room 210, Minneapolis, MN 55455, rafaelmonsanto@hotmail.com.

Conflict of interest: None

Financial disclosures: None

Introduction

Since the 16th century, many researchers have dedicated to study the temporal bone in depth.[1–4] In the late 19th century, the growing need to understand the physiopathologic basis of many diseases affecting the ear, associated with the popularization of the processing methods of human tissues, has stimulated otolaryngologists and pathologists to establish research facilities dedicated to the development and study of otopathology.[1–5] Over time, the studies published by these laboratories have documented the pathophysiologic basis of most pathologic conditions and diseases affecting the ear.[1–5] The objective of this study is to briefly describe the history, current situation, and future perspectives of the otopathology laboratories in the United States.

Histopathologic analysis of human temporal bones

The tissue processing methods of temporal bone specimens has substantially evolved in the last 3 centuries, following the introduction of new technology and equipment.[3–5] Microscopic study of the middle and inner ear was made possible with the development of an advanced processing method, which allow maximum preservation of structures: fixation of harvested temporal bones with formalin, decalcification with ethylenediaminetetraacetate (EDTA), dehydration using increasing concentrations of alcohol, and embedment in celloidin.[2–6] The embedded temporal bones (Fig.1A) are serially sectioned at 20 micrometers using a sliding microtome, and the sectioned samples are later stained and mounted in glass slides (Fig. 1B). Processing a temporal bone is a crafty task, involving many possible artifacts that may damage the valuable specimens; therefore, only well-trained and experienced technicians can recognize those flaws and prevent irreparable damage to the bones.[6] To achieve such level of skill, years of extensive training are needed – it is unlikely one will become an expert by using written material only.[5–7]

Professors Stacy Guild,[8] in the 1920s, and Harold Schuknecht,[4] in the 1960s, developed methods for cytologic descriptions and quantifications of the inner ear structures. Over the years, technologic advances have further broadened the possibilities regarding study of temporal bones: today, it is possible to scrutinize archived and fresh sections under different microscopy modalities (such as transmission, electron, and differential interference contrast/ Nomarski microscopes),[4,9] to perform immunostaining, immunohistochemistry, polymerase chain reaction (PCR), molecular biologic assays, proteomic analysis, and to virtually manipulate and perform 3-D reconstructions of the specimens.[3,8,10–12]

Otopathology laboratories in the United States

In 1924, Dr. Samuel J. Crowe and Dr. Stacy R. Guild stablished the first Otopathology Laboratory in the United States, at Johns Hopkins University.[8] Since its foundation, the laboratory at Hopkins University published several papers demonstrating histopathologic correlates of several ear diseases.[8] One of Dr. Guild's trainee was Harold Frederick Schuknecht (1917–1996), who later promulgated the further development of otopathology research at Henry Ford Hospital and Harvard University.

Professor Schuknecht established the otopathology laboratory at Massachusetts Eye and Ear Infirmary (Harvard University), in 1961.[4,11] The contributions of Dr. Schuknecht and his peers had a great impact in the practice of otology and otopathology in the latter half of the 20th century.[4,11] Over the last 4 decades, the archived temporal bone collection at Harvard University has grown substantially (it contains now over 2000 specimens), and their studies approached several aspects of temporal bone anatomy and pathology.[11]

In parallel, in the 1960s, an otopathology laboratory was founded in California – the "Temporal Bone Laboratory of the House Research Institute".[13] Over the years, the laboratory has published several papers demonstrating pathologic changes secondary to many conditions affecting the ear, especially otosclerosis and Meniere's disease.[13] The laboratory at the House Ear Institute has later merged with the "Temporal Bone Laboratory at University of California", giving rise to the "NIDCD National Temporal Bone Laboratory".[13,14] The laboratory at UCLA, directed by Professor Fred Linthicum, currently contains over 2000 human temporal bones.[13]

