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Quantitative Assessment of Vestibular Otopathology in Granulomatosis With Polyangitis: A Temporal Bone Study

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Objective: To investigate the temporal bone histopathology of vasculitis, especially in the vestibular organs, in granulomatosis with polyangitis (GPA).

Methods: Using light and differential interference contrast microscopy, we examined 12 human temporal bones from six deceased GPA patients and 12 histopathologically normal human temporal bones from six deceased age-matched patients.

Results: In the GPA group, three patients had undergone tympanostomy tube placement. Two of them had suffered mixed hearing loss; one, sensorineural hearing loss; and one, conductive hearing loss. Of the 12 specimens in the GPA group, the granulation tissue invaded the round window niche in seven; cochlear hair cells were not preserved in five. Hemosiderin was deposited in the stria vascularis in eight specimens, in the ampulla or semicircular duct in 10, and in the vestibule in three. The spiral ligament showed severe loss of cellularity in two specimens. In the GPA group, type I vestibular hair cell density was significantly decreased; however, type II vestibular hair cell density did not significantly differ between the GPA group and the control group.

Conclusion: Our histopathologic findings in human temporal bone specimens of GPA patients delineated changes in the tympanic membrane, middle ear cavity, round window membrane, organ of Corti, stria vascularis, spiral ligament, ampulla, semicircular duct, and vestibule. Type I vestibular hair cell density significantly decreased in the GPA group, as compared with the control group.

Key Words: Granulomatosis with polyangitis, vertigo, vestibule, hair cells, temporal bone.

Level of Evidence: N/A

INTRODUCTION

Granulomatosis with polyangitis (GPA), also known as Wegener's granulomatosis, is a systemic autoimmune disease. A combination of granulomatosis and polyangitis, it primarily affects the upper airway, lower airway, and kidney (with less frequent involvement of other organs). Otologic findings, such as otitis media, hearing loss, vertigo, and facial paralysis, are seen in 56% to 60% of GPA patients.[1,2] Most (85%) GPA patients have problems with the nose or paranasal sinuses.[3,4]

Only a few reports have described cochlear findings in temporal bones from deceased GPA patients; to the best of our knowledge, no previous studies have investigated the changes in the vestibular sensory neuroepithelium. In this quantitative study, we investigated histopathologic changes in the middle ear cleft and inner ear, especially in the vestibular organs, in temporal bones donated by deceased GPA patients.

MATERIALS AND METHODS

Specimens

We examined 12 human temporal bone specimens (eight from the collection of the University of Minnesota and four from the collection of the Massachusetts Eye and Ear Infirmary and Harvard Medical School) donated by six deceased GPA patients (the GPA group). We also analyzed those patients' medical histories. Their mean age was 59.5 ± 14.1 years (range, 36 to 72 years). In addition, we examined 12 histopathologically normal human temporal bone specimens from six age-matched patients (the control group). Their mean age was 61.8 ± 14.7 years (range, 36 to 78 years). In both facilities as well, the temporal bones had been removed at autopsy, fixed with 10% formalin, decalcified with ethylenediaminetetraacetic acid, and embedded in celloidin. Then, each temporal bone was serially sectioned in the horizontal plane at a thickness of 20 μm, with every tenth section stained with hematoxylin and eosin. We examined the specimens under light

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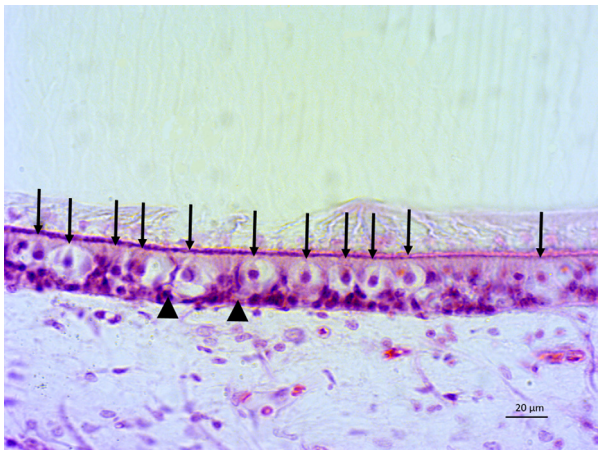

