7th International Symposium on

Meniere's Disease
and Inner Ear Disorders

Ménière's Disease and Inner Ear Disorders:
Update 2015

Edited by
Maurizio Barbara

PROCEEDINGS OF THE 7TH INTERNATIONAL SYMPOSIUM ON MÉNIÈRE'S DISEASE AND INNER EAR DISORDERS

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Table of Contents

Genetics/Proteomics

Vestibular issues

Imaging

Dehiscence syndrome

Clinical issues

Treatment

Intratympanic treatment

Surgery

Cochlear and vestibular implant

Preface

For a mono-thematic meeting that gathers all the experts in the field every five years, one would expect to find many novelties and clarifications that had the right time to develop and to be validated. We all know that this would be unlikely to occur for Ménière's disease, one of the most intriguing, somewhat frustrating pathologies that, over more than 150 years since its first description, has kept scientists and dedicated professionals busy for any development that would be helpful for curing our affected patients.

This book contains a collection of contributions that were delivered at the VII International Symposium on Ménière's disease and Inner Ear Disorders, held in Rome on October 17 to 20, 2015, and that were addressing many of the known, but also some of the unknown aspects of this inner ear disorder. Among them, a particular interest has been solicited by the *genetic issue* involving the inner ear in general and that will surely represent in the future one of the new pathogenetic and diagnostic lines to pursue for the study of the Ménière population. A great impact is also foreseen by the routine application of specifically-designed *imaging procedures* that would enable to confirm the presence of endolymphatic hydrops as the real pathognomonic finding of the disease, and to follow-up the affected subjects during its clinical course, along with the role played by the different therapeutical approaches for it. The treatment of Ménière's disease seems also to have taken a straightforward direction towards the *intratympanic delivery* of drugs, where steroids appear to be promising molecules in this regard, without running particular risks for an additional inner ear damage, as it could be possible when ototoxic molecules are used.

Noteworthy is also the possibility for a self-administered local *pressure treatment* to positively act on the disease, allowing to avoid to resort to any surgical procedure for its resolution. The final part of the contributions that have highlighted the Meeting, and consequently this book, is devoted to the rehabilitation of the affected population from the most frequent irreversible damage caused by a long-lasting disease, *i.e.*, deafness. The proposal of *cochlear implantation*, not only in the classical bilateral cases that would be rare for this disease, but also for the unilateral forms related to a single-sided deafness, is clearly seen as a promising tool to apply for restoration of the binaural auditory function, a problem that, for many years, has been left neglected and untreated.

A real novelty for this monothematic Meeting is represented by the report of a possible *vestibular prosthesis* that would enable mostly the bilaterally-impaired subjects to restore their balance disorder.

In summary, with all the limitations that a book containing contributions from a Meeting nowadays carries in term of scientific impact, it is the editor's wish to promote it so as it could serve, in analogy with the publications of the previous

Meetings, as a guide for the young colleagues who wish to get knowledge of Ménière's disease, as a cue for the development of new research lines, or – at least – as a memory of those fascinating days spent in the Eternal City to place on your crowded, hopefully not dusty, shelf.

Prof. Dr. Maurizio Barbara
President of the VII International Symposium

CAUSE, PATHOGENESIS AND SYMPTOMS OF MÉNIÈRE'S DISEASE

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Meniere's disease (MD) is a variable complex disease, which in most cases is managed medically. In one out of eleven patients in our clinic, the disease progresses into intractability and the patient becomes incapacitated and can lose his or her job and ability to drive a car. This is where surgery becomes a necessary option. A basic tenet in medicine is that conservative treatment, whether medical or surgical precedes more radical or destructive treatment. This is especially true for MD. If we can understand a disease and in lay terms explain it to the patient, this becomes an important part of the treatment process. For decades many, and some currently have used the defeatist term 'idiopathic endolymphatic hydrops', to describe MD as a mantra. 'Idiopathic' refers to a disease of unknown cause. MD is neither idiopathic, nor is its pathological correlate, endolymphatic hydrops. Rather it is a clinical entity, which renders the patient difficulty and distress.

My favorite term and concept towards understanding of a disease is, 'pathogenesis', which applies to all otological diseases, in general and in particular within this context to MD. A simple dictionary definition of 'pathogenesis', is 'the development of a diseased condition'. A more specific definition includes the etiology of the disease and the processes or mechanisms which transpire towards the pathological state, which when accompanied by symptoms, for example deafness, vertigo, etc., leads the patient to seek consultation with a physician. There is much we do not know regarding this complex disease, but clinical and research observations provide evidence which allows us to better understand this disease.

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In 1984, at a Collegium Meeting in Basil, Switzerland, with some trepidation I presented a paper describing the etiology, pathogenesis and pathophysiology of symptoms in MD. The paper, published in 1985, was entitled, 'The cause (multifactorial inheritance) and pathogenesis (malabsorption of endolymph), of Meniere's disease and its symptoms) (mechanical and chemical)'.[1] While it was discussed by Schuknecht; Bill House and the professor in Basil, they did not challenge its basic tenets. The most important cause was genetic, but I was unable to find other publications discussing the genetics of MD at that time. Subsequently, a Pub Med Research revealed more than 90 published articles describing the relationship of genetics to MD. The majority of these studies especially in recent years appeared in basic science journals such as *American Journal of Genetics*; *Human Genetics* or *International Journal of Immunogenetics.*

As we have described and published in the past, there are diseases that can predispose to endolymphatic hydrops and Meniere's disease. These include advanced otosclerosis with otosclerosis obstructing the vestibular aqueduct,[2,3] chronic inactive otitis media and mastoiditis,[4] syphilis,[5] delayed MD symptoms and endolymphatic hydrops from childhood measles or mumps.[6] In unpublished observations, I have witnessed a number of patients who had sudden deafness (usually due to a viral endolymphatic labyrinthitis), who years later developed increased pressure; tinnitus and vertigo, who responded well to medical, and in some cases surgical therapy.

The majority of patients who have no observable or related disease as a precursor to MD have, I believe, a genetic predisposition. In the aforementioned article published in 1985 there were two major findings, which lead to the concept of inheritance.

I reported the findings of 500 patients with MD. Colleagues, residents and fellows routinely have asked about a family history in patients with otosclerosis, but would not ask about a family history in patients with MD, as we did. We observed that upwards to 20% of patients had a family history. A recent preliminary study of ours shows 17.6% or 67 out of 380 patients demonstrating inheritance (unpublished data). Klockars and Kentala described similar results.[7] It is important for all otologists to ask about a family history in patients with MD.

After my publication in 1985, Birgerson *et al*.[8] in 1987 describes a genetic investigation of familial MD, and later Morrison *et al*.[9] in 1994, and in other publications described genetic aspects of MD. Subsequently, many articles have appeared in genetic journals, *e.g*., Fung *et al*.[10] in 2002; Frykholm *et al*.,[11] 2006; Klar *et al*.,[12] 2006; Hietikko *et al*.,[13] 2012; Requena *et al*.[14] in 2014.

The next most important finding from that study and confirmed in thousands of cases to date are multiple anomalies found during endolymphatic sac surgery definitely seen in the vast majority of cases. These findings are not seen in normal mastoids or in many mastoidectomies for chronic mastoiditis to date. The findings include hypopneumatization of the mastoid, a sigmoid (lateral) sinus that is not lateral, but medial and anterior, hypo-development of the aditus and supra pyramidal recess and most importantly Trautmann's triangle, that plate of bone separating the sigmoid sinus from the posterior semi circular canal

which also separates the mastoid from the posterior cranial fossa. Trautmann's triangle may be oriented in a more vertical than horizontal orientation, but most importantly Trautmann's triangle is often reduced in size and can be absent in too many cases. In these patients the sigmoid sinus abuts against the bony wall of the solid angle containing the posterior semi-circular canal. In these cases it can be very difficult to gain access to the dura containing the endolymphatic sac beneath the solid angle.

What else could possibly explain these consistent anomalies except congenital genetically induced findings since birth? Thus inheritance becomes the most important etiological factor in MD.

After etiology the pathogenesis, I believed and still believe, is endolymph malabsorption. Why? Endolymphatic hydrops and the longitudinal flow of endolymph was demonstrated in animals in 1927 by Stacy Guild,[15] plus many studies by Valvassori and Dobben[16] demonstrating hypo-development of the vestibular aqueduct and endolymphatic sac and peri-aqueductal hypopneumatization around the vestibular aqueduct by Sando and Ikeda,[17] plus published examples of tumors and fractures involving the vestibular aqueduct.[18] In addition, stiffness of the endolymphatic sac and dura is commonly seen in MD patients and especially findings from endolymphatic sac revision cases all causing symptoms of MD.

While radial flow of endolymph is always important in chemical homeostasis of the membranous labyrinth in MD, I strongly believe longitudinal flow predominates. Pathology of obstruction and endolymph malabsorption seems logical, while recognizing other functions of the endolymphatic sac. In fact, I believe the reason the endolymphatic sac enhancement is successful in so many cases is a reversal of pathogenesis.[19]

Pathophysiology of symptoms of MD (vertigo, deafness, pressure, tinnitus, loudness intolerance), can be assessed and determined by animal studies; observations of patients clinically and in sequential histopathological studies in patients with MD. Any explanation of symptoms must take into account the many vagaries of MD symptoms including violent tornado-like episodes of vertigo; positional vertigo which occurs commonly in patients with MD, feeling of constant imbalance or disequilibrium and drop attacks which Bill House once told me he thought were due to rupture of the membranous labyrinth. Other symptoms, such as progressive fluctuating sensorineural hearing loss, pressure and tinnitus which can be severe also need to be accounted for.

Years ago at many meetings, it was enjoyable to hear the arguments and debates between Schuknecht from Harvard and Tonndorf from Columbia University. Those arguments are reminiscent of the debates between Gore Vidal and William F. Buckley. Now our discussions seem more subdued. Schuknecht believed symptoms of MD were due to rupture of the membranous labyrinth, while Tonndorf believed they were due to physical or mechanical alterations. Besides hydrops of pars inferior, we see hydrops of the pars superior, as well, with utricle enlargement. I recall one temporal bone in our collection where the utricle significantly bulges into the horizontal semicircular canal affecting the crista ampularis with likely physical effects. Membrane ruptures are seen in our

laboratory and other cases studied in the literature in some, but not most cases. This would have allowed for potassium rich endolymph to bathe the basal surface of hair cells as well as the eighth cranial nerve endings. Repeated exposure to toxic potassium levels could cause vertigo episodes and a decline in hearing.[20] Thus the pathophysiology of symptoms of MD results from the interaction of physical (mechanical) and chemical factors occurring simultaneously where one factor can dominate at a given point in time.

Reissner's membrane consists of separated endothelial cells, and a study of that membrane in our laboratory, MD cases show a stretching of the membrane to a much greater distance between cells.[21] By combining the concepts of Schuknecht and Tonndorf and including passage of electrolytes (potassium and sodium) across a permeable stretched or ruptured membrane, both chemical and mechanical factors are at play and explain not only a sudden symptom, but also the intervening chronic symptoms of MD.

As mentioned, studies indicate that up to 20% of MD are familial, suggesting a significant part of MD is inherited.[7] Although familial MD did not pursue classic Mendelian inheritance, it seemed to be transmitted in an autosomal dominant pattern, from a study by Oliveira and Braga[22] in 1992, with a penetrance of 60% and worsening of the symptoms with successive generations and an earlier age of onset.[11,23] Despite reports of familial cases,[10,11,13,24] no causative gene or genes have been found in large series of cases.

MD is a complex multifactorial disease and, as a consequence, the genetic contribution to MD is also complex. The single gene or the genes, potentially implicated may not be sufficient to determine the disease by themselves, so, in future research, we have to consider with great attention the combined effect of environmental factors on a susceptible genetic background and the possibility of gene interactions.

Today exome sequencing or even genome-wide studies have gained an elevated level of definition from recent technological advances in sequencing and in sequence analysis. The true problem of genome-wide association is mainly the costs of the evolving methods of statistical analysis in addition to the collection of larger well-defined case control groups.

Conclusion

Whereas our observations and studies are noted herein, other observations and studies can be more comprehensively observed in an invited publication in the Lancet.[19] From the above observations including thousands of patients treated medically and surgically and other observations regarding the etiology and pathogenesis and pathophysiology of MD, perhaps you can see why my publication 30 years ago, *The cause – multifactorial inheritance and pathogenesis; malabsorption of endolymph of Meniere's disease and its symptoms, mechanical and chemical*", is more valid than ever.

Acknowledgement

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PATHOLOGY

MÉNIÈRE'S DISEASE: HISTOPATHOLOGICAL ANALYSIS, NOVEL ULTRASTRUCTURAL, MOLECULAR AND MRI FINDINGS

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Abstract

In this study we performed a systematic histological and an ultrastructural analysis, complemented with molecular and immunohistochemical analysis in vestibular endorgans from labyrinthectomy in Ménière's disease (MD). Histological studies demonstrated varying degrees of degeneration, with the cristae more affected than the macula utricle. A monolayer of epithelial cells in the cristae was consistently observed; in contrast, the maculae utricle was better preserved. We also detected a pronounced basement membrane thickening in the crista as compared to the maculae utricle. Additional findings included hair cell and supporting cell vacuolization, stereocilia loss, and edema in the stroma. Transmission electron microscopic (TEM) analysis of these specimens showed disorganization of the basement membrane underneath the epithelia and normal morphology of nerve terminals and myelinated fibers. Further analysis of the vasculature showed novel ultrastructural changes in the blood labyrinthine barrier (BLB) not previously reported in the otology literature. In the BLB of capillaries in the vestibular endorgans stroma we found increased transcellular vesicular transport across vascular endothelial cells, pericyte detachment and perivascular basement membrane disruption in the stroma. Severe cases showed in addition, vacuolization of endothelial cells and/or complete atrophy. Ultrastructural alterations in the BLB from Meniere's specimens correlate with

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the stromal edema. These results suggest that alterations of the BLB maybe a contributing factor in the underlying physiopathology of MD. Recent studies using cellular and ultrastructural changes match well with our molecular biological studies in similar type of specimens. We have detected changes in the expression of several critical inner ear proteins: specifically down and upregulation of aquaporin 4 and 6 respectively (both expressed in supporting cells), upregulation of cochlin (expressed within the stroma) and a decrease in collagen IV and laminin-1b, important structural basement membrane and perivascular basement membrane proteins. Recently, objective quantitative criteria for EH using delayed intravenous gadolinium 3D fluid attenuation inversion recovery (3D-FLAIR) MRI has been developed. Using this technique, we found that the degree of EH strongly correlated with auditory hearing loss in MD subjects. MRI studies demonstrated increased gadolinium enhancement in the MD perilymph, indicative of BLB permeability. In conclusion MD is a complex syndrome that involves histological and molecular substrates that need to be further investigated to provide novel treatments for this debilitating disease.

Introduction

Meniere's disease is a disabling syndrome characterized by fluctuating hearing loss, recurrent episodic vertigo, aural fullness and tinnitus. It is estimated to afflict 15 to 46 individuals per 100,000 population per year.[1] Although the most prominent pathological correlate of Meniere's disease is endolymphatic hydrops[2], the presence of hydrops may be an epiphenomenon[3]. Endolymphatic hydrops, the dilation of the membranous labyrinth, may be the end result of a multitude of causes including inflammatory, metabolic, genetic or autoimmune disorders.[4] The pathophysiology of both endolymphatic hydrops and the associated symptoms of Meniere's disease, remain unknown. Several studies suggest that Meniere's disease is closely associated with dysfunctional inner ear blood flow[5], oxidative stress and perturbation in fluid dynamics[6]. To understand the molecular pathology of Meniere's disease we have investigated the changes that occur in vestibular endorgans obtained from at surgery from patients with Meniere's disease using classical histology, transmission electron microscopy, immunohistochemistry and real-time PCR. There was a significant anatomical deterioration of the sensory epithelia hair cells and supporting cells and thickening of the basement membrane (BM) underneath the sensory epithelia, and deterioration of perineural and perivascular BMs[7]. We also have described the localization of several basement membrane proteins in the normal human cochlea and vestibule from normal aging individuals.[8] In comparison to normal, there was a significant upregulation of cochlin within the stroma, and a dowregulation of collagen-IV and laminin-B2 in the basement membrane of the MD inner ear.[9]

Given the nearly universal finding of EH in Meniere's disease, the overproduction of endolymph or the underabsorption of endolymph secondary to a disrup-

tion of fluid homeostasis has been theorized to be due to altered expression of water channels.[10] Aquaporins (AQPs) play a fundamental role in regulating fluid homeostasis in all living organisms, including bacteria, plants, and animals.[11] We have proposed that Meniere's disease pathophysiology maybe secondary to an alteration of AQP expression in the inner ear, causing an accumulation of endolymphatic fluid or EH.[12,13] Utricular maculae from subjects with intractable Meniere's disease demonstrated that AQP4 expression was significantly decreased and AQP6 expression was significantly increased compared with both acoustic neuroma as well as postmortem normative. The AQP6 expression exhibited a loss of polarity, being spread throughout the cell, rather than being polarized to the apical supporting cell. [13] In the cerebrum, the loss of a polarized AQP4 expression appears to be a principal component of pathological cerebral edema. Aquaporins have specific roles in the blood brain barrier, regulating the flow of fluids, and an alteration in expression likely affects permeability of the blood labyrinthine barrier (BLB). In this report we describe the ultrastructural changes in the blood labyrinthine barrier in the macula utricle obtained from patients with Meniere's disease. We also summarize our major findings on the histopathological and ultrastructural findings in Meniere's disease. These results support our theories on the role of the BM and BLB in MD pathology[7].