In the late 1960s, Professor Michael Paparella (who was one of Dr. Schuknecht residents at Henry Ford Hospital) was named the head of the newly founded "Otopathology Laboratory" at the University of Minnesota.[5] Professor Paparella and his peers published, over the years, several papers approaching histopathologic aspects of pathologic and physiologic conditions of the ear.[5] They also performed translational studies, collaborating with the otolaryngology, pediatric, and other departments of the University of Minnesota and other institutions as well – those studies gave clinical meaning to many histopathologic findings demonstrated in human temporal bones.[5] Many otolaryngologists, researchers and medical residents from several countries have actively contributed with those studies – since its inauguration more than 30 years ago, more than 200 fellows have used the archived temporal bone collection to perform research or even to complete their PhD dissertation.[5] Today, the collection at the University of Minnesota contains more than 2,000 human temporal bones; it houses the largest otitis media collection in the world (Fig. 2).[5,7,9]

In the 1960s and 1970s, many other temporal bone laboratories initiated their activities, reaching their peak in the 1980s – by then, in the United States alone, 28 laboratories were open and producing scientific knowledge for the otology/neurotology community.[6,14] Those laboratories were considered by some authors as "gold mines" for studying ear diseases: the studies involving otopathology provided, over the years, the grounds on which the knowledge of almost every pathologic condition affecting the ear has been built.[6,7]

Otopathology laboratories: current situation

From the 28 otopathology laboratories established in the USA in the 1980s,[6,14] only 3 are fully operating today: the otopathology laboratories at Harvard Medical School, University of Minnesota, and University of California (Fig. 3) (for a full list of active and inactive laboratories, see https://www.tbregistry.org/About-Us/Where-We-Are).[6,7] The history of the inactive/closed institutions, as well as their temporal bone archives, are kept by the "National Temporal Bone, Hearing and Balance Pathology Resource Registry" ("The Registry"), a non-profit organization founded in 1992 by the National Institute on Deafness

and Other Communication Disorders (NIDCD) of the National Institutes of Health (NIH).[14] The Registry maintains temporal bone sections and information of approximately 7,821 cases from 23 different laboratories.[14] In addition, the Registry actively prospects and enrolls possible temporal bone donors, and keeps a 24-h nationwide network that coordinates collection of temporal bones after a donor's death. Merchant et al.[4] estimated, in 2008, that over 5,000 individuals have been recruited as temporal bone donors by the Registry, with retrieval of over 700 temporal bone specimens. The harvested specimens are randomly distributed to the currently active facilities in the United States.[14]

Although the Registry has solved many issues regarding the enrollment of donors, distribution of the specimens and maintenance of current collections, several otopathology Laboratories become inactive in the past decades, and 2 of the 3 remaining laboratories are in risk of closing.[6,7] The reasons for the "crisis"[6] in otopathology are complex: they include critical shortage of trained and committed scientists dedicated to otopathology research, difficulty competing for funding, and escalating costs of the materials used in the processing of temporal bones – processing a pair of human temporal bones may cost $3.000[7]; in addition, it may take up to a year to process a single temporal bone.[3–6] Thus, although there is financial support for retrieval of temporal bone specimens, the costs for maintaining the laboratories and allowing preparation of the specimens for preliminary pathologic study before a hypothesis-driven grant request is submitted to NIH are not sufficiently covered by the current available funding mechanisms.[6]