Fig. 1. Horizontal section of a human temporal bone, seen under differential interference contrast microscopy. Vestibular hair cells types I and II in the macula of saccule. *Thin arrows* type I vestibular hair cells; *Arrow heads* type II vestibular hair cells

and differential interference contrast microscopy (Axiocam, Carl Zeiss X-ray Microscopy, Pleasanton, CA, USA).

This study was approved by the institutional review board of the University of Minnesota (#0206M26181).

Qualitative Histopathologic Aassessment

We assessed the tympanic membrane, epitympanum, mesotympanum, round window membrane and niche, facial nerve, internal auditory canal, cochlea (outer and inner hair cells, spiral ligament, stria vascularis and spiral ganglion cells), semicircular canals, utricule, and saccule.

Vestibular Hair Cell Density

We assessed the density of vestibular hair cells using the criteria described by Merchant.[5,6] Using differential interference contrast microscopy at high magnification, we distinguished vestibular cells by their morphologic characteristics. We used that method to measure the density of type I and II hair cells in each vestibular sense organ. Type I cells are flask-shaped, with a spherical nucleus, and are surrounded by a nerve chalice. Type II cells are shaped like a cylinder, with a cylindrical nucleus, and do not have a nerve chalice (Fig. 1).

Vestibular hair cell counts were made in all potions of the sensory epithelium of each vestibular organ in which the plane of section was perpendicular to the surface of the sensory epithelium. In the saccular macula, in which all of the sections through the saccule was perpendicular, counts were performed on the three middle sections.[5,6] Because the anterior semicircular canals were opened before perfusion, we did not analyze them in this study.

We calculated the vestibular hair cell density, defined as the number of hair cells per 0.01 mm^2. To determine surface area, we multiplied the thickness of the section (20 µm) by the length of the sensory epithelium where the count was made. We separately counted type I and type II hair cell types that had a visible nucleus within an area 500 µm wide. In human vestibular neurosensory epithelium, type I and type II hair cells are not homogeneously distributed and there exists significant regional differences.[7] Further, in our specimens substantial postmortem artifacts exist. In order to minimize these effects, we randomly selected 10 sites of 50 µm from the specimens that were suitable for cell counts.

To avoid double-counting in our raw hair cell counts, we corrected our calculated hair cell density by using Abercrombie's formula: $Hi = hi \times t/(t+d)$ where Hi = the corrected density of hair cells, hi = the raw density, t = the thickness of the section (20 µm), and d = the mean value of nuclear diameters in 250 vestibular hair cells. That formula results in a correction factor of 0.82 for type I hair cells and of 0.87 for type II hair cells.[8]

Statistical Analysis

Results of our quantitative vestibular assessment are presented as the mean ± standard deviation (SD). To compare the GPA group and the control group, we used the nonparametric Mann-Whitney U test (SPSS 22.0 for Windows, SPSS Inc, Chicago, IL, USA). We defined significance as $P < .05$.

RESULTS

There was no difference in specimen in both facilities. Of the six patients in the GPA group, three had undergone tympanostomy tube placement for serous effusion in the middle ear. In all, two had suffered mixed type; one, sensorineural; and one, conductive hearing loss (Table I). We unfortunately did not have available any clinical vestibular testing data.

Of the 12 temporal bone specimens in the GPA group (Table II), the granulation tissue invaded the round

TABLE I.
Summary of Clinical Findings.