Methods

The methodology of inner ear tissue processing for light and electron Microscopy has been described in detail by our group.[7] Human specimens: The University of California, Los Angeles Institutional Review Board (IRB) approved the use of archival human temporal bones in this study (Protocol # 10-001449-AM-00002).

Results and discussion

1. Histopathological and ultrastructural studies in the sensory epithelia. Both light and electron microscopy showed alterations in the vestibular hair cells and BMs. We found that neuroepithelial degeneration, hair cell and supporting cell deterioration, correlate with an associated thickening of the underlying basement membrane.[7] Figures 1a and 1b show the sensory epithelia from a normal and Meniere's disease macula utricle. Electron microscopy demonstrated perivascular basement membrane thickening and endothelial cell cytoplasm vacuolization in both cristae and utricular maculae. Figures 1c and 1d demonstrate the ultrastructural changes in the sensory epithelia and basement membrane in a Meniere's disease specimen. These results show that not only the subepithelial sensory epithelium basement membrane is affected but also the perivascular basement membranes within the stroma is afflicted by degenerative changes.
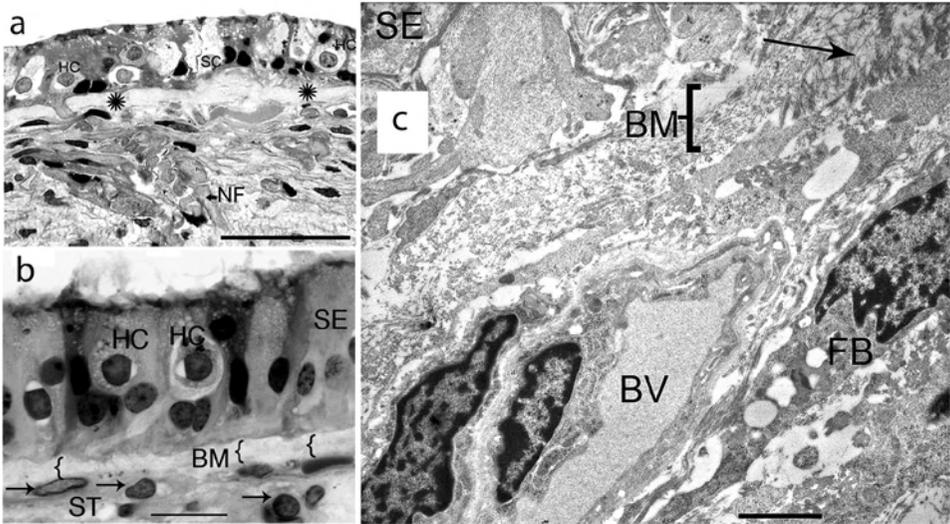
Fig 1. Light and TEM findings in the crista and macula utricle from a Meniere's disease patient. (a) The sensory epithelium in the crista ampullaris is disorganized. Stereocila is absent at the luminal portion (LU). The stroma (ST) appears normal. There is loss of hair cells (HC). HCs show perinuclear vacuoles and the supporting cell cytoplasm occupy the area of the HC loss. Thickening of the basement membrane is prominent (asterisk). (b) The macula utricle from another MD patient. The BM shows mild thickening (asterisk and {}. (c) TEM micrograph of the BM in the epithelial stroma. The BM is pathologically thick with disorganized collagen-like fibrils (arrow) Blood vessel appears normal (BV) at this magnification. FB: Fibroblast. Bar in a = 50 um, b = 10 um. c = 4 um.
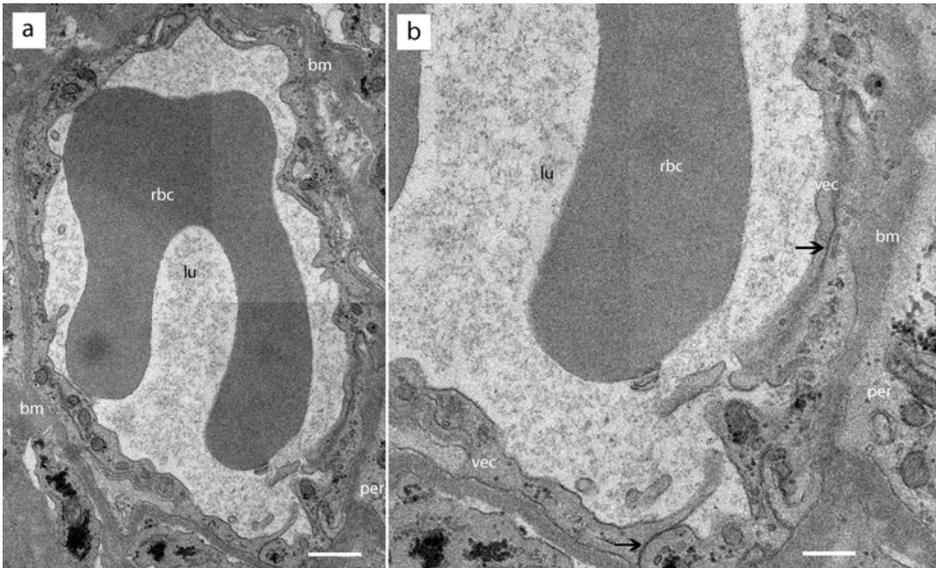
Fig 2. TEM in blood vessel located in the macula utricle stroma from a normal subject with documented normal auditory and vestibular function. (a) low power and (b) high power magnification. Normal (vec) and pericyte processes (per) are seen, bm is also normal, rbc: red blood cell, lu:lumen. Thin arrow point to normal tight junction. Bar in a 2 μm, b = 300nm.
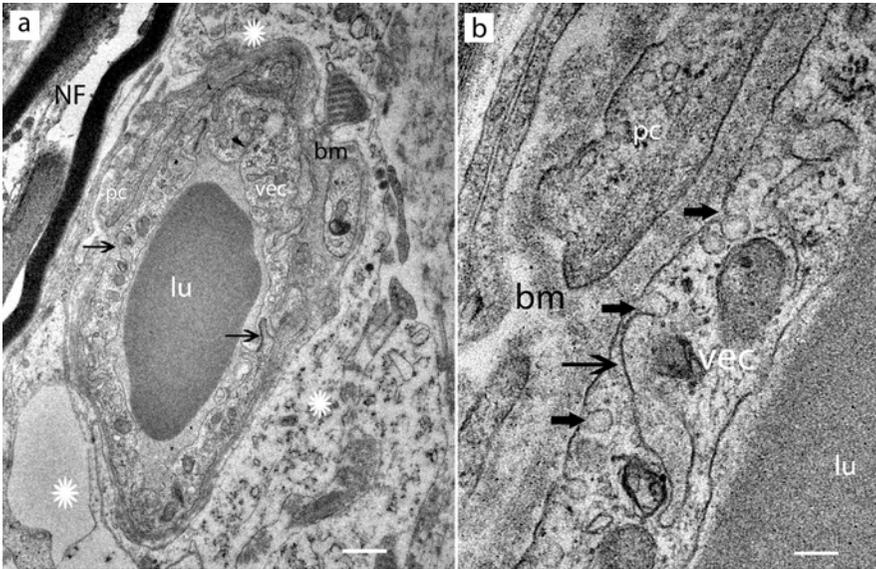
Fig 3. TEM in blood vessel located in macula utricle stroma from a normal and MD patient. (a) low power. Asteriks indicate extracellular matrix with signs of edema. (b) High magnification. Abundant open caveolar flask-like structures that touch the abluminal membrane of the VECs are seen (arrowheads). Thin arrow point to normal tight junction. The border of the basal lamina (BL), which is shared between endothelial cells and a pericyte, is also deteriorated. Bar in a = 2μm, b = 250 nm.

2. Ultrastructural organization in the BLB in normal and MD. Ultrastructural analysis of the BLB of the capillaries in the vestibular endorgan stroma, revealed endothelial cell damage, pericyte detachment, and increased transcellular vesicular transport across vascular endothelial cells. The normal BLB is characterized by vascular endothelial cells, joined by tight junctions (Fig 2a and 2b). In Meniere's specimens there is pericyte detachment and perivascular basement membrane disruption (Fig 3a and 3b). Severe pathological cases demonstrated vacuolization of endothelial cells and/ or complete atrophy (Fig 4a and 4 b). Ultrastructural alterations in the BLB of MDs specimens showed stromal edema. The cellular and molecular organization of the blood labyrinthine barrier in the inner ear of animal models (mouse and guinea pig) is beginning to be elucidated.[6] To our surprise studies on the ultrastructure and molecular biology in the human inner ear vasculature and specifically in the BLB are almost non-existent either the normal or otopathology i.e. Meniere's disease. The BLB is critical for the maintenance of epithelial ion transport systems and the regulation of fluid volumes of the inner ear and damage to the BLB would likely be associated with altered fluid homeostasis, possibly related to the formation of EH.

3. Imaging Studies: We have recently reported that EH and possibly the increased permeability of the contrast in the perilymph on MRI can be reversible with the use of diuretics correlating with clinical improvement, offering a
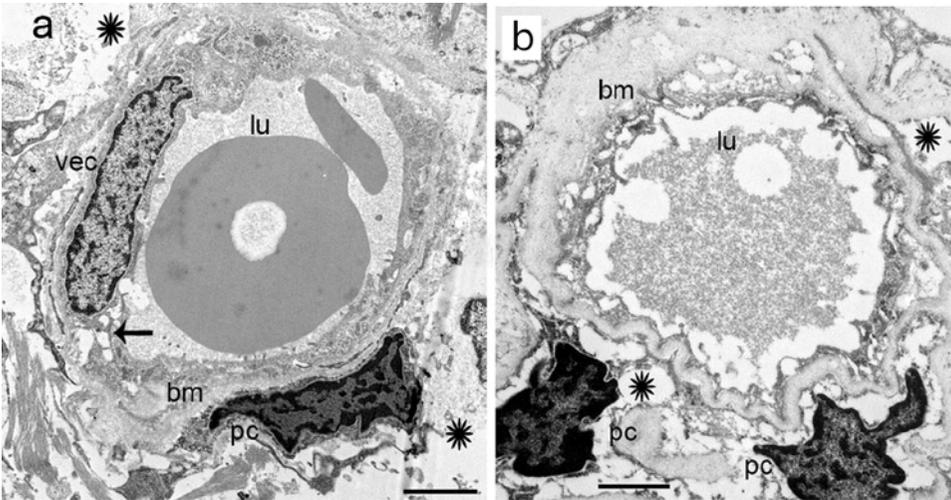
Fig 4. Blood vessels in Meniere's disease. (a). Blood vessel shows an almost normal morphology; the stroma shows signs of edema (asterisks). There is mild vacuolization on VECs and pericytes and edema in the stroma (asterisks). (b) blood vessel from another patient with major atrophy in the stroma i.e. edema, perivascular membrane (bm) is thick and pericytes are atrophic. c. Bar (a) and c= 2μm, b and d= 1 μm.

potential target for pharmacological intervention in Meniere's disease. [14,15] Thus, MD and other otopathologies which may be associated with dysfunctional inner ear blood flow may exert further disruptive effects by altering the vascular permeability of the blood-labyrinthine barrier.[6] Many centers and our group have developed an objective quantitative criteria for EH using delayed iv gadolinium 3D-FLAIR MRI. [16-] The vestibular endolymphatic space (VES) / vestibule ratio can be used as a quantitative indicator of degree of EH.[14] The degree of EH strongly correlated with hearing loss in MD (Spearman = 0.89, p = .0003). Figure 5 illustrates the imaging methodology. 3D maximum intensity projection images (MIPs) from a heavily T2-weighted 3D-FLAIR sequence obtained at a 4-hour delay after double-dose intravenous gadolinium administration is used. Illustrated is imaging of a 60 year-old man with unilateral right-sided definite MD. The vestibular endolymphatic structures (VES) are outlined in blue, and the vestibule is outlined in yellow. The normal left side shows a VES/vestibule ratio of 25%, whereas the right side, with MD, demonstrates a dilated utricle and saccule, effacing the vestibular perilymph, with a VES/vestibule ratio of 67%. Of note is the increased contrast signal intensity seen in the perilymph of the cochlear basal turn on the MD side, indicative of increased permeability of the BLB in the MD ear, commonly noted in the MD ear. The increased permeability of the BLB noted in MRI studies correlates with the histopathological findings of BLB ultrastructural degenerative changes noted on a cellular level. Such microvascular damage may be associated with dysfunctional cochlear blood flow[26], which may lead to microvascular ischemia may result in basement
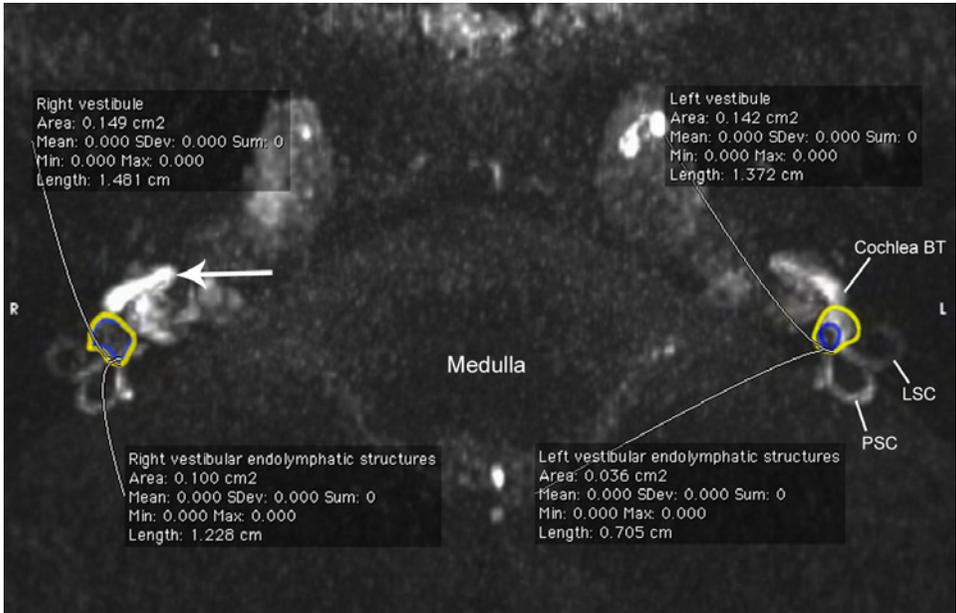
Fig 5.

membrane damage and perivascular fibrosis seen in vestibular endorgans from intractable MD[7] and may also cause the otolithic membrane damage.[11] Micro-vascular ischemia of the BLB may also be related to the spiral ligament atrophy noted in archival temporal bone studies of MD. [27] We propose that increased permeability and damage to the BLB appears to be part of the histopathology and pathophysiology of MD.

Conclusions

Overall these results are indicative of pathological alterations in the BLB vascular endothelial cells and the BM composition in Meniere's disease, however, the primary or main cellular target(s) for these alterations in MDs remain to be identified. Ultrastructural studies and immune-electron-microscopic studies are being conducted in our laboratory to identify the distribution of key BMs proteins and the ultrastructural alterations of the BLB in the human vestibule and the cochlea in normal and pathological conditions.

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THE UTRICULO-ENDOLYMPHATIC (BAST) VALVE – CLINICAL SIGNIFICANCE

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The utriculoendolymphatic (UE) valve was first described by Bast in 1928. It allows the controlled egress of endolymph from the pars superior of the labyrinth (utricle and semicircular canals) to the pars inferior (saccule and cochlea). Its presumed function is to protect the pars superior from sudden changes of pressure in the pars inferior. A reverse function may occur if sudden changes occur in the pressure of the endolymph in the pars superior. Clinical and operative conditions of the inner ear are reviewed in light of these functions.

The valve is located in the antero-inferior wall of the utricule at the orifice of the utricular duct consisting of an inner valve lip and outer membranous wall.[1]

The UE valve develops in association with the appearance of the mammalian pars inferior (auditory system) and in humans, it develops with the formation of the less ancient part of the inner ear (pars inferior): cochlea and saccule.