Due to the combination of the difficulties involved in the processing of temporal bones and the decrease in the number of active temporal bone laboratories, concerns were raised regarding the future of otopathology.[6,7] In 2010 and in 2012, Professors Michael Paparella, Richard Chole, Michael McKenna and Joseph Nadol reported that whilst there is (limited) funding for hypothesis driven otopathology research, none is directed to cover the costs of temporal bone collection and processing.[6,7,15] Those researchers convened, in 2012, an "Otopathology task force", aiming to circumvent the funding issues by proposing several actions to be taken: (1) to actively prospect renewable and stable funding sources directed to basic expenses of otopathology research; (2) encourage expansion of existing facilities and establishment of new laboratories; and (3) institute an annual lectureship to report the current status and discuss the future of otopathology.[15] Since then, a few private companies and individual efforts, in conjunction with existing organizations dedicated to support ear research (such as the NIDCD and the "International Hearing Foundation"), addressed additional financial resources to otopathology laboratories.[6,15,16] Despite those initiatives, some of the remaining temporal bone laboratories are still striving to remain financially stable; Thus, insufficient funding seems to constitute the most urgent matter regarding the future of otopathology in the United States.[6,7,15] We agree with the recommendation of other authors[6,7,15] that renewable and stable funding sources directed to cover the basic costs of the remaining otopathology facilities with processing and studying human temporal bones are needed. That should be part of a long-term commitment by funding agencies (such as the National Institute on Deafness and Other Communication Disorders - NIDCD) and otolaryngology organizations (such as the American Academy of Otolaryngology-Head and Neck Surgery, the American Otological Society, and the American Neurotology Society) to

protect and ensure continuity of the activities of the few remaining Human Temporal Bone laboratories.[6]

Next steps and future perspectives

While light microscopic study of human temporal bones has profoundly influenced the knowledge of otologic disorders (and will continue to be important in the future), significant gaps remain in the understanding of their basic molecular and pathophysiologic mechanisms: for example, from over 100 genetic loci and at least 64 genes identified in association with non-syndromic hearing loss, only 3 have been well characterized at the otopathological level: DFNA9, DFNA17, and DFNB1.[4,14,15] But several new developments raised hopes of filling those gaps in the near future: adapted techniques for molecular biologic analyzes (immunohistochemistry, electron microscopy, PCR, real time-PCR, cDNA microarrays, RNA and gene expression, proteomic analysis, in-situ hybridization, and more) are now feasible in archived temporal bone specimens.[3,7,8,12–14] In addition, the prospective enrollment of potential donors performed by the Registry enables collection of biologic material from the patients, such as biopsy, blood and oral swab samples, which may be preserved for later molecular studies.[4,11,14] Therefore, molecular biologic analyses, in conjunction with otopathologic evaluation, may lead to a broader understanding of basic pathophysiologic mechanisms of several syndromic and non-syndromic disorders affecting the ear.

Another promising possibility brought by the Registry's enrollment system is selection of potential donors who underwent ear surgery. Acquisition of specimens with detailed clinical histories and surgical descriptions may yield additional post-operative information, suggest technical refinements, and indicate possible surgical prognostic factors.[6,11,15] This is a matter of great interest, considering the scarcity of well-documented specimens from donors who underwent several common otologic procedures, such as cochlear implants and surgery for otosclerosis and Meniere's disease. The cochlear implant surgery constitutes a clear example of how otopathologic studies may be the underpinning of further enhancements in surgical procedures: more than 100.000 cochlear implants have been inserted in the United States so far, but less than 200 specimens are available for otopathologic evaluation.[14] Otopathologic studies of implanted specimens (in association with detailed descriptions of the procedures and hearing/performance tests) may provide answers to many unsolved questions, including: (1) which electrode array model and/or surgical technique allow less traumatic surgery and best preservation of cochlear architecture (less inner ear inflammation, fibrosis, ossification, and damage to the sensorineural structures) and (2) which anatomic, histologic and surgical factors associate with operative and postoperative complications, residual hearing preservation, and functional results.[3,8,11,13–16] Thus, otopathologic evaluation of temporal bones from donors who underwent ear surgery may generate technical and surgical enhancements and development of more pathology-oriented procedures. [3,4,6,8,11,13–16]

Taken together, continued study of human temporal bones is warranted for several reasons: [4,6] (1) mechanisms of several diseases (including genetic mutations) affecting the ear, auditory and vestibular disorders have not yet been elucidated; (2) findings of temporal bone

studies will help to develop new pathology-oriented treatments, interventions and surgical procedures; (3) they allow comparative analysis between temporal bones affected by specific ear diseases and specimens from donors with normal (or age-appropriate) hearing and balance function; and (4) the prospective enrollment process conducted by the Registry has brought new possibilities for translational and basic research.