Patient	Age/Sex	Determinnants of GPA Diagnosis	Non-otologic organ Involvement	Hearing Loss	Vertigo	Facial Paralysis	Treatment for GPA	Lorcal Treatent
1	71/M	Kidney and muscle boipsy	Renal	Mixed	NR	No	Prednisone	Bilateral tubes
2	57/F	Lung biopsy	Nasal, pulmonary, renal	Mixed	NR	No	Cyclophosphamide and Steroid	Bilateral tubes
3	72/M	Lung biopsy	Pulmonary, renal	Yes Type NR	NR	NR	Cyclophosphamide and Methylprednisolone	-
4	52/M	Autopsy	Pulmonary, renal	NR	NR	NR	NR (treated in the past)	-
5	36/M	Nasal biopsy	Nasal, renal	Conductive	NR	NR	None	Bilateral tubes
6	69/F	Nasal biopsy	Nasal, renal	Sensorineural	Yes	NR	None	No

GPA indicates granulomatosis with polyangitis
NR = not recorded.

TABLE II.
Summary of Histopathologic Findings.

Patient	Side	Tympanic Membrane	Tympanic Cavity	Round Window Niche	Round Window Membrane	Facial Nerve	Internal Auditory Canal	Hair Cells	Stria Vascularis	Spiral Ligament	Cochlear Neuron Population	Semicircular Canals	Vestibules
1	Right	-	+	-	-	-	-	+	+	+	+	+	+
	Left	+	+	-	-	-	-	+	+	+	+	+	+
2	Right	+	+	+	+	-	-	-	+	-	+	+	-
	Left	+	+	+	+	-	-	-	+	-	+	-	-
3	Right	+	+	+	-	-	-	+	+	-	+	+	+
	Left	+	+	-	-	-	-	-	+	-	+	-	-
4	Right	-	-	-	-	-	-	-	-	-	-	+	-
	Left	-	-	-	-	-	-	-	-	-	-	+	-
5	Right	-	+	+	+	-	-	-	-	-	-	+	-
	Left	-	+	+	+	-	-	-	-	-	-	+	-
6	Right	-	-	+	+	-	-	+	+	-	+	+	-
	Left	-	-	+	+	-	-	+	+	-	+	+	-

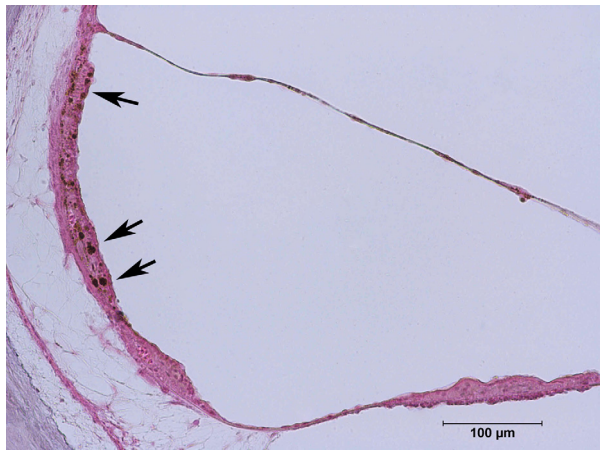

Fig. 2. Case 1. The scala media in the right ear in the middle turn of the cochlea. Hemosiderin deposition was seen *(arrows)*.

window niche in seven of them. Cochlear hair cells were not preserved, and/or the organ of Corti was atrophic, in five specimens. The vessels of the stria vascularis were thickened or occluded, and/or hemosiderin was deposited, in eight specimens (Fig. 2). The spiral ligament showed severe loss of cellularity in two. Hemosiderin was deposited, and/or inflammatory cells were present, in the ampulla or semicircular duct in 10. In the right ear of patient 6, the ampulla of the posterior canal was partially collapsed, with areas of hemorrhage in the perilymphatic space of the vestibule. Hemosiderin was deposited in three utricles and one saccule (Fig. 3).

In the GPA group, the mean density of type I vestibular hair cells was significantly decreased, as compared with the control group (lateral semicircular canal: $P = .005$, posterior semicircular canal: $P = .010$, utricle: $P = .012$, saccule: $P = .001$) (Fig. 4). However, the mean density of type II vestibular hair cells did not significantly differ between the two groups (lateral semicircular canal: $P = .318$, posterior semicircular canal: $P = .204$, utricle: $P = .163$, saccule: $P = .087$).