Its presumed function according to Schukrect and Belal is to permit occasional egress of endolymph from the pars superior to the endolymphatic duct to be processed in the endolymphatic sac; while preventing an excessive loss of endolymph with the possible consequence of membrane distortions and interference with the motion mechanics of the vestibular sense organ (Fig. 1).[2]

The valve is meant to protect the humoral and anatomical integrity of the pars superior (more primitive and ancient) balance system, from the pathological disorders (developmental, disease or trauma) involving the pars inferior auditory system (Fig. 2).

Diseases of pars inferior include developmental anomalies like scheibe cochleosaccular degeneration, inflammatory diseases (*e.g.*, mumps), traumatic problems either accidental or operative and degenerative diseases like otosclerosis, presbyacusis with ruptured basilar membrane.

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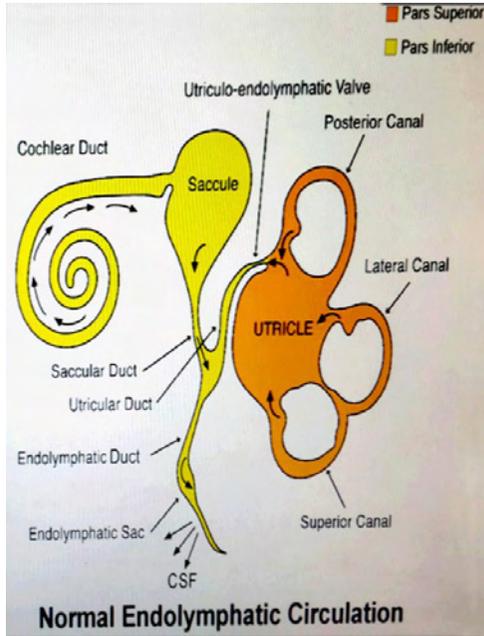
Fig. 1. Normal Endolymphatic Circulation.
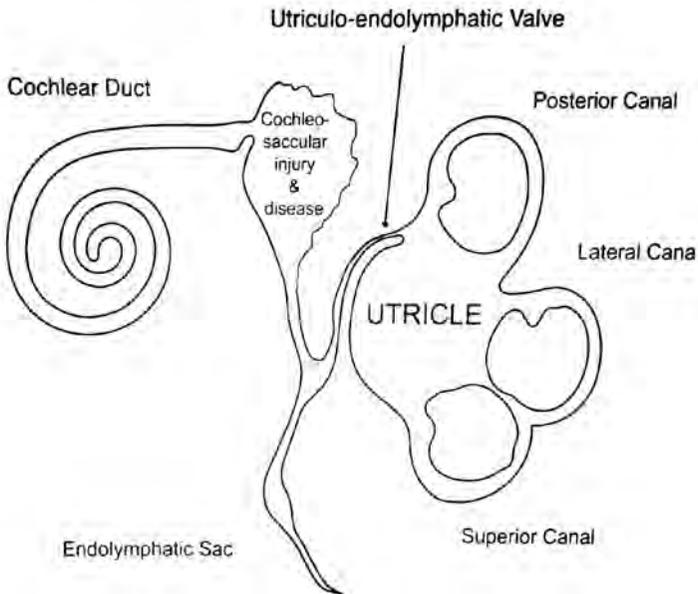
Fig. 2. Bloked UE valve in reaction to injury or disease in the pars inferior of the endolymphertic system (cochlea or saccule).
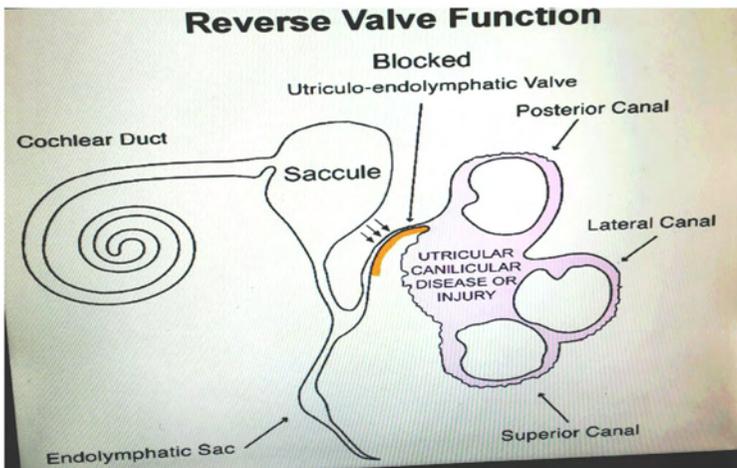
Fig. 3. Blocked UE valve in reaction to injury or disease in the pars superior of the endolymphertic system (utricle and semicircular canals).

Realistically, this protective function of the valve can work in reverse direction, *i.e.*, to protect the pars inferior (cochlea and saccule) in case of trauma or disease of the pars superior (utricule and semicircular canals).

Diseases of the pars superior may include developmental anomalies like superior canal dehiscence, inflammatory diseases like petrous bone cholesteatoma, traumatic problems accidental or operative like fenestration operation, and vascular lesions (*e.g.*, anterior vestibular artery occlusion).

Blocked UE valve occurs in reaction to volume, pressure or biochemical changes in the endolymph and may play a part in the symptomatology and treatment of Ménière's disease, results of intratympanic drug therapy, endolymphatic shunt surgery, and Meniett device. Also,it plays a part in percentage of dizziness after cochlear implantation, clinical presentations of superior canal dehiscence and results of its surgical management.

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PATHWAYS TO THE INNER EAR

GENTAMICIN DISTRIBUTION IN THE EAR FOLLOWING LOCAL APPLICATIONS

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1. Introduction

It has been widely assumed that intratympanically-applied gentamicin primarily enters perilymph of the ear through the round window (RW) membrane. Resulting concentration gradients of drug along scala tympani (ST) have been quantified[1] and hair cell losses have been shown to be greater in the basal turn relative to the apex.[2] However, recent functional studies with gentamicin applications targeted to the RW or stapes have demonstrated that gentamicin entry at the stapes also occurs.[3] In the present study, we have directly measured gentamicin entry into perilymph of the vestibule and into ST and have used a computer model of the inner ear fluids to quantify the relative entry at the RW and stapes following local gentamicin applications to the RW niche.

2. Methods

Perilymph gentamicin kinetics was established in experiments on guinea pigs, anesthetized with isofluorane in oxygen. Experimental protocols were approved by the Animal Care Committee of Washington, under protocols 20101035 and 20130069. The inner ear was exposed by a lateral approach, giving access to the RW niche and to the lateral semi-circular canal (LSCC).

Elimination kinetics was first established by loading perilymph with 500 μg/ml gentamicin in artificial perilymph, injected for one hour at one μL/min from a pipette sealed into the LSCC. As the cochlear aqueduct provides the outlet for flow, this loads the entire perilymphatic space with drug. Perilymph was

sampled immediately (zero delay) or one, two or four hours after injection. A sequential sampling method was used to collect perilymph, in which 10×2 μL samples were taken successively from the LSCC. Initial samples originate from perilymph of the LSCC and vestibule and later samples originate from scala vestibuli (SV), ST and finally CSF. Each sample was diluted and analyzed with a particle enhanced turbidimetric inhibition immunoassay (Beckman Coulter).

In subsequent experiments, 20 μL of 40 mg/ml gentamicin solution (Merck, Refobacin) was applied to the RW niche. Application of this volume results in a portion of the solution running anteriorly in the middle ear, contacting the stapes footplate and possibly apical parts of the cochlea. Perilymph was sampled by the same sequential sampling procedure from the LSCC as described above, collecting perilymph one, two or four hours after drug application. Only a portion of the available data is presented due to space limitations.

3. Results

Following perilymph loading of gentamicin by LSCC injection, perilymph was sampled either immediately or with delays of one, two or four hours. The decline of gentamicin with time for perilymph originating in the vestibule was extremely slow, falling progressively to 60% of the injected concentration over a four-hour period. In contrast the concentration decline was far more rapid for samples originating from ST, falling to 20-30% of the injected concentration over the four-hour period. Computer simulations of these experiments showed that gentamicin loss from perilymph was dominated by concentration decrease in the basal part of scala tympani (ST). A similar decrease of dextran marker at the base of ST was previously reported.[4] The decline is caused by two types of perilymph interaction with CSF. The first is a perilymph dilution resulting from a slow (30 nL/min) volume entry of CSF into ST. The second is a fluid exchange process in which respiratory pressure-induced fluid oscillations drive a perilymph/CSF oscillation across the cochlear aqueduct as a result of RW membrane compliance. The present results with gentamicin show that perilymph kinetics is completely different in the vestibule compared to the basal part of ST, with much faster drug loss from ST. Subsequent analysis of perilymph gentamicin concentrations following RW niche applications took into account these kinetic differences.

In separate experiments, gentamicin was applied to the RW niche and perilymph was again sampled sequentially, collecting ten individual samples. As the fluid samples originate from different regions of the ear, this method allows gentamicin entry rates at the RW and stapes to be quantified. The left panel of Figure 1 shows calculated sample curves from our computer model for different proportions of entry at the RW and stapes keeping the total entry (both sites combined) constant. This calculation takes into account kinetic differences between different regions of the ear derived in prior experiments. The shape
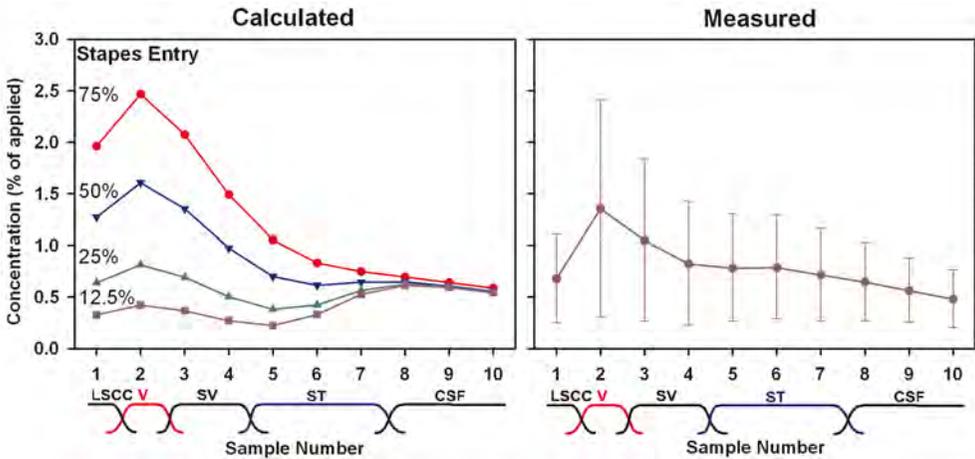
Fig. 1. Left: Calculated concentrations of sequentially-collected perilymph samples from the LSCC as the relative proportions of gentamicin entry at the RW and stapes are varied. Initial samples originate from the LSCC and vestibule, followed in sequence by SV, ST and CSF. While levels in the vestibule are highly dependent on entry at the stapes, changes in entry at the RW have lesser influence on ST as concentrations there are more influenced by perilymph-CSF interactions and local diffusion between ST and SV. Sample curves are a sensitive indicator of relative entry rates. Right: Mean (± SD) sample curve of five experiments in which samples were collected one hour after gentamicin was applied to the RW niche. Highest gentamicin concentrations were present in samples originating in the vestibule, with lower levels in samples originating from ST. The measured data are consistent with less than 50% of gentamicin entering at the stapes.

of the sample curve can be used as an index of how much gentamicin enters by each route, primarily by comparing differences in concentrations between samples originating in the vestibule (samples 1-2) and ST (samples 6-7). The right panel of Figure 1 shows measured sample data summarized for experiments in which perilymph was sampled one hour after gentamicin application to the RW niche. There is considerable variation of the overall amount of entry between animals, similar to that seen in prior studies.[1] In most animals, higher gentamicin concentrations were present in the vestibule compared to ST, confirming the fact that gentamicin was entering vestibular perilymph directly near the stapes. An analysis of these experiments showed that calculated sample curves were a best fit to the measured data when 53% of gentamicin entered at the RW, 39% entered at the stapes and 8% entered apical regions through the thin bony otic capsule. The small amount entering at the apex was included to account for the observed curve shapes.[5] Each of these values is the mean from simulating the five experiments individually. Figure 2 schematically summarizes entry rates and resulting perilymph concentrations.
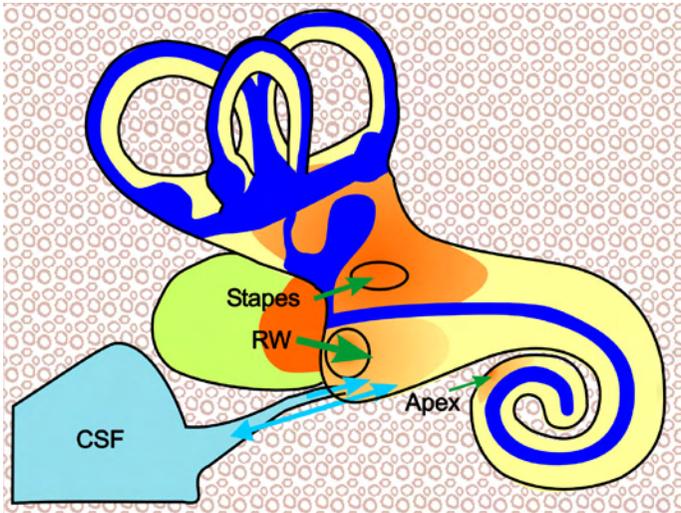
Fig. 2. Schematic of gentamicin entry into the guinea pig ear with local, intratympanic applica-
tion. Gentamicin enters predominantly at the RW membrane, but perilymph levels are lower in
ST because of CSF-perilymph interactions (CSF volume entry and pressure-induced fluid oscil-
lations across the aqueduct). Entry is lower at the stapes but higher concentrations are achieved
in vestibular perilymph as losses from this region of the ear are less. A small entry through the
thin bony capsule into apical perilymph also contributed. Cochleotoxicity is thought to result
from gentamicin in SV rather than from the lower amount in ST.

4. Discussion

These data confirm indirect data from a prior report[3] that gentamicin enters peri-
lymph of the vestibule directly in the vicinity of the stapes as well as through
the RW into ST. Although analysis shows that less gentamicin enters via the
stapes than via the RW membrane, perilymph gentamicin levels reach substan-
tially higher levels in the vestibule than in ST due to lower ongoing losses there.
The resulting higher concentrations in vestibular perilymph almost certainly
contribute to the preferential vestibulotoxicity of gentamicin.

However, the cochleotoxicity of gentamicin cannot be assumed to result from
gentamicin entry into ST through the RW membrane. When applied specifically
to the stapes footplate, auditory thresholds were increased substantially more than
when the same amount of drug was applied to the RW membrane.[3] Although this
could have resulted from higher ST drug levels being achieved with applications
to the stapes (if entry at the stapes occurred far more readily than at the RW),
the current data do not support this. Calculations with entry only at the stapes
suggest that ST concentration would be extremely low due to the losses occurring
there. Furthermore, it has been shown that fluorescent gentamicin (gentamicin-
Texas red; GTTR) accumulated in the hair cells when applied systemically
while simultaneously washing ST with artificial perilymph.[6] In contrast, GTTR
did not accumulate in hair cells when GTTR-containing solution was perfused

only through ST. This leads to the conclusion that GTTR primarily enters hair cells from the endolymphatic side, probably through their non-specific cation transduction channels. In the context of locally-applied gentamicin, this raises important questions with respect to how gentamicin reaches, and subsequently damages, cochlear hair cells. It is possible that the lower drug levels observed in ST, combined with limited entry into hair cells from ST perilymph may render the ST route insignificant. Instead, the possible entry of gentamicin from scala vestibuli to endolymph and from there to the hair cells needs to be considered as an explanation for the functional data.[3] This pathway has not previously been considered and gentamicin passage from SV to endolymph (which would undoubtedly be opposed by the endocochlear potential) has not been documented. But if cochleotoxicity is a consequence of gentamicin levels in the vestibule and SV, this would suggest that attempts to target delivery specifically to the stapes or to block entry at the RW membrane may not be effective remedies to reduce cochleotoxicity. It is appreciated that extrapolation of guinea pig data to humans is problematic as the interactions between perilymph and CSF measured in guinea pigs may not occur to the same degree in humans due to the narrower cochlear aqueduct. Nevertheless, a cautious approach needs to be taken in human studies and it should not be assumed that a more efficient delivery to the stapes will necessarily result in lower cochleotoxicity

5. Acknowledgement

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DRUG DELIVERY VIA THE OVAL WINDOW

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Recently it has emerged that the oval window (OW)/stapes footplate is an important pathway for drug delivery to the inner ear. This was suggested by MRI scanning, where we observed that gadolinium-derived contrast agents (GDCA) can be seen within both the vestibular and the cochlea within one hour when applied to the round window/stapes niche of experimental animals.[1] We went on to test this directly by assessing the concentration of the solute trimethylphenylammonium (TMPA) within the fluids of the inner ear after its selective application to either the RW or the OW.[2] Perilymph TMPA levels were measured using ion-selective microelectrodes sealed into the otic capsule or in perilymph samples collected from the lateral semi circular canal. Analysis of these data indicated that large portions of GDCA and TMPA entered the inner ear via the OW.