Conclusion

Our study briefly describes the history, current situation and future perspectives of otopathology in the United States. The remaining active facilities are still producing new basic and translational research every year. Molecular biologic assay techniques and new microscopy and computer equipment have broadened the possibilities for temporal bone studies. However, the lack of funding for running the few remaining human temporal bone laboratories seem to constitute the most urgent matter regarding the future of otopathology in the United States. A long-term commitment from funding agencies and otolaryngology organizations and also stable and renewable funding sources directed to cover basic expenses of the laboratories are needed.

Acknowledgments

Source of Funding: This project was funded by NIH NIDCD U24 DC011968, International Hearing Foundation, Starkey Hearing Foundation, Lions 5M International, Coordenadoria de Aperfeiçoamento Pessoal de Nível Superior (CAPES), and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)

We are grateful to Ms. Mary E. Knatterud, PhD, Thais Abrahão, M.D., PhD student, and Grace Park, B.S., for critically reviewing the manuscript. We also thank our supporters: National Institute on Deafness and Other Communication Disorders (NIDCD) of the U.S. National Institutes of Health (NIH); the International Hearing Foundation; the Starkey Hearing Foundation; the Lions 5M Hearing Foundation; Coordenadoria de Aperfeiçoamento Pessoal de Nível Superior (CAPES); and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)

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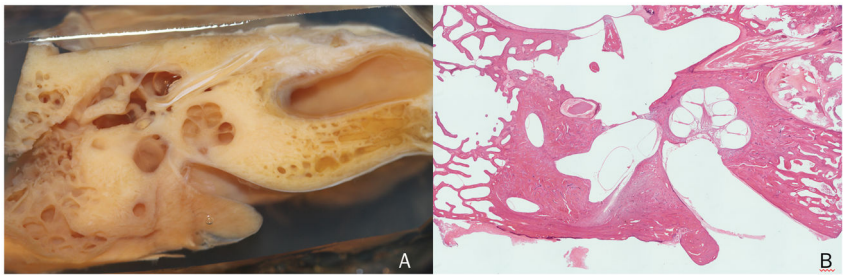

Figure 1.

A: A temporal bone specimen (epitympanic view) embedded in celloidin, ready for horizontal sectioning. B: A representative horizontal section of a human temporal bone, stained with Hematoxylin and Eosin (x1).

Figure 2.
Photographs of (A) the archived human temporal bone collection at the University of Minnesota and (B) archived temporal bone slides

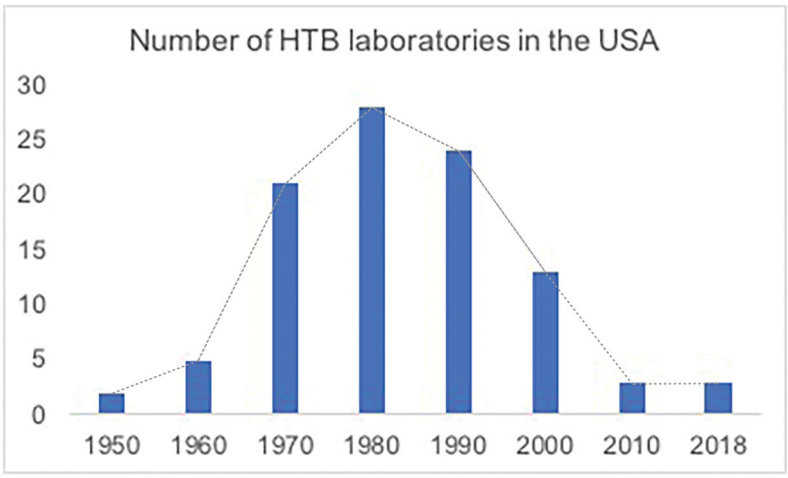

Figure 3.
Number of active Human Temporal Bone Laboratories in the United States over the decades