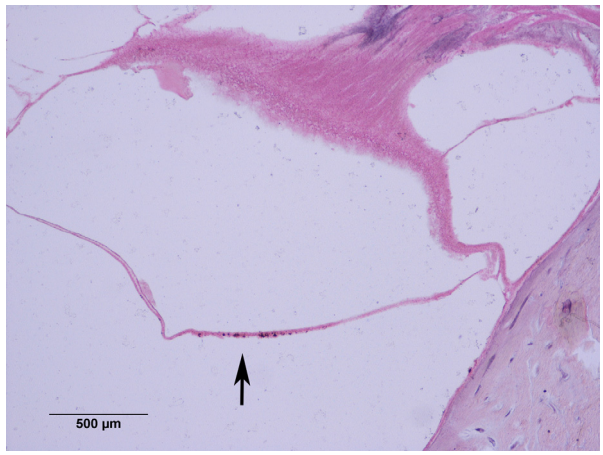

Fig. 3. Case 1. The utricle in the left ear. Hemosiderin deposition was seen *(arrow)*.

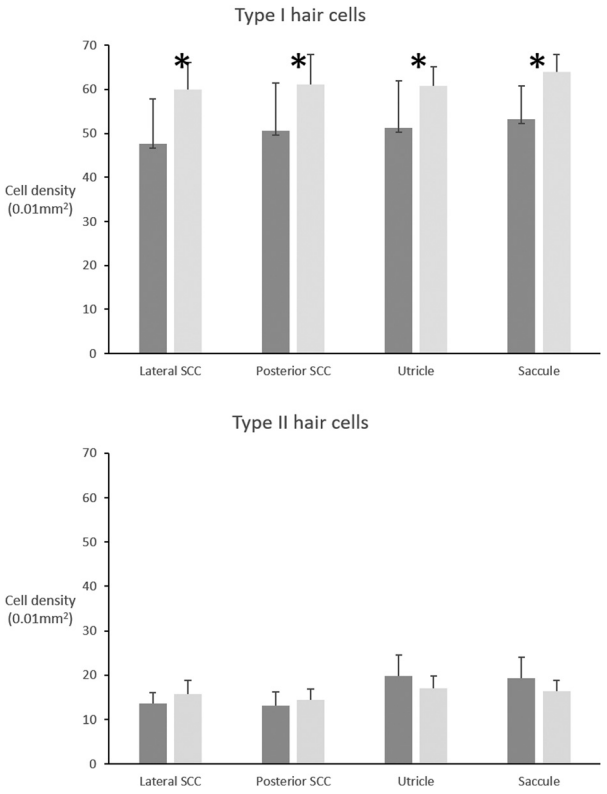

Fig. 4. A diagram showing the density of vestibular hair cells (upper, type I; lower type II) between the 2 groups (dark grey, granulomatosis with polyangitis patients; grey, control patients) in our study. SCC, semicircular canal. *Statistically significant difference between groups ($P < .05$).

DISCUSSION

It has been known that granulation tissue and bone absorption in the middle ear, with invasion of the inner ear through the round window have been commonly encountered and may cause hearing loss of any type: conductive, mixed, or sensorineural in GPA cases.[9] In those with conductive hearing loss, otitis media with effusion is the most common otologic manifestation[10,11]; it results from eustachian tube obstruction and/or nasopharyngeal involvement.[12] Because GPA can involve the middle ear and mastoid cavity,[9] granulation tissue can also be the cause of conductive hearing loss.