We have turned our attention to potential therapeutic ramifications of these findings, and reason that it might be possible to preferentially target either the vestibular system or the cochlea by delivering drug to either the OW or the RW, respectively. In the treatment of Ménière's disease (MD) this might mean that an optimal dosage of gentamicin could be delivered to the vestibule, potentially reducing the concentration in the cochlea thus reducing the risk of inadvertent ototoxicity. We have applied a high concentration of gentamicin onto either the OW or RW, and found that gentamicin applied to the OW induced significantly higher levels of ototoxicity.[3] Our new data now show that targeted application of a lower dosage of gentamicin on the OW can result in a reduction of vestibular function while avoiding elevations in hearing threshold. In these experiments, gentamicin (1 uL of 5 mg/mL) was applied directly onto the OW in adult guinea pigs. One and two weeks following this procedure, short latency vestibular

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evoked potentials (VsEP) responding to brief bone-conduction stimuli were recorded with an electrode located in the facial nerve canal. Auditory function was assessed by recording auditory evoked potentials (AEP) in response to pure tone pips (2-32 kHz) using the same electrode. Two weeks after treatment, there was a 64% reduction in VsEP amplitude without elevations in AEP threshold in gentamicin-treated animals.

It is still too early to know whether the risk of ototoxicity during local gentamicin therapy for MD can be reduced by targeting a low dosage of gentamicin onto the OW. If this were to be attempted clinically, it could be achieved by injection of gentamicin to the OW under direct vision with an endoscope, or potentially with self-gelling polymers injected through the tympanic membrane. However, the presence of mucosal folds could obstruct the passage of drug to the oval window niche, and this might influence whether liquid or gel-like preparations were best to be employed.

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ENDOLYMPHATIC SAC

A NEW MURINE MODEL FOR MÉNIÈRE'S DISEASE – VASOPRESSIN-INDUCED ENDOLYMPHATIC HYDROPS

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1. Introduction

Ménière's disease (MD) is a disorder of the inner ear characterized by intermittent episodes of vertigo, fluctuating sensorineural hearing loss, tinnitus and aural pressure. It is assumed that endolymphatic hydrops (EH) is the pathological feature most descriptive of MD. Currently, there is no universally accepted explanation for the underlying pathophysiology of this disease, although the histopathological findings in MD have been described in extensive studies on temporal bones. EH can be induced experimentally in animals, but the disease with its clinical manifestations is not reproducible in the laboratory and can only be studied in patients. A number of methods have been developed to simulate an animal model of MD. Since its introduction by Kimura and Schuknecht in 1965,[1] surgical induction of EH in the guinea pig (by obliterating the endolymphatic duct and sac) has become the standard model for the study of MD. The procedure has been readily adopted by some investigators as it reliably produces both histological EH and hearing loss. However, the model does not produce anything resembling attacks of vertigo, even though a predictable low-tone hearing loss can ensue.[1,2] We have developed a more suitable animal model, having a closer resemblance to the pathophysiological process in MD.[3] To devise a more complete animal model for MD, we used vasopressin (VP) to induce excess production of endolymph, since VP concentration in the blood of MD patients is increased[4] and VP causes EH in guinea pigs[5,6] and rats.[7] This procedure will give us more complete knowledge concerning the pathogenesis of MD.

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2. Materials and methods

Adult CBA/J or ICR mice were treated by daily subcutaneous injection of 50 µg/100g/day VP for five days up to eight weeks. Animals were sacrificed for histological examination immediately after the last VP injection, or six or eight weeks after 14 days or eight weeks VP treatment for the investigation of re-covery. Morphological analyses were performed of the cochlea, vestibular end organs and endolymphatic sac (ES).[3]

3. Results

3.1. *Effects of VP administration to the inner ear*

In the ears of VP-treated mice, all cochleae showed mild to severe EH, depend-ing on the duration of VP treatment. The severity of hydrops was greater in two weeks VP treated (2w) animals than in five days VP treated (5d) animals, and increased in eight weeks VP treated (8w) animals. In the 8w animals, Reissner's membrane expanded into the scala vestibule and touched the bony partition between the turns in the severely hydropic cochlea. The loss of nerve fibers, spiral ganglion cells, and outer hair cells were observed in some cochleae. In this case, the degeneration of spiral ganglion cells was generally severe in the lower turns. Considerable intercellular edema of the stria vascularis was also observed.

The vestibular parts also showed signs of EH related to the duration of VP treatment. The severity of EH was marked in the ampullae in general. In 5d animals, the saccules and ampullae showed signs of EH, whereas the utricles showed no signs of hydrops. EH was observed in utricles, saccules and ampullae of 2w and 8w animals. In 8w animals, some ampullar membranes were attached to the bony wall.

3.2. *Endolymphatic sac*

In the control ears, the intermediate portion of the ES was easily identified by its cylindrical cells, which protrude into the lumen as irregular papillae. The epithelial lining of the distal portion of the ES was cuboidal. In the intermediate portion, numerous widened lateral intercellular spaces (LISs) were seen between the epithelial cell linings of the ES. In the distal portion, LIS was not so widened compared with the intermediate portion. In the VP-treated ears, the lumen of the distal portion was markedly dilated. The epithelial cells became thinner and the LISs were collapsed. Inside the lumen, the ES contained clear endolymph without macrophages. The intermediate portion was also dilated. The LISs were collapsed and the looseness of the perisaccular tissue was no longer observed. The epithelial cells were often of the cuboidal or flat type.

3.3. Recovery of the inner ear after the end of VP treatment

Six weeks after the two weeks or eight weeks after the eight-week administration of VP, cochleae showed reduced signs of EH, being almost identical with the normal controls. However, loss of spiral ganglion cells, nerve fibers and hair cells were still observed in 8w animals. The utricles, saccules and ampullae also showed reduced EH, being almost identical with the normal controls in the 2w animals, while attachment of the ampullar membrane was still noted in some 8w animals. The ED and ES showed an almost normal ES lumen in 2w animals, while less though significant dilation was noted in the 8w animals.

4. Discussion

Although obliteration of the ED and ES in the guinea pig is the ideally available procedure for eliciting EH, this model is not perfect for MD, because following such procedures these animals rarely show episodic vestibular symptoms. Furthermore, this model requires the surgical obstruction of the ED and ES.[1,2,8] Several modifications of this model, which produced hydrops in varying degree, were still too destructive and were not physiologically accurate models for MD. In our present study, we succeeded in producing hydrops without any surgical procedures. Another interesting point was that we could induce EH in the mice. In mice, surgical obliteration of the ES is difficult because of its size and location. However, mice do have several advantages as an animal model, compared with the guinea pig. Mice are now widely used for inner ear research, have a number of antibodies available, and also have more technical advantages for the investigation of genetic problems.

   In the present investigation, we used VP to induce EH. It has recently been suggested that hormones such as VP, aldosterone and natriuretic peptide may be involved in homeostatic mechanisms in the inner ear.[4] The VP concentration in the blood of MD patients is increased,[4] and administration of VP causes EH in the guinea pig[5,6] and rat.[7] Furthermore, aquaporin (AQP)2 and type-2 VP receptor (V2R) were found in the inner ear. These findings suggest that VP may play a critical role in endolymph homeostasis and may be intimately involved in the development of MD.[4] The present study revealed that long-term administration of VP induced severe EH in mice. Reissner's membrane expanded into the scala vestibule and touched the bony partition. In the surgical destruction model, it takes a long time to induce severe EH (two months postoperatively).[1,2] We also found that it takes a long time to induce severe EH by VP administration. Loss of nerve fibers, spiral ganglion cells, and outer hair cells were also observed in some long-term VP-treated cochleae. In the surgical destruction model, degeneration of the sensory cells and spiral ganglion was observed at the apical turns one month postoperatively; degeneration increased in frequency in this region and involved the lower turns as survival time lengthened.[1,2] In human temporal

bone studies in MD patients, similar findings were demonstrated.[10] The present findings may thus indicate that our model may be closer to clinical MD.

Concerning ways to induce EH by VP, one suggested mechanism is excess production of endolymph in stria vascularis and dark cells. VP increased the influx of water from the perilymph into stria vascularis via AQP2 expressed on the basal cells, thus leading to EH.[4] Up-regulated expression of AQP2 mRNA in the stria vascularis increases water permeability to endolymph, resulting in an increased production of endolymph.[7] Another possible mechanism can be a reduced absorption of endolymph in the ES. VP reduced water re-absorption in the ES, resulting in EH.[5,7]

We have also demonstrated that EH caused by VP recovered following the cessation of VP administration. The EH gradually increased as the VP administration was lengthened, but gradually recovered after the cessation of VP. In contrast, there was no recovery from the degeneration of cochlear hair cells and loss of spiral ganglion cells. These characteristic findings (*i.e.*, the recovery from EH) in the present model are regarded as superior to the surgical destruction model. Concerning the mechanisms of MD, it has been suggested that stress is closely related to the increase in plasma VP in patients with MD. Since plasma VP concentrations are known to be increased by various kinds of stress in humans, the elevated plasma VP level preceding an attack seems to partially reflect a patient's stress condition. Early stress control may improve the prognosis for MD dramatically.[4] Together with the present findings and the previous reports, increased VP due to stress may play an important role in MD and may also indicate the possibility that early treatment (including stress relief) may actually cure MD, whereas delay in treatment may lead to permanent hearing loss.

5. Conclusion

Our new mice model is, non-surgical, a more suitable animal model, having a closer resemblance to the pathophysiological process in MD, which can be widely used for the MD research.

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ION TRANSPORT IN ENDOLYMPHATIC SAC EPITHELIAL CELLS ON THE BASIS OF EXPERIMENTAL ANIMAL RESEARCHES

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1. Introduction

The endolymphatic sac (ES) is believed to play an important role in the homeostasis of the endolymphatic system. However, the ion transport system of the ES has not yet been clarified. Endolymph has a higher concentration of Na^+ and K^+ than perilymph. Both endolymph and perilymph have unique ionic compositions. *In-vivo* electrophysiological studies demonstrate the presence of active Na^+ and Cl^- outflow from the ES into the extracellular space, suggesting that Na^+ and Cl^- are dominant ions in the ES.[1,2] Based on recent research results in experimental animals (rodents), the ion transport system in the ES epithelial cells is outlined.

2. Molecules relating to ion transport in ES epithelial cells

The ES is divided into three parts on the basis of their morphological features: proximal, intermediate and distal portions. The morphological findings imply that the epithelial cells in the intermediate portion may be more functional than those in the other portions. The epithelial cells in the intermediate portion consist primarily of two types: mitochondria-rich cells and ribosome-rich cells. Molecules relating to ion transport in ES epithelial cells in the intermediate portion are shown in Table 1. The type of cells with each molecule remains to be identified.

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Table 1. Molecules relating to ion transport in ES epithelial cells of the intermediate portion.

Molecules	Cell localization
Ion channels	
Na$^+$ channel (amiloride-sensitive)[21]	apical membrane
Epithelial sodium channel (ENaC)[10]	apical membrane
K$^+$ channel (outward delayed rectifier)[3]	basolateral membrane
Non-selective cation channel (ATP-activated)[8,9]	apical membrane
Cystic fibrosis transmembrane conductance regulator (CFTR)[10]	apical membrane
ATPases	
Na$^+$-K$^+$-ATPase[22]	basolateral membrane
H$^+$-ATPase[13,14]	apical membrane
Carbonic anhydrase[13,16,17]	cytoplasm, membrane
Ion exchangers	
Cation exchanger: Na$^+$-H$^+$ exchanger[15]	apical membrane
anion exchangers	
Cl$^-$-HCO3$^-$ exchanger(SLC4A2)[14]	basolateral membrane
Pendrin (SLC26A4)[13]	apical membrane
Ion cotransporters	
Bumetanide-sensitive Na$^+$-K$^+$-2Cl$^-$ cotransporter 2 (NKCC2)[11]	apical membrane
Thiazide-sensitive Na$^+$-Cl$^-$ cotransporter (TSC)[12]	apical membrane

3. Electrophysiological profile on ion transport in ES epithelial cells

Previous reports on ion transport in the ES (including cation, anion and acid/base transports) are summarized below.

- The resting membrane potential of approximately -60 mV in the ES epithelial cells;[3]
- The presence of DC potential in ES endolymph (ESP) of + 15-20 mV;[1,2]
- Higher Na$^+$ and lower K$^+$ concentrations of ES endolymph, and the presence of active Na$^+$ outflow from the ES into the extracellular space;[2]
- Lower Cl$^-$ concentration than that in the extracellular fluid, and the presence of Cl$^-$ outflow from the ES into the extracellular space;[1]
- K$^+$ and Na$^+$ are permeable ions, but Cl$^-$ is a negligible ion in the ES isolated epithelial cells;[4]
- Stronger activity of Na$^+$, K$^+$-ATPase and higher Na$^+$ permeability in ES mitochondria-rich cells;[5]
- ES endolymph has a weak acidity.[6,7] H$^+$-ATPase may be involved in acidification in ES endolymph.[7] Acid-base transport may be one important part of the ion transport system in ES.

4. Ion transport property in ES epithelial cells

The Na^+ imaging study[5] demonstrates that mitochondria-rich cells in the ES have a higher activity of Na^+, K^+-ATPase and a higher Na^+ permeability, suggesting that molecules relating to Na^+ transport may locate in mitochondria-rich cells. Mitochondria-rich cells in ES have a characteristic property of Na^+ absorption from a viewpoint of electrochemical gradient, a high Na^+ permeability, and a high activity of Na^+, K^+-ATPase (Fig. 1). Na^+ enters the cell across the apical membrane through ion channels and ion transporters driven by an estimated electrochemical driving force of approximately 140 mV. K^+ enters the cell across the apical membrane through the non-selective cation channel[8,9] driven by an estimated electrochemical driving force of approximately 20 mV. Na^+ is removed across the basolateral membrane by Na^+, K^+-ATPase. K^+ is brought into the cell by the pump, and subsequently diffuses out through the basolateral K^+ channel (outward delayed rectifier),[3] which is involved in the maintenance of negative intracellular potential. The model is similar to that found classically in several other Na^+-absorbing epithelia.

Molecules relating to Cl^- transport are an ion channel (CFTR),[10] ion cotransporters (NKCC2[11] and TSC[12]) and a Cl^--HCO_3^- ion exchanger (pendrin)[13] in the apical membrane, and a Cl^--HCO_3^- exchanger (SLC4A2)[14] in the basolateral membrane. CFTR has been shown to co-localize with ENaC.[10] It is speculated that Cl^- transport follows Na^+ transport and acid/base transport because Cl^- has to be transported across the apical membrane against the large electrochemical gradient around 75 mV.
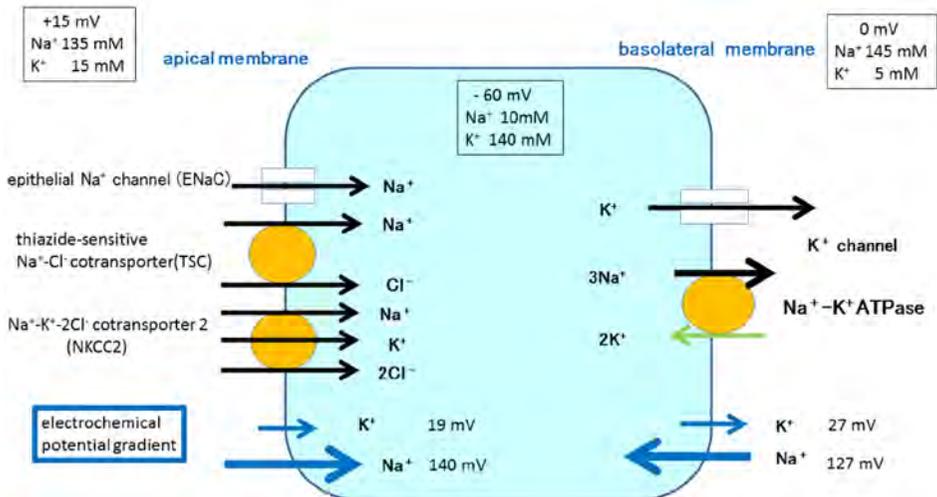
Fig. 1. Na+ transport model with electrochemical driving force in ES mitochondria-rich cells.

Molecules relating to acid/base transport are H^+-ATPase,[13] Na^+-H^+ exchanger (NHE)[15] pendrin in the apical membrane,[13] Cl^--HCO_3^- exchanger (SLC4A2) in the basolateral membrane,[14] and intracellular and membrane-bound carbonic anhydrase.[7,16,17] H^+-ATPase, pendrin, carbonic anhydrase have been shown to localize in the same type of ES epithelial cells.[13] For the maintenance of acidity in ES lumen the inflow of H^+ into the lumen needs to be larger than the inflow of HCO_3^-. NHE in the apical membrane, which is presumed to be active due to large Na^+ inflow into the cell, besides H^+-ATPase may be largely involved in the acidity of ES endolymph.

5. Regulation of Na^+ transport in ES

Concerning regulation of Na^+ transport in ES, the following several lines of evidence support the hypothesis that aldosterone may regulate Na^+ transport in ES, resulting in endolymph volume regulation:

- The presence of mineralocorticoid receptors (MRs) in the ES epithelial cells;[18]
- The presence of 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), which enables aldosterone to bind selectively to MRs by converting cortisol (corticosterone) into inactive metabolites.[19] The presence of 11β-HSD2 is essential in aldosterone target tissues;
- The presence of ENaC in the ES epithelial cell;[10]
- The presence of TSC in the ES epithelial cells.[12] TSC, which had been accepted to have a specific localization in distal convoluted tubule of the kidney until TSC was observed in ES epithelial cells;
- The antagonist of aldosterone, canrenoate, intravenously produced a decreased ESP change with no change in the endocochlear potential.[20]

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AUDIOLOGICAL ISSUES

ON THE ROLE OF DEPLETIVE TESTS:
A REVIEW ANALYSIS

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Ménière's disease (MD) is an inner ear disorder of unknown cause that is characterized by fluctuating hearing loss, tinnitus, aural fullness, and episodic rotatory vertigo. The MD course is quite variable, and its etiology is largely unclear. The MD pathological feature is considered to be endolymphatic hydrops.[1] On the ground that MD could be the aural counterpart of glaucoma, being both associated with increased fluid pressure, Klockhoff and Lindblom in 1966[2] have proposed to test the possibility of reducing the intralabyrinthine pressure via an osmotic dehydration produced by glycerol, so that the glycerol test is largely used as non-invasive diagnostic procedure. The standard glycerol test consists of a per-orally administration of a single dose of glycerol (1.5 g/Kg body weight) on an empty stomach and to compare on pure-tone audiograms the threshold levels obtained before administration with that after one, two and three hours. The criteria of their study were a significant threshold improvement since one hour after glycerol intake with a maximum auditory improvement after two to three hours; an average auditory gain of 17 dB in the range 125-8000 Hz; an improvement in speech perception characterized by increase of speech intelligibility from 60% to 86%, and an improvement of loudness from 70 to 55 dB. According to these criteria they were able to define positivity of the test in the early, fluctuating stages of the disease and a negative one in the advanced stages.

Other investigators have reported on different timings for the analysis of a depletion test, such as a 27% of positivity after one hour and 74% after two hours,[3] or maximum effect after three hours,[4] whilst later on Klockhoff confirmed the previous experience of the maximum auditory improvement after two hours.[5]

Regarding the frequency range affected by the depletion test, Klockhoff and Lindblom identified it at the low frequencies; Snyder at 1000 Hz while decreasing toward the high frequencies;[3] and Swanson[6] confirmed the maximum effect at low frequencies with a relative decrease towards the high frequencies.

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From the above factors it may be possible to summarize as favorable indicators the low-tone hearing loss;[2] the 25-40 dB minimum hearing loss required in the range between 250-4000 Hz;[3] and the absence of any ack near the performance of the test.[7]

Recent investigations have reported that the homeostasis of endolymph is normally maintained without significant volume flow, leading to a local transport of ions with a water equilibrating system[8] owing to the osmotic gradient. New mechanisms for possible actions of glycerol may then be resumed in passive and active mechanisms. By passive mechanism, glycerol not only increases the osmotic pressure of the fluid in which is dissolved, *i.e.*, blood, but also the plasma osmolarity in cochlear capillaries of the stria vascularis, inducing endolymphatic diffusions into blood circulation and reducing pressure in hydropic labyrinth. By active mechanism glycerol would activate the ionic pump, increasing in the endolymphatic sac the secretion of the glycoproteins that would favor the endolymph absorption.

In clinical practice, the term *glycerol test* can be regarded as an 'umbrella term' for describing the effects that follow the administration of several different osmotic and diuretic drugs in reducing the hearing threshold.[9] For instance for the urea, which metabolizes to only a very small extent with a slow penetration into the inner ear, when orally administered at the dose of 25g/body weight, diluted in 100-200 ml of fruit juice, it has been reported 60% of positivity in MD patients.[10] When using for depletive test ethanol or mannitol, positive results were observed only occasionally, since mannitol induces a smaller rise in serum osmolality than glycerol due to less and slower penetration than glycerol, with vascular effects mostly consisting in vasodilatation.[11] Regarding Isosorbide, its effects have been described to last till four hours after oral administration, with 50% of positivity in MD.[12] Acetalazolamide, an inhibitor of carbonic anhydrase, influencing various cellular processes[13] and by reducing the degree of the experimentally endolymphatic hydrops.[14] An impletive effect has also been described for this drug when administered intravenously.[15] Furosemide has been shown to induce its potent natriuretic action on the saccule, as shown by a caloric test 40 minutes after its administration by the improvement of vestibular evoked myogenic potential (VEMP) amplitude.[16]

Nowadays, the interest for glycerol (or depletive tests) is focused on objectivating audiological and vestibular correlates with sensitivity and specificity on test models. It has become clear that the best method to correlate the functional early hearing and vestibular improvement after drug administration glycerol will be to associate to the subjective pure tone and speech audiometry assessment also more objective correlates. In this regard, in the recent years among these latter have been proposed electrocochleography (EcochG), otoacoustic emissions and distortion products (DPOAE), vestibular evoked myogenic potential (VEMP) and MRI.

Coats *et al.* found a highly significant relationship between an enlarged Summating Potential (SP) and a positive glycerol test.[17] Fukuoka has compared gadolinium contrast-enhanced MRI, glycerol test, and the ECochG SP/AP ratio, showing hydrops positivity in 88-90% at the MRI, 55% at the glycerol test and 60% at EcochG.[18]

When pure-tone audiometry and distortion products and otoacoustic emissions were compared before and one, two and three hours after glycerol intake, a prevailing positivity of audiometric findings was found.[19]

The comparison of pure tone audiometry with VEMPs before and two hours after glycerol intake has also shown that to an improvement of the auditory threshold of 50% corresponded a significant amplitude increase of VEMP p1-n1 in 39,3%.[20]

In the last years, Basel has proposed a new method to evaluate in the pure tone audiometry the threshold shift during the glycerol test, for obtaining a greater sensitivity.[9] Instead of considering all the frequencies' shift, he introduced the Aggregate Thresholds Shift concept (ATS), *i.e.*, the individual evaluation the pure tone audiometry results: at 125 Hz; at 500-1000-3000 Hz; and at 4000-6000 Hz, noticing that the greatest positive effect was localized in the low-middle range. According to these criteria, the false positive rate would be 4,5% for a 30-40 dB shift and only 0.8% for a 50 dB shift.

Conclusions

The use of a standard glycerol test still has a value in the diagnostic of MD since it can be helpful for assessing the stage of the disease and, eventually, the most appropriate initial medical treatment. However, in order to increase its sensitivity and specificity it would be mandatory to also use objective tests other than the simple subjective puretone or speech audiometry. The routine use, for example, of MRI will in this regard contribute to definitely exclude all the other conventional tests used for the diagnostic process in case of MD.

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KINESIOLOGY TAPING AND KINETIC TRAINING IN REHABILITATION OF SOMATOSENSORY VERTIGO

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Abstract: In otoneurological clinical practice, cervical vertigo is one of the most common vestibular disorders.

Purpose: The aim of this study was to evaluate the effectiveness of kinesiology taping in patients with cervical vertigo syndrome, based on an objective assessment of spinal conduction from the balance system sensory receptor (SEP test), the postural control based on both cranio-corpography (CCG test) and Freyss' dynamic stabilometry and subjective study of neck mobility and questionnaire.

Material and method: The research covered 60 patients. Through medical examination all the patients were diagnosed with vertigo of somatosensory etiology (disturbed somatosensory evoked potentials – SEP on a cervical level). The group was divided into two subgroups: I, in which kinesiology taping was applied, using strips by Nitto Denko Kinesiology Tape / K-Active Tape; and II, kinetic therapy treatment (somatosensory training). Somatosensory evoked potential (SEP) test, craniocorpography (CCG), Freyss' stabilometry, manoeuvrability of the neck and subjective self-evaluation by the patients were performed before and after therapy.

Results: The results were compared between the groups. With regard to the SEP test, waves N13, P11 and P27 latency measurement results obtained after rehabilitation did not show any statistically important differences, as compared between groups and before and after therapies. In the case of the CCG examination it was demonstrated that both examined groups showed the improvement in static (Romberg test), not differing between each other. A significant difference was also observed on Freyss' platform after rehabilitation: the somatosensory training

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was more effective. Manoeuvrability of the neck improved after both therapies. In both groups, self-evaluation of the patients was better after rehabilitation. *Conclusions*: In patients with cervical vertigo, neither the kinesiology taping therapy nor somatosensory training had an impact on the objective study of SEP. But posturographies – CCG and Freyss' stabilometry – revealed improvement; the same in both groups in the static part of CCG and improvement after somatosensory training (in group II) on the dynamic Freyss' platform. The neck was better movable, the vertigo was less frequent, less intense and lasted shorter after both types of rehabilitation.

1. Introduction

In clinical practice, cervical vertigo is one of the most common vestibular disorders. Neuhauser reported, that vertigo/dizziness had a prevalence of 22.9% in the last 12 months and an incidence of 3.1% of respondents in Germany.[1]

Deep sensory conduction disorders seem to be the most important cause. An incredible wealth of proprioceptors, proven to exist in soft tissues of cervical spine, results in a high likelihood of damaging them, which in turn will manifest itself in balance disorders.[4, 5]

Cervical vertigo is a peculiar form of vertigo, and a detailed vestibular diagnostics is fundamental for the diagnosis, choosing the type of rehabilitation, and further treatment.

Nowadays, physiotherapy gives access to diverse forms of therapy, helping both the treating and the treated parties to fight system dysfunctions. One of the examples is kinesiology taping, which is now being rediscovered (although the method has been well-known internationally since the 1970s). Kinesiology taping is a therapeutic method developed in Japan, which consists of taping (parts of) the body with special cotton strips designed specifically for this technique. Kinesiology taping was developed with the purpose of relieving pain and providing support to accelerate recovery of overstrained soft tissues, and its suggested effects include proprioceptive facilitation, inhibition of pain, normalization of muscle tone, oedema therapy enhancement, and blood circulation improvement.

The aim of this study was to evaluate the effectiveness of kinesiology taping in patients with cervical vertigo syndrome, based on an objective assessment of spinal conduction from the balance system sensory receptor (SEP test), a postural control based on a cranio-cartography (CCG test), a course of reaction in disturbed somatosensory function based on Freyss dynamic stabilometry and analysis of the impact of the cause of cervical vertigo on the results of the applied rehabilitation method.

2. Material and method

The research covered 74 patients (aged 18 to 66). Through medical examination all the patients were diagnosed with vertigo of somatosensory etiology (disturbed somatosensory evoked potentials – SEP on a cervical level) or of mixed etiology (disturbed SEP and vasogenic cervical disorders), and such a division created two groups of subjects depending on a somatosensory component (38 people, aged from 19 to 64, average age 45.[6] and on a cervicogenic mixed etiology (36 people, aged from 18 to 66, average age 44.[8] In each of the groups, kinesiology taping rehabilitation was applied. In the somatosensory etiology group, 25 females and 13 males were subjected to rehabilitation, and in the mixed etiology group 25 females and 11 males. A clinical control group consisted of 61 people (24 females and 37 males) having no otoneurological disorders. Participants from the control group were aged between 22 and 62 (average age 47).[10]

All patients were subjected to kinesiology taping rehabilitation. Strips by Nitto Denko Kinesiology Tape / K-Active Tape were used. One type of application was used – a technique following a whiplash injury, where bases were stuck in the area of upper angles of shoulder blades with the head in a natural position, in bending position tails with a 25% strip tension were applied. Time of application was between 8 and 15 days (average 12.6 days).

Fig. 1. Type of application used on the cervical spine.

To check the efficiency of kinesiology taping, a series of special tests were performed before launching the rehabilitation and after the therapy: somatosensory evoked potential (SEP) test, craniocorpography (CCG), Freyss' stabilometry test.

SEP tests were performed on the Ratia-Alvar Centor C device, through percutaneous electrical stimulation of the median nerve of the right upper limb in the carpal tunnel (Figure 2A+B).
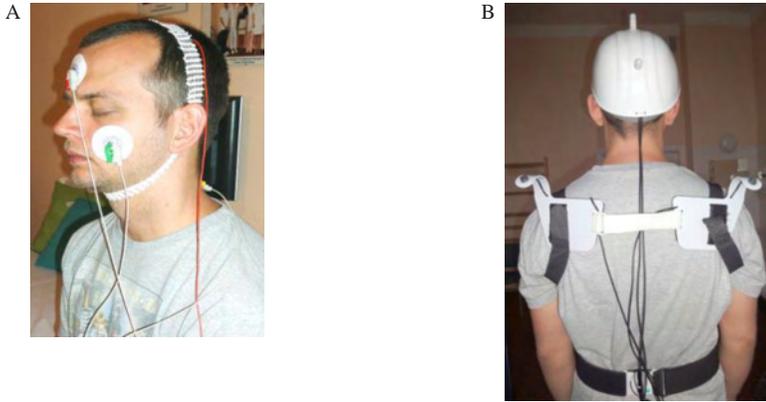
Fig. 2a. SEP tests on the Ratia-Alvar Centor C device

During CCG, the patients stand and then move in place (Romberg's and Unterberger's tests). Freyss' stabilometry comprises four sequences, 20 seconds each. At the first stage, the platform's mobility was set in the sagittal plane body position. The patient is standing still for 20 seconds with his or her eyes open, and then for 20 seconds with the eyes closed (Figure 4). The third and fourth test sequence considered mobility in the frontal plane; as in the previous case, the test was carried out with and without visual control.

Fig. 4. The patient is standing still for 20 seconds with the eyes closed

3. Results

With regard to the SEP test, waves N13, P11 and P27 latency measurement results obtained after kinetic rehabilitation did not show any statistically important differences, as compared with the group treated with Kinesiology Taping method (Table 1).

Table 1. Comparison of SEP between kinetic rehabilitation and kinesiology taping groups.

Changes	Kinesiology Taping		Kinetic rehabilitation		Sign. Levene's test	Variety statistics		df	Sign. t-test	95% confidence	
	Mean	SD	Mean.	SD						min	max
N13	-0,09	1,30	-0,57	1,36	0,639	equal	1,81	105	0,072	-0,04	1,00
P11	0,027	1,714	-0,175	1,564	0,455	equal	0,61	105	0,545	-0,456	0,859
P27	-0,54	3,11	0,08	3,99	0,138	equal	-0,90	105	0,371	-1,99	0,75

In the case of the CCG examination it was demonstrated that two of the examined groups improved after rehabilitation. With regard to Freyss' stabilometry, the differences between the groups were observed (Table 2).

Statistically significant differences were obtained with:
- Change of the mean speed of the platform (Vmean); the improvement after kinetic therapy is higher than after kinesiology taping.
- Change in the maximum range of platform Amax, with a side-to-side (R-L) mobility and eyes open; in kinetic rehabilitation the results were better.
- Change of Amax and mean speed of the head with a left-to-right (R-L) platform mobility, without any visual control; after kinetic training the results were better.

Self-evaluation of the patients was better after rehabilitation in both groups.

4. Discussion

The results did not show any statistically significant changes in the latency of somatosensory potentials between kinesiology taping and kinetic rehabilitation. Academic literature lacks information on the SEP test in patients who had undergone taping treatment, in particular in patients with cervicogenic vertigo. Analyzing sensory conduction among sportsmen and in the group of people with sedentary lifestyle, Bulut *et al.* noticed a decrease in the potential latency values and a simultaneous decrease in their range after work-out on a treadmill.

Table 2. Comparison between groups on the base of Freyss' stabilometry.

Changes		Taping Mean	Taping SD	Kinetic rehabilitation Mean	Kinetic rehabilitation SD	Levene's test	Variety Equal (eq)	Statistics	df	t-test	95% confidence min	95% confidence max
Forward-back, eyes open	Amax.	-0,0265	0,1104	-0,0431	0,0720	0,167	Equal (eq)	0,86	103	0,392	-0,0217	0,0549
	Amean.	-0,0365	0,1368	-0,0536	0,1006	0,159	eq	0,69	103	0,490	-0,0318	0,0659
	Vmean	0,0013	0,0313	-0,0043	0,0218	0,931	eq	1,00	103	0,320	-0,0055	0,0166
Vmean head.		-0,00778	0,05557	-0,00690	0,05646	0,894	eq	-0,08	103	0,938	-0,02297	0,02122
Forward-back, eyes closed	Amax.	-0,0551	0,1502	-0,0776	0,1779	0,436	eq	0,70	103	0,486	-0,0414	0,0864
	Amean	-0,030	0,164	-0,065	0,135	0,907	eq	1,16	103	0,249	-0,025	0,096
	Vmean.	-0,00984	0,05145	-0,01238	0,05834	0,385	eq	0,23	103	0,815	-0,01891	0,02399
Vmean head	Amax	-0,0162	0,0769	-0,0410	0,0971	0,262	eq	1,45	103	0,149	-0,0090	0,0586
Right-left, eyes open		-0,045	0,136	-0,111	0,157	0,919	eq	2,29	103	0,024	0,009	0,123
	Amean	-0,01619	0,14198	-0,01667	0,14228	0,950	eq	0,02	103	0,987	-0,05566	0,05662
Vmean.		0,0038	0,0276	-0,0174	0,0319	0,216	eq	3,62	103	0,000	0,0096	0,0328
Vmean head	Amax.	-0,0090	0,0598	-0,0150	0,0699	0,688	eq	0,47	103	0,642	-0,0193	0,0312
Right-left, eyes closed		-0,009	0,219	-0,122	0,173	0,251	eq	2,79	103	0,006	0,033	0,192
	Amean	0,0005	0,1785	-0,0057	0,1567	0,676	eq	0,18	103	0,855	-0,0610	0,0734
	Vmean.	0,0073	0,0646	-0,0136	0,0685	0,921	eq	1,58	103	0,116	-0,0053	0,0470
Vmean head		-0,008	0,185	-0,126	0,355	0,110	eq	2,22	103	0,029	0,012	0,223

Results pointed out the significance of intense and systematic physical exercise in shaping somatosensory potentials.

Haavik-Taylor *et al*. describe a significant decrease in the range of waves N20 and N30 after manipulation treatment on dysfunctional cervical spine joints. It may prove the existence of temporary impact on changes in cortical plasticity, illustrated by a decrease in the range of somatosensory potentials.

It may be presumed that kinesiology taping influences the surface structures of the neck too shallowly to interfere with the structures of the core, as was the case with manual therapy.

In this paper, a combination of the Freyss' stabilometry with the use of the mobile platform and the CCG test, particularly while marching – seems to give exhaustive answers to questions concerning the condition of 'multisensory' balance.

Craniocorpography results obtained through this research showed that the results in the tested groups changed significantly after therapy. Alpini *et al*. described the CCG test of mobile sequence in patients diagnosed with whiplash injury, as compared with healthy individuals. They found out that the subjects demonstrated abnormal motor strategies, mainly decreasing head stability, reduced body movement, paradoxal excessive stabilisation, referred to as the collar effect. Soto Varela *et al*. analysed the CCG of Romberg's test in patients with cervical vertigo, labyrinth pathology and in healthy individuals. It turned out that head movements were as much as 67% more frequent in the group with cervical vertigo than in patients with labyrinth pathology (27%) and in healthy individuals (31%).

In the case of Freyss' stabilometry, the results were better for individuals not treated with kinesiology taping. Osinski *et al*. noted the improvement in posture coordination on Freyss' platform in patients with proprioceptive vertigo who underwent neurosurgical fixation of the cervical spine, but only in a particular period: from one month to one year after surgery.

Sitko *et al*. describe the results of both physiotherapy and manual therapy in patients with cervicogenic vertigo.[20]

An objective influence on cervical mechanoreceptors was not confirmed in this study. Still, other aspects of kinesiology taping may play a role in the observed postural control improvement in patients with purely somatosensory vertigo. González-Iglesias *et al*. reported reduced pain and improved cervical spine mobility after this kind of therapy, which can make a difference for shaping the posture and motility in individuals with the organic, but solely bone-joint-muscle cause of the vestibular syndrome.[22]

There is no denying that a multitude of application methods, non-invasiveness, and high availability of the method as well as broad therapeutic indications make kinesiology taping a quite universal tool for handling dysfunctions in multiple systems. Yet, in the case of cervical vertigo therapy, it seems that this method should be considered supportive to primary treatment, while leaving room for continuation of research and experiment.

5. Conclusions

1. In patients with cervical vertigo, the applied kinesiology taping and kinetic therapy did not have any impact on the shaping of objectively assessed conduction (SEP) from the balance system sensory receptor through the cervical spine up to the sensory cortex.
2. Significantly improved results of the static and dynamic test carried out with the use of CCG in patients both after kinesiology taping and kinetic therapy were noted.
3. The final results of Freyss' dynamic stabilometry remained significantly better in the group of individuals after kinetic therapy.

6. Acknowledgement

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THE USEFULNESS OF OPK PARAMETERS ANALYSIS FOR SACCADIC AND SMOOTH PURSUIT DISTURBANCES INTERPRETATION

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Abstract: The idea of the study was to estimate if optokinetic tracking consisted of saccadic and smooth pursuit eye movements. The authors analyzed parameters of these three eye movements, comparing them with each other, to find the correlations between disturbances of the saccades, smooth pursuit and optokinetic nystagmus (OKN) parameters in 50 patients with central vestibular disorders and in 50 healthy subjects. Functional MRI was performed in the patients during these three visual stimulations to search for the structural fusion.

1. Introduction

Rapid eye movement and sinusoidal tracking are generated in the same centers of the central nervous system (CNS) as optokinetic reflex and in some patients one can observe the coexistence of the pathological results of these tests.

The aim of the study was the analysis of possible correlation between pathological results of the neuro-otological tests described above.

2. Material and methods

Fifty patients (31 female, 19 male, aged from 34 to 56 years) representing central vestibular disorders − VBI (vascular insufficiency), multiple sclerosis, arterial

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sclerosis, arterial hypertension, post-concussion syndrome − were examined using VNG in the scope o saccadic test, smooth pursuit test and optokinetic nystagmus. Hypermetry, hypometry, normal saccadic recording, symmetry of smooth pursuit, asymmetry of the optokinetic nystagmus (amplitude- A-, frequency- f- and slow phase velocity- v-) were analyzed and compared.

3. Results

3.1. Results in patients

1. In 17 (34%) patients: saccaddes hypermetry, unilateral disturbances of eye-tracking and asymmetry of OKN (on one side higher A, lower f and lower v and its distortion) coexisted.
2. In 15 (30%) cases: normal saccadic movement, bilaterally disturbed eye-tracking and bilateral distortion of OKN were stated.
3. In six (10%) patients: saccadic hypermetry, normal eye-tracking, asymmetry of OKN (one side higher A, lower f and distortion of v) were observed.
4. In nine (18%) patients: saccadic hypometry, unilateral eye-tracking pathology and OKN asymmetry (lower A) coexisted.
5. In four (8%) cases: normal saccadic movements, correct smooth pursuit and symmetrical OKN were recorded.

3.2. Results in the healthy group

The healthy group consisted of 50 subjects (33 female, 17 male, aged from 32 to 67 years).
1. In 23 (46%) persons: normal saccadic movements (no more than 15-20% over target), correct smooth pursuit and OKN symmetric reaction were observed.
2. In 13 (26%) persons asymmetry of OKN, hypometry of saccades and normal eye-tracking coexisted.
3. In 14 (28%) subjects: hypermetric saccades, smooth pursuit distortion coexisted with asymmetry of OKN.

4. Conclusion

The question was, is it possible, using OKN recording results, to define the possible pathology in saccadic and smooth pursuit eye movement? The results of the study suggest this possibility in CNS vestibular pathology.

5. Acknowledgement
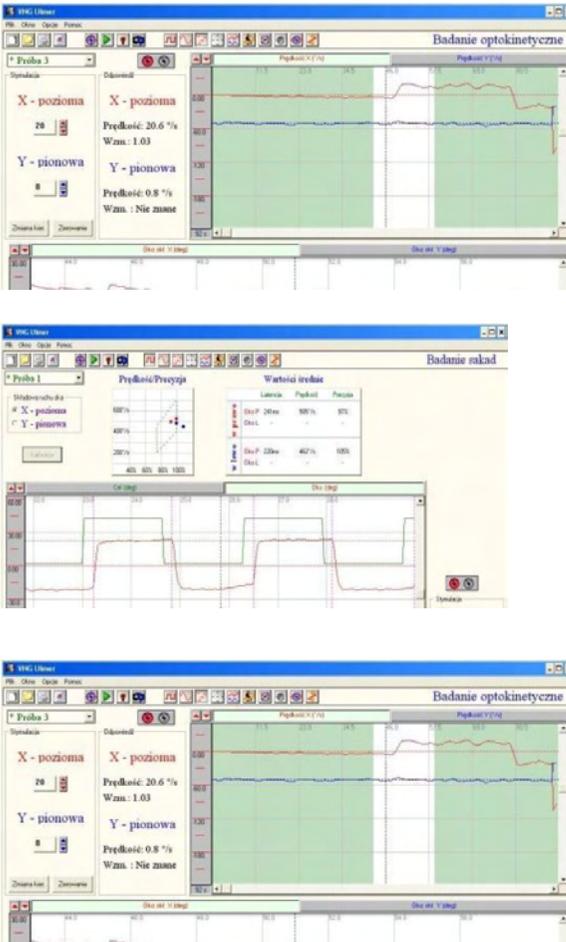
Fig. 1. Patient, group 1: saccadic hypermetry, unilateral disturbances of eye-tracking and asymmetry of OKN.

EFFECTS OF BODYTILT ON MULTIFREQUENCY ADMITTANCE TYMPANOMETRY[1]

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1. Introduction

Hydrops and abnormalities of inner fluid pressure are involved in some otological diseases such as Ménière's disease (MD). However, demonstrating abnormal perilymphatic or endolymphatic pressure is challenging. Mutifrequency admittancemetry (MFA) has proven its interest in MD since the width of conductance tympanograms increases in MD patients outside an attack compared to controls.[2] To confirm that the increase in conductance width is due to hyperpressure and not hypopressure in these patients tested outside an attack, we assessed the effect of changes in inner ear fluid pressure due to body tilt on the results of multifrequency admittancemetry tympanograms.

2. Materials and methods

A MFA including conductance (G) tympanogram at 2 kHz and resonance frequency (RF) measurements were performed in 20 volunteers (40 ears) free of otological or neurological disease. The measures were collected in three different positions: vertical, supine and Tredelenburg positions in order to modify the inner ear pressure. The MFA testing was performed in both ears immediately after each change in position.

MFA was recorded using a Grason-Stadler (GSI 33) otoadmittancemeter version 2. RF was determined with the frequency sweep technique.

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3. Results

Changes in inner ear fluid pressure due to body tilt induced an increase in the width of G tympanograms. In the vertical position the mean value was 141.7 ± 56.5 DaPa, in the supine position it increased to 158 ± 58.3 DaPa, and increased even more in the Tredelenburg position (20°) with a mean of 184 ± 69.6 DaPa ($p < 0.01$)(Fig. 1).

RF also increased in the Tredelenburg position.
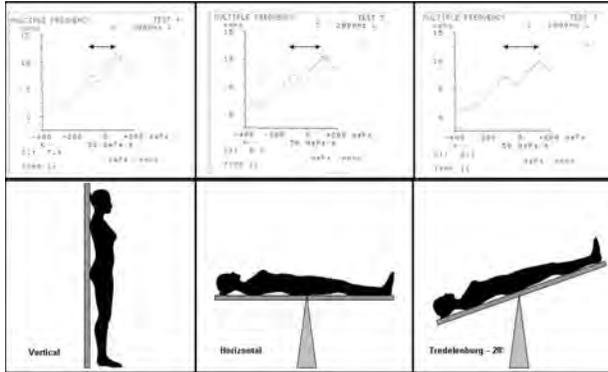
Fig. 1. MFA testing in different positions (A), showing widening of G tympanograms at 2 kHz when the patient is supine or in the Tredelenburg position compared to being vertical (B).

4. Discussion

These results demonstrate that the supine and Tredelenburg positions, which increase CSF pressure, can also increase the peak width of G tympanograms. Even though the same increase was demonstrated in MD patients outside an attack in a previous study, we now hypothesize that hyperpressure in inner ear fluids may occur even between attacks.[2] This and other studies performed in MD patients suggest that MFA is a suitable tool for detecting inner ear fluid hyperpressure since it demonstrates an increase in the width of G tympanograms. This increase observed in MD patients and the correlation between hydrops observed on MRI scan and MFA results in the study by Kato *et al.* also support this hypothesis.[3]

5. Conclusion

We conclude that the increased width of G tympanograms in MD patients outside an attack may be due to an increase in inner ear fluid pressure.

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OTOACOUSTIC EMISSIONS IN SUDDEN SENSORINEURAL HEARING LOSS

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1. Introduction

The reported high rate of 32-65% spontaneous recovery of idiopathic sudden sensorineural hearing loss (ISSNHL),[1-3] together with the variable response observed among patients receiving the same treatment make it difficult to study the indications and benefits of the various treatments suggested, some of which having potentially significant side effects.[4,5] This emphasizes the need for early prognostic criteria that may predict outcome. The identification of prognostic factors on presentation could possibly enable prediction of spontaneous recovery on one hand, and risk for permanent or progressive hearing loss requiring maximal treatment and long-term follow-up on the other hand.

Several prognostic factors have been previously suggested to predict ISSNHL outcome. These included the patient's age, gender, audiogram shape, severity of the hearing loss on presentation, the time elapsed from onset to the commencement of treatment, presence of vertigo, hypertension, accompanied tinnitus, and hearing acuity in the contralateral ear.[2,6-12] However, no consistent correlations were reported between any of these variables and the prognosis of ISSNHL.

Otoacoustic emissions (OAEs) are an objective neurophysiological test reflecting the function of the inner ear outer hair cells (OHCs). The evoked OAEs which include the transient evoked otoacoustic emissions (TEOAEs) and the distortion products otoacoustic emissions (DPOAEs), are both commonly used

in clinical practice.[13,14] For the TEOAE, click stimuli is mostly used. This is a broad band noise which activates wide regions of the basilar membrane, and reflects the OHCs activity at the threshold level. The DPOAEs are thought to reflect the OHC activity at the supra-threshold level.[13,15] Thus, although both evoked OAEs measure the OHCs function, they reflect different ranges of their activity.[16] OAEs and the behavioral audiometry results are significantly correlated. TEOAEs are most of the time absent in ears with pure tone thresholds greater than 20-25 dBHL, and usually no DPOAEs can be recorded when the pure tone threshold exceed 40-50 dBHL.[14,17-21]

In contrast to these commonly accepted limits for OAE's recordings, Sakashita *et al.*[22] first reported recordable TEOAEs among patients suffering from ISSNHL in the face of pure tone threshold worse than 35 dBHL with implication for better hearing outcome. Several studies follow investigating the role of OAEs in the prediction of ISSNHL outcome.[22-29]

Previous studies have yielded contradictory results for OAEs as related to ISSNHL outcome. Possible explanations are major differences in methodology, co-existing confounding parameters and the employment of variable criteria for hearing improvement and OAEs detectability.

The purpose of the present study was to investigate the role of TEOAEs and DPOAEs in the prediction of ISSNHL outcome in a prospective study employing currently accepted guidelines for patients' diagnosis, treatment and follow-up[30-33] while avoiding the methodological drawbacks of previous studies.

2. Patients and methods

Fifteen patients suffering from unilateral ISSNHL were included in an open-label prospective study. All met the following inclusion criteria: (1) pure tone bone conduction threshold elevation > 30 dB HL over at least three contiguous frequencies as compared to the contralateral ear; (2) sudden onset of the hearing loss evolving within three days or less; (3) cause of hearing loss unknown; (4) no hearing fluctuation; (5) symmetric hearing before the onset of ISSNHL according to a previous audiogram or patient's report; (6) no previous episode of ISSNHL; (6) normal otoscopy and type-A tympanometry; (7) air-bone gap < 15 dB HL; (8) retrocochlear lesion ruled out by magnetic resonance imaging (MRI) with and without gadolinium enhancement.

The study participants were treated by oral Prednisone 60 mg/day for five days followed by tapering down of the steroid dosage over additional seven days. When no significant improvement in hearing was observed on the 12th day post-presentation follow-up audiometry, the patients were further treated by twice a week intratympanic injections of Dexamethasone 10 mg/ml for the following two weeks (a total of four treatment sessions).

On presentation and before the commencement of treatment, all patients had audiological and OAEs evaluations including pure tone air (AC) and bone

conduction (BC) audiometry over the frequency range of 0.25–8 kHz and 0.5-4 kHz respectively, speech audiometry, tympanometry, TEOAEs and DPOAEs recordings. Identical evaluations were carried out after seven days, 14 days and three months. The intervals of the follow-up evaluations were based on the finding that the final hearing level following ISSNHL is reached within the course of therapy in 54.5% of patients and in 97% by three months.[31,32]

All audiological and OAEs evaluations were carried out in a soundproof audiometry booth by the same certified clinical audiologist (R.Z.).

The study protocol and procedures were approved by the local institutional review board. All subjects signed an informed consent form describing the purpose of the research and the subject's role.

As ISSNHL refers to sensorineural hearing loss, the BC thresholds were taken into account for calculating hearing outcome. The BC pure tone average thresholds of the three contiguous frequencies with the maximal hearing loss on presentation were calculated (BC-PTA). Hearing improvement was related to the hearing level of the contralateral unaffected ear following the recently published clinical practice guidelines for sudden hearing loss of the American Academy of Otolaryngology Head and Neck Surgery.[30] This enables unified outcome measure when pre-ISSNHL audiometries, that otherwise could have been used as a reference, are missing.

Hearing improvement was calculated employing the following equation:[29,34]

Hearing improvement (HI) = (HL _pre – HL_post / HL_pre – HL_contra) * 100%

HL_pre: BC-PTA on presentation; HL_post: BC-PTA on the follow-up visit; HL_contra: BC-PTA of the same frequencies in the contralateral ear.

Hearing improvement was defined as significant when HI > 50%.[35]

In addition to HI percentage, hearing acuity was classified according to the American Academy of Otolaryngology Head and Neck Surgery guidelines in terms of class A, B, C and D hearing.[33]

TEOAEs were considered as detectable for TEOAEs SNR> 3 dB SPL in the presence of signal reproducibility > 60%. DPOAEs were considered as detectable for DPOAEs SNR > 3 dB SPL. For the purpose of the present study, TEOAEs and DPOAEs were considered present when detectable in at least one frequency.

Changes in the OAEs SNRs as found in the follow-up evaluations were calculated for each individual frequency as the difference between the SNR at the follow-up and on presentation.

2.1. Statistical analysis

The number of patients having significant HI as related to the presence or absence of detectable OAEs was compared by the Fisher exact test.

Differences in the proportions of gender, vertigo, tinnitus and audiogram shapes between the significant hearing improvement (SHI) and no hearing improvement (NHI) groups were compared by the Fisher exact test.

Comparisons between the SHI and NHI groups in patients' age, time interval to the commencement of treatment, initial BC-PTA and hearing in the contralateral ear were performed either by the Student's unpaired two-tailed t-test or the non-parametric Mann-Whitney test, according to the Shapiro-Wilk normality test results.

Normal distribution of the audiometry and OAEs test results in the baseline and follow-up evaluations was tested by the Shapiro-Wilk normality test.

The magnitude of HI as related to the presence or absence of OAEs was compared either by the Student's unpaired two-tailed t-test or the non-parametric Mann-Whitney test, according to the normality test results.

The correlations between changes in the BC-PTA and OAEs SNR were analyzed by Pearson product-moment or Spearman rank correlations according to the normality test results.

Univariate analysis of variance of HI on the third month follow-up with the detectability of TEOAs and DPOAEs, magnitude of HI and BC-PTA in the seventh day follow-up was carried out in order to examine the amount of variance in the final HI which might be explained by each of these parameters.

P values < 0.05 were considered statistically significant.

3. Results

Fifteen patients, 11 males and four females, participated in the study. The mean age was 57.6 ± 16.2 years (mean $\pm$ standard deviation) ranging from 30 to 86 years. The right and left ear were involved in seven and eight cases respectively. The mean delay from ISSNHL onset to the commencement of treatment was 5.1 ± 3.6 days (mean $\pm$ standard deviation) ranging from one to 15 days. The average BC-PTA on presentation was 83.3 ± 25.3 dB HL, and 59 ± 38 dB HL on the completion of the study. The results of hearing and OAEs evaluations are detailed in Table 1.

On presentation, three and five patients had detectable TEOAE and DPOAE in at least one frequency respectively. No significant differences were found in the proportion of detectable TEOAEs or DPOAEs on presentation between the significant and non-significant HI groups on the third month follow-up (two-tailed Fisher's exact test). On the seventh day follow-up, TEOAEs could be recorded in seven and DPOAEs in six patients. Significantly larger proportions of detectable TEOAEs ($p < 0.001$, two tailed Fisher's exact test) and DPOAEs ($p < 0.005$, two-tailed Fisher's exact test) were found among patients having significant HI on the three months follow-up (Figs. 1, 2).

The sensitivity of recordable TEOAEs on the seventh day post-presentation follow-up towards the prediction of significant HI reached 71% (95% confidence interval 29.1-96.3%) and the specificity 100% (95% confidence interval 63.1-100%). For the DPOAEs the corresponding values for the sensitivity and specificity were 83% (95% confidence interval 35.9-99.6%) and 100% (95%

Table 1. Audiometry, and OAEs evaluations results.

Patient Nº	BC-PTA (dBHL)/ Hearing class Baseline	TEOAEs/ DPOAEs Baseline	HI (%)/ Hearing class Seventh day	TEOAEs/ DPOAEs Seventh day	Treatment	HI (%)/ Hearing class Third month
1	110/D	-/-	0/D	-/-	OS, ITS	0/D
2	90/D	-/-	13/D	-/-	OS, ITS	16/D
3	57/C	+/+	18/B	+/+	OS, ITS	100/A
4	48/B	-/+	0/B	-/+	OS, ITS	0/B
5	73/D	-/+	51/B	+/+	OS, ITS	65/A
6	92/C	+/+	14/C	+/-	OS, ITS	8/C
7	73/C	+/+	92/A	+/+	OS	95/A
8	110/D	-/-	0/D	-/-	OS, ITS	0/D
9	52/C	-/-	16/B	+/+	OS, ITS	84/A
10	110/D	-/-	0/D	-/-	OS, ITS	13/D
11	50/C	-/+	84/A	+/+	OS	84/A
12	110/D	-/-	11/D	-/-	OS, ITS	43/D
13	60/C	-/-	67/B	+/-	OS, ITS	0/C
14	105/D	-/-	16/D	-/-	OS, ITS	13/D
15	110/D	-/-	0/D	-/-	OS, ITS	0/D

BC-PTA: Bone conduction pure tone average thresholds of the three contiguous frequencies with the maximal hearing loss; HI: Hearing improvement; Minus sign: Non-detectable OAEs; Plus Sign: Detectable OAEs; OS: Oral steroids; ITS: Intratympanic steroids.
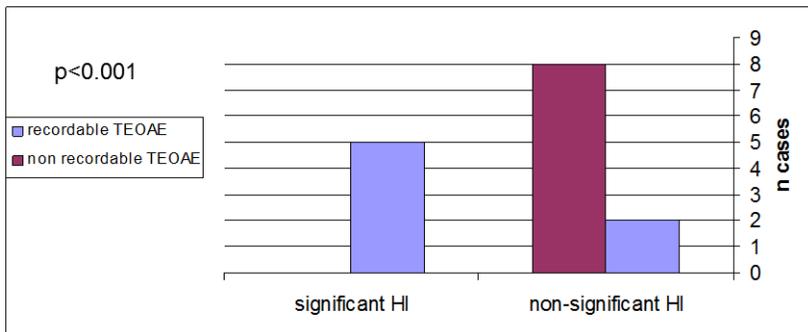
Fig. 1. Number of patients with significant (> 50%) and non-significant (< 50%) hearing improvement as related to TEOAEs detectability on the seventh day follow-up. Recordable TEOAEs were significantly associated with significant hearing improvement on the third month follow-up (p < 0.001, two-tailed Fisher's exact test).

confidence interval 66.4-100%) respectively. In comparison, the sensitivity and specificity of hearing improvement > 50% on the seventh day audiometry towards the prediction of significant final HI were 75% (95% confidence interval 19.6-99.4%) and 82% (95% confidence interval 48.2-97.7%) respectively.
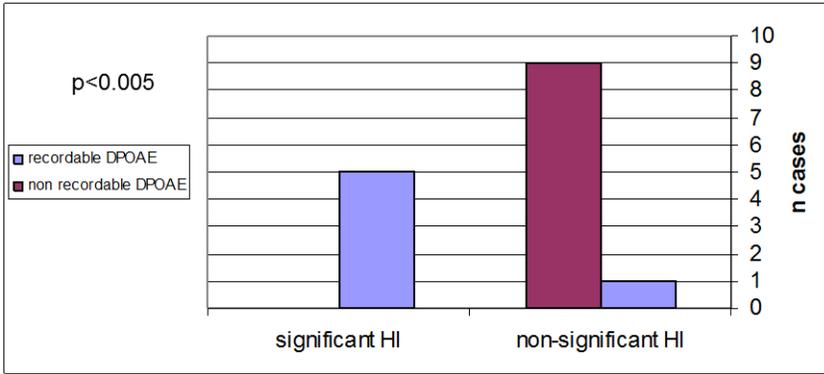
Fig. 2. Number of patients with significant (> 50%) and non-significant (< 50%) hearing improvement as related to DPOAEs detectability on the seventh day follow-up. Recordable DPOAEs were significantly associated with significant hearing improvement on the third month follow-up (p < 0.005, two-tailed Fisher's exact test).

On the third month follow-up examination, five patients had significant HI (> 50%) while in ten HI was categorized as non-significant (< 50%). No significant differences were found between the two groups in age, gender, time elapsed from presentation to treatment, presence of vertigo and tinnitus, audiogram shape, and contralateral ear hearing. BC-PTA on presentation was significantly higher in the group of patients with non-significant hearing improvement on the final follow-up (94.5 ± 22.8 dBHL vs. 61 ± 11.3 dBHL in the significant HI group; p < 0.05 Mann-Whitney test).

The average HI on the third month follow-up among patients in whom TEOAEs could be recorded on the seventh day follow-up was 62 ± 41% (mean ± standard deviation) compared to 11 ± 15% only when the TEOAEs were missing (P < 0.001, Student's unpaired two-tailed t-test, Fig. 3). For recordable and missing DPOAEs on the seventh day follow-up, HI reached 71 ± 37% vs. 10 ± 14% respectively (p < 0.001, Student's unpaired two-tailed t-test, Fig. 4). No significant differences in the third month follow-up HI were found between patients with recordable and missing TEOAEs and DPOAEs on presentation.

The final HI was significantly correlated with TEOAEs SNR improvement on the seventh day follow-up at 3 kHz (Rho = 0.65 p < 0.01, Spearman rank correlation). No significant correlation was found between the final HI and SNR improvement on the seventh day for any of the DPOAEs f2 frequencies. No consistent correlations were found between the HI in any of the follow-up evaluations and the OAEs SNR changes on the same visit.

Univariate analysis of variance of HI on the third month follow-up with the detectability of TEOAs and DPOAEs, HI and BC-PTA in the seventh day follow-up evaluation was carried out in order to examine the amount of variance in the final HI which might be explained by each of these parameters and to find out which parameters might predict ISSNHL hearing outcome.
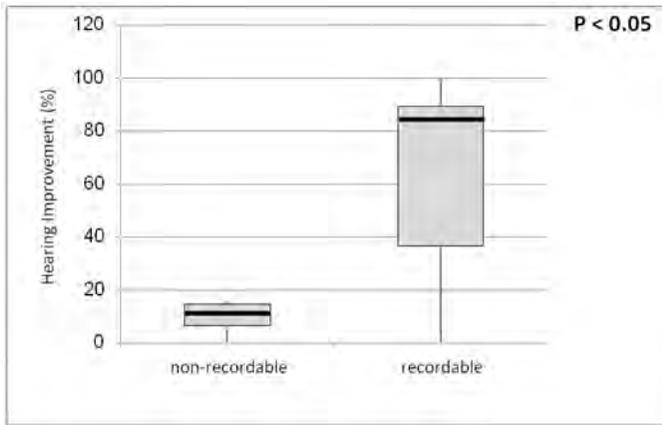
Fig. 3. Whisker's plots of hearing improvement percentage on the third month follow-up as related to the detection of TEOAEs on the seventh day follow-up. Hearing improvement was significantly higher among patients with detectable TEOAEs ($P < 0.05$, Student's unpaired two-tailed t-test). The boundary of the box closest to zero indicates the 25th percentile, the solid line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers above and below the box indicate the 90th and 10th percentiles.
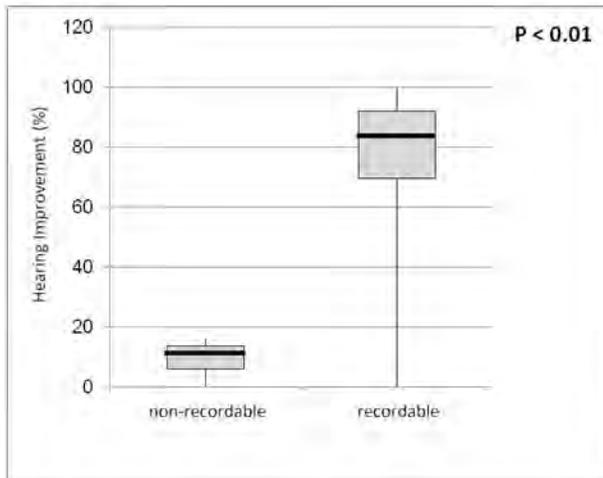
Fig. 4. Whiskers plots of hearing improvement percentage on the third month follow-up as related to the detection of DPOAEs on the seventh day follow-up. Hearing improvement was significantly higher among patients with detectable DPOAEs ($P < 0.01$, Student's unpaired two-tailed t-test).

One-tail analysis showed significant contribution of both TEOAEs ($p < 0.05$) and DPOAEs ($p < 0.01$) and their interaction ($p < 0.01$) towards less than 50% HI on the third month follow-up while the effects of HI and BC-PTA in the seventh day follow-up evaluation were not statistically significant.

4. Discussion

The main finding of the present study is that lacking of any OAEs responses on the completion of seven-day oral steroidal treatment for ISSNHL might predict unfavorable hearing outcome as measured three months post-presentation. Undetectable OAEs was associated with non-significant HI with specificity of 100% for both TEOAEs and DPOAEs protocols. Significantly larger number of patients having detectable OAEs on the seventh day follow-up met the criteria for HI greater than 50% three months post-presentation. Also, the average final HI was significantly larger for these patients. The sensitivity of TEOAEs and DPOAEs towards the prediction of significant HI reached 71% and 83% respectively.

Two of the 15 patients recovered their hearing to that of the contralateral ear after the oral steroid course. The remaining 13 patients were treated by intratympanic Dexamethasone injections. Seven patients had no OAEs responses on the seventh day follow-up and all of them stayed in the same hearing impairment class. Six patients had detectable OAEs, and three of them had significant HI on the final follow-up moving from hearing class D and C to A. Three patients with detectable OAEs stayed in class B and C.

For the 13 patients treated by IT steroids, presence of OAEs response in at least one frequency on the seventh day follow up predicted hearing improvement > 50% on the final follow-up with sensitivity of 50% and specificity of 100%. Thus, non-detectable OAEs might predict failure of the salvage IT steroids protocol.

Our results corroborate with previous studies reporting prediction of hearing outcome by TEOAEs[36] and DPOAEs[22] when conducted during the treatment of ISSNHL. Lalaki *et al.*[36] found that TEOAEs could be recorded within three days from the commencement of treatment among almost all patients who finally recovered their hearing. Chao and Chen[22] reported significantly higher DPOAE's amplitudes among patients who recovered their hearing as recorded five days from the beginning of treatment. Hoth *et al.*[27] have found significant correlations between earlier detectability of TEOAEs and DPOAEs and later PTA improvement.

The early detectability of TEOAEs and DPOAEs, preceding PT hearing improvement, might be explained by the high sensitivity of these testing modalities in the detection of changing outer hair cells activity. It has been previously suggested that the outer hair cells might recover earlier than the inner hair cells and supporting cochlear structures.[37] Thus, partial resolution of the outer hair cells function which cannot yet support PT threshold decrement might be detected by OAEs testing to be followed by further recovery eventually leading to a clinically significant hearing improvement.

The presence of OAEs in the face of non-significant HI on the third month follow-up in some patients, as demonstrated by the lower than optimal sensitivity values found, imply that ISSNHL might also involve auditory organ and pathways lesions beyond the outer hair cells.[38]

Opposing the seventh day follow-up results, both TEOAEs and DPOAEs testing before the commencement of oral steroidal treatment failed to predict hearing outcome. Thus, OAEs evaluation cannot support patient's management decisions on the presentation of ISSNHL.

In agreement with our results, several previous studies have failed to find significant correlation between TEOAEs[24,26,27,36] or DPOAEs[26-28] on presentation and final hearing outcome. The univariate analysis of variance showed that neither hearing improvement percentage nor the pure tone thresholds seven days post-presentation can predict the future hearing outcome. These results corroborate previous publications reporting that final hearing level is reached within the course of therapy only in 54.5% of patients suffering from ISSNHL.[31,32]

5. Conclusions

Our results suggest potential role of TEOAEs and DPOAEs evaluation conducted following the systemic steroidal therapy in the prediction of ISSNHL prognosis. The study results are restricted because of the small number of participants, thus a larger-scale research is required to confirm the conclusions of the present study

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A COMPARISON OF ECOCHG, VEMP, VNG AND ROTARY CHAIR RESULTS IN PATIENTS DIAGNOSED WITH MÉNIÈRE'S DISEASE

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1. Introduction, background and purpose

The diagnostic battery for evaluating patients suspected of having Ménière's disease (MD) may include a variety of tests, including: electrochleography (ECochG), vestibular evoked myogenic potentials (VEMP), videonystagmography (VNG), and sinusoid harmonic motion/rotary chair (RC). However, the efficacy and accuracy of using these particular tools in diagnosing MD has not been well-studied. Also in need of further investigation are comparisons of these procedures to determine the most accurate, efficient and cost-effective one(s) for identifying MD in patients suspected of having this disorder.

In 2013, Seo and colleagues studied the correlation among pure tone average, ECochG and VEMP in individuals diagnosed with MD and found a significant difference in the pure tone average and ECochG compared to healthy adults.[1] VEMP-testing did not demonstrate a significant difference between these two groups. Wang *et al.* correlated the results of audiometry, VNG (air calorics) and VEMP testing in individuals diagnosed with MD.[2] Their results showed caloric abnormalities in 75% of patients with demonstrated unilateral weakness. There was a linear regression when comparing audiometry with unilateral weakness, the worse the hearing, the weaker the caloric. VEMP results were abnormal in only 38% of these patients.

While the sensitivity and specificity of ECochG for diagnosing MD has improved considerably in recent years,[3] comparisons of these values between ECochG and the other tests listed above have not been reported. Thus, the purpose of this study was to perform these comparisons with the goal of identifying

which particular test (or combination of tests) was the most sensitive and specific to MD. Such information may lead to more accurate and less time-consuming (and therefore more cost-effective) protocols for diagnosing MD.

2. Methods

The study was conducted through a retrospective chart review of patients from 2011 to 2013 who were diagnosed with MD by an otolaryngologist at the University of Kansas Medical Center. The subjects were referred for the specific test battery based on displayed signs and symptoms consistent with MD, including tinnitus, and/or aural fullness, and/or fluctuating hearing loss and/or vertigo. All testing was completed by audiologists in the University of Kansas Medical Center. ECochG was performed in the Hearing and Speech Department and RC, VNG and VEMP testing were completed in the Otolaryngology Department.

ECochG was performed in the manner described by us in several publications (*e.g.*, see Refs. 3-6). In brief, our recordings are made from the tympanic membrane (TM), using a commercially available TM electrode and electrode cable distributed by Sanibel, and either the Interacoustics Eclipse or Bio-Logic NavPro AEP unit. The components of interest included the cochlear summating potential (SP) and the whole nerve action potential (AP) of CN VIII to broadband click stimuli, from which the SP/AP amplitude and area ratios were derived (also as described by us numerous times[3-8]). Amplitude ratios greater than 0.40 and area ratios greater than 2.0 to 90 nHL clicks were considered as positive findings for endolymphatic hydrops.

VNG tests included assessment of gaze nystagmus, spontaneous nystagmus, head positional testing, body positioning testing including Dix-Hallpike maneuver, and calorics. VNG was determined to be abnormal if significant nystagmus (more than six degrees/sec) was apparent or if nystagmus was less than eight degrees/sec, and/or a 28% unilateral weakness was noted during caloric testing (indicating a peripheral site of lesion).

C-VEMP testing was performed using an air conducted stimulus at a presentation level of 100 dB HL. Thresholds and amplitude ratios for each ear were obtained and VEMPs were determined to be abnormal if thresholds were less than 70 dB and/or amplitude ratios between ears were greater than 40%.

Since many MD patients also undergo RC testing, we included this procedure among our comparisons. Phase, gain and symmetry were plotted against normative values using commercially available software. RC results were deemed to be abnormal if there was low gain at any frequency and/or phase lead was present and/or asymmetry was noted (indicative of peripheral lesion).

The authors compiled retrospective data from over 250 patient charts; however, only 30 individuals had the unique test battery administered that was necessary for this study. All participants were seen at the KUMC between 2011 and 2013, and were between the ages of 31-79 years. Patients who had inconclusive

ECochG results due to the amount of hearing loss or artifact were not included in this study, as were patients with abnormal Auditory Brainstem Responses (ABR) results indicative of retrocochlear pathology.

Data collected for each subject included: physician diagnosis; gender; normal/abnormal results for each test (and sub-test); affected ear(s)/side. The Freeman-Halton extension of the Fisher exact probability test using a two rows x four column contingency table was utilized, providing the authors with information about the interaction of the diagnostic tests. Additionally, a post-hoc analysis computation (Bonferroni correction) was used by computing chi- square for each combination of tests. Sensitivity and specificity were also calculated for ECochG, VNG, VEMP and RC by analyzing the true and false positives and negative decisions of each of the tests in comparison to the positive or negative diagnosis of MD. Sensitivity was calculated by dividing the true positive results over the total positive results. Specificity was calculated by dividing the true negative over the total negative results.

3. Results and discussion

The following represents basic demographic information for the subjects in this study:

- Ages ranged from 31-79 years;
- Of the patients, 46.7% were female (n = 14); 53.3% were male (n = 16);
- 33.3% of patients were diagnosed with bilateral MD, 23.3% were diagnosed with MD on the left side and 43.3% were diagnosed with MD on the right side.

Raw data from the data collection sheet comparing number of abnormal results per diagnostic test are as follows: ECochG: 29/30; VNG: 26/30; VEMP 15/30; RC 17/30. Asymmetry in the VEMP was present in 13 of the 30 subjects diagnosed with MD and the VEMP response was absent in two of the 30 subjects. 30% of the patients were also diagnosed with benign paroxysmal positional vertigo (BPPV), while d the diagnoses for 33.3% of the patients included migraine-associated vertigo.

The Fisher Exact Probability Test revealed a statistically significant ($p < 0.01$) difference between the diagnostic tests; however, it was unspecified as to which tests were different. Post-hoc analysis using Bonferroni correction chi-square on each pair of diagnostic tests revealed a statistically significant difference between ECochG and RC, ECochG and VEMP, VNG and VEMP and VNG and RC. The overall sensitivity/specificity percentages for each test are as follows:

- ECochG: 95.2/82.7
- VNG (calorics): 72.7/66.7
- VEMP: 50.0/66.7
- RC: 58.0/33.3

While a thorough discussion of our results is restricted in this manuscript due to do page limits, it is obvious from our findings that ECochG emerged at the most sensitive and specific test for MD among the four procedures compared in this study. The specificity/sensitivity values that we observed of 95.2%/82.7% are very similar to the ones reported for ECochG alone by Almomani *et al*.[3] of 92%/83%. Of importance to this finding is that both SP/AP amplitude *and* area ratios were included in our measurements. It is likely based on various reports in the literature that using the amplitude ratio alone would have yielded a sensitivity value similar to the other tests (*i.e.*, less than 70%), while specificity would have remained high. VNG caloric responses represented the second most sensitive/specific test for MD, and there were no significant differences between ECochG and VNG for this purpose. Significant differences were noted between ECochG and VNG versus VEMP and RC, however.

4. Summary and conclusions

While ECochG, VNG, VEMP and RC remain as important diagnostic tests for patients with symptoms of inner ear dysfunction, ECochG is the most sensitive and specific one for MD, followed by caloric VNG. Thus, both ECochG and VNG should be routinely included in the diagnostic workup for patients suspected of having MD/endolymphatic hydrops. Furthermore, the value of performing VEMP and RC on patients suspected of having MD is questionable unless sites of lesion other than or in addition to the cochlea also are suspected. Of particular note is the importance of measuring/deriving both the SP/AP amplitude and area ratios in the evaluation of the electrocochleogram. When this latter value is included, the sensitivity of ECochG for identifying MD jumps from approximately 60% to over 90%.

Future research in this area should include a multi-center study to provide a better sample of the true MD population. Our data base is relatively small and the physicians from whom we receive referrals all value the information gained about their suspected MD patients from the four tests we compared. To our knowledge, a standardized diagnostic protocol that includes these particular procedures has not been widely adopted. In addition, despite being an important tool and long-standing tool for helping to diagnose MD, the approaches/procedures for recording, measuring and interpreting ECochG continue to vary considerably among clinicians who practice this technique on their patients. Finally, ECochG and the other tests compared in this study are also used to help diagnose other hearing-/vestibular-related disorders such as Auditory Nerve Dysynchrony Disorder, and Semi-circular canal dehiscence. Sensitivity/specificity comparisons among the various diagnostic tests for these conditions also need to be completed.

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ELECTROCOCHLEOGRAPHY AND CERVICAL VESTIBULAR EVOKED MYOGENIC POTENTIAL TEST IN MÉNIÈRE'S DISEASE

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Abstract

Introduction: Ménière's disease (MD) is a chronic inner ear dysfunction without any definite diagnostic tools. The goal of this study was to evaluate the results of electrocochleography (ECoG) and cervical vestibular evoked potential (cVEMP) tests in adult patients with definite MD and find correlations between the results of these tests.

Patients and methods: after excluding patients with previous history of otologic surgery or injections or neurologic disorders, 62 patients were enrolled. Click sound stimulation for ECoG and tone burst sound stimulation (500 Hz) for cVEMP test were performed. A summation potential (SP) to action potential (AP) ratio larger than 0.4 was accepted as elevated ECoG. In addition, absent wave, elevated threshold, or abnormal morphology was considered negative cVEMP test. All tests were done in non-active phase of disease according to clinical findings.

Results: 58% of patients were female; mean age of patients was 43.7 years; mean duration of the disease was 46.8 months; 75.8% of patients had elevated ECoG and of the patients in whom the disease was on the right side according to AAO-HNS guideline (29 patients), 79.3% had elevated results while in patients with left side clinical disease (27 patients), the elevated ratio was 66.8%. The results for cVEMP test are as follows: 71.0% had negative cVEMP overall, while in 58.6% of patients with right-sided disease and 77.3% of those with left-sided disease, test results were negative. Correlation coefficient of both tests were not

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statistically significant. No correlation between age or duration of disease and test results was found.

Conclusion: as both tests had some beneficial help for diagnosing MD, none of them can be used as a diagnostic modality. Combination of both tests for diagnosis may be helpful, as they did not have significant correlation.

1. Introduction

Ménière's disease (MD) which is classically defined by fluctuating sensorineural hearing loss, episodic vertigo, and tinnitus or aural fullness, has very challenging aspects.[1] Despite more than 150 years after its description by the French physician Prosper Ménière,[2] many questions about its pathophysiology and consequently its diagnosis and management remain unanswered. This leads to performing a lot of research efforts to clarify this vague status.

Vestibular evoked myogenic potential (VEMP) which was applied in clinical setting in 1992 by Colebatch and Halmagyi, is a response in muscles to sound or electric stimulation.[3] It seems that VEMP is a good indicator of saccular and inferior vestibular nerve function especially in a clinical setting.[4] Saccular involvement as a second frequent site after cochlea in MD lead to common use of this test.[5] Nevertheless, VEMP cannot be an effective diagnostic test. On the other hand, electrocochleography (ECoG), which is a measurement of the electrical potentials generated by the cochlea and auditory nerve may be normal in some patients and is not a diagnostic modality by itself.[6]

In a study presented by Murphy *et al.*, only the superiority of ECoG in comparison to VEMP for diagnosis was showed.[7] Wu *et al.* assessed some audiologic test results in MD separately.[8] Presence of a few studies about both these tests in patients with MD on one side and lack of evaluation of diagnostic capability and correlation of these two tests on the other side motivated the current study.

2. Materials and methods

Patients with the diagnosis of MD who came to the clinic of Otorhinolaryngology at the Amir Alam hospital (a tertiary and teaching hospital) in 2014 were assigned as the population study.

Patients with definite MD according to the American Academy of Otolaryngology – Head and Neck Surgery (AAO-HNS) criteria[9] were included. Those with any other otologic disorders or surgery including intratympanic injections, or those who had musculoskeletal or neurologic disorders, were excluded. Finally and according to the sample size calculation, 62 patients enrolled in the study. In addition to primary audiometric evaluation, electrocochleography and cervical VEMP (cVEMP) tests were performed in all participants in a non-active phase of the disease according to clinical findings.

Table 1. Distribution of abnormal ECoG* and cVEMP** test results according to clinically involvement of each ear in Ménière's disease.

Clinically ear involved (n)	Abnormal ECoG results (%)				Abnormal cVEMP test results (%)			
	Right	Left	Both	None	Right	Left	Both	None
Right side (29)	62.1	0.0	17.2	20.7	27.6	6.9	31.0	34.5
Left side (27)	7.4	63.0	3.7	25.9	0.0	44.4	25.9	29.6
Both side (6)	16.7	0.0	50.0	33.3	16.7	0.0	83.3	0.0

* ECoG: electrocochleography; ** cVEMP: cervical vestibular evoked myogenic potential.

Cervical VEMP testing was performed with tone burst stimulation sound (500 Hz and 95 dB). Electric response was considered negative or abnormal if there was no wave, increased stimulation threshold or decreased amplitude, or abnormal morphology.

Electrocochleography was done with sound click stimulus and considered as elevated or abnormal when SP to AP ratio was greater than 0.4 and others classified in the not-elevated group including patients who had not shown any wave due to significant sensorineural hearing loss.

For data analysis, chi square and Fisher exact tests were applied for dichotomous variables. Correlation between variables was assessed with Pearson and Spearman's rho.

3. Results

Of the 62 patients in the study, 58% were female. Mean age of the patients was 43.7 ± 13.1 years. Mean follow-up and duration of disease were 46.8 months, while median was 30 months (range: 1-240).

As patients were classified by ear involvement according to AAO-HNS criteria, 29 patients had right-sided ear disease, 27 had left-sided disease, and six patients suffered from bilateral disease. ECoG results showed elevated ratio in 75.8% of all patients, while cVEMP test results were considered in abnormal group in 71.0% of all participants. The difference between two tests were not statistically significant (p value: 1.00). Equally, sub-group analyses according to side involvement did not show statistically significant differences. More details about test results are shown in Table 1.

Overall results of both tests are as follows: normal results of both tests in 6.5%, elevated ECoG along with normal cVEMP test results in 22.6%, negative cVEMP test with normal ECoG in 17.7%, and abnormal results in both tests in 53.2%. In evaluation of the test results for each ear, clinically right side involvement was seen in 35 patients, whereas 33 patients had clinically left side pathology (patients with bilateral involvement were categorized in both groups for this analysis). For each ear, abnormal test results on that side were analyzed, specifically abnormal output in both tests on the same side was assumed as one

Table 2. Distribution of abnormal Ipsilateral ECoG* and/or cVEMP** test results according to clinically involvement of left or right ear in Ménière's disease.

Clinically ear involved (n)***	Ipsilateral abnormal ECoG (%)	Ipsilateral abnormal cVEMP (%)	Ipsilateral abnormal ECoG and cVEMP (%)	Ipsilateral abnormal ECoG or cVEMP (%)
Right side (35)	77.1	65.7	45.7	97.1
Left side (33)	63.6	72.7	48.5	87.9

* ECoG: electrocochleography; ** cVEMP: cervical vestibular evoked potential; *** Patients with bilateral ear involvement included in both group and for each side, data of abnormality on that side were considered for analysis.

parameter and another parameter was ipsilateral abnormality in any of these two tests. More details can be seen in Table 2.

As the cVEMP test may show abnormal results by itself in patients older than 60 years, all analyses for this test were done in a subgroup which only included patients younger than 60 years and results did not show any statistically significant differences.

Correlation coefficient of both tests were not statistically significant (p value: 0.82). Similarly, no correlation between age or duration of disease and any of two test results were seen. In addition, analyses in sub groups led to similar results.

4. Discussion

Electrocochleography and cVEMP are two audiovestibular tests proposed as complement for diagnosis of MD.[1] The significance of these tests as diagnostic tests are unclear and not included in diagnostic criteria until now. This study shows that patients with definite MD can have positive test results especially if two tests are applied and the result is considered positive when any of two tests shows abnormality (97% and 88% for right and left side disease respectively). Lack of correlation between the results of two tests may enhance the mentioned diagnostic application.

A few studies can be found which mainly focus on these tests in MD. Murphy *et al.*[7] assessed superiority of ECoG in comparison of VEMP in evaluation of MD in 117 patients. In their analysis, 27% of patients had abnormal ECoG and VEMP test results and none of them had abnormal VEMP in the presence of normal ECoG findings. On the other hand, the current study showed that 53.2% of patients had abnormal results in both tests and also normal ECoG along with abnormal cVEMP test were found in 17.7%. Moreover, Murphy found that 73% of patients had abnormal ECoG with normal cVEMP test, while in this study, only 22.6% showed these results. These differences in results may be explained by the difference in study population. The current study only evaluated