In our GPA group, patient 5 had conductive hearing loss due to middle ear lesions without any cochlear pathologies. Patients 1 and 2 had mixed-type hearing loss, clearly showing tympanic cavity and/or tympanic membrane lesions, cochlear hair cell losses, and cochlear neuron degeneration. In patients who have not progressed to complete deafness, sensorineural hearing loss that is due to the interaction between the middle ear and inner ear or to dysfunction of microcirculation in the inner ear can be reversed with immunosuppressive treatment.[2,13–15] Patient 6 had sensorineural hearing loss with no evidence of any tympanic cavity lesions, but did have hair cell loss and cochlear neuron degeneration.

For the patients in our study, we unfortunately did not have available any laboratory test results with regard to proteinase 3 anti-neutrophil cytoplasmic antibodies (PR3-ANCA) and myeloperoxidase anti-neutrophil cytoplasmic antibodies (MPO-ANCA). Other investigators found that the presence of granulation tissue and thickened mucosa in the middle ear and tympanic membrane was more common in patients with positive PR3-ANCA test results, but that patients with positive MPO-ANCA test results predominantly had otitis media with effusion.[15,16]

In our study, we did not observe progression of granulation from the middle ear into the inner ear through the round window membrane, although that sequence has been previously reported.[9]

Even though the exact mechanisms of sensorineural hearing loss in GPA patients are not clear, studies describe several mechanisms including vasculitis, deposition of the immune complexes in the cochlea, or toxic effects of inflammatory byproducts passing through the membranes into the inner ear.[1,17,18] In addition or instead, it might be due to pressure on the acoustic nerve by granulomatous lesions.[11,19] We confirmed that GPA can also affect the inner ear, including the stria vascularis and capillaries,[13,15] as previously reported by others. In eight specimens in our GPA group, the vessels of the stria vascularis showed vasculitis, were thickened or occluded, and/or hemosiderin was deposited. In two specimens, the spiral ligament showed severe loss of cellularity. In five specimens, hair cells were not preserved and/or the organ of Corti was atrophic.

In our GPA group, hemosiderin was deposited in the semicircular canal in 10 specimens and in the vestibule in three. Only one patient had suffered clinical vertigo; that patient's specimen showed partial collapse in the ampulla of the posterior canal on the right side, with areas of hemorrhage in the perilymphatic space of the vestibule. Although vertigo is uncommon in GPA patients,[18] it can be caused by immune complex deposition or acute hemorrhage in the vestibular portion, and/or central nervous system involvement caused by polyneuritis.[20] The reason for rare vertigo in those patients is likely due to the bilateral slow disease process in vestibular system that triggers central compensation. However, if an acute hemorrhage or asymmetrical involvement occurs, then clinical vertigo may be obvious. Regarding vestibular hair cells, type I vestibular hair cell density was significantly decreased in our GPA group, as compared with our control group. Such a decrease also occurs in patients with systemic lupus erythematosus (SLE), a chronic inflammatory autoimmune disease.[21]

None of our patients had facial nerve palsy. Nor did we find any evidence of vasculitis in the facial nerve. In the literature, facial nerve palsy has been reported in 8% to 10% of patients, usually associated with otitis media.[22,23]

Our study confirmed that GPA patients have more cochlear symptoms than vestibular symptoms. Schuknecht et al.[24] reported that temporal bone specimens from an aged cat, dog, and human (all suffering from slowly progressive deafness) revealed atrophic changes in the cochlea and saccule, with a normal utricle and normal semicircular canals. Their study demonstrated that the

pars inferior (cochlea and saccule) abides by the biologic rule that the phylogenetically newer systems in an organism are more susceptible to injury. The cochlea and saccule likely share a common denominator that makes them jointly susceptible to certain genetic alterations, viral diseases, and aging.

CONCLUSION

In this study, we described the histopathology of temporal bone specimens from deceased GPA patients. Our otologic findings included otitis media, hearing loss, and vertigo. Our histopathologic findings delineated changes in the tympanic membrane, middle ear cavity, round window membrane, organ of Corti, stria vascularis, spiral ligament, ampulla, semicircular duct, and vestibule. Type I vestibular hair cell density was significantly decreased in the GPA group, as compared with the control group.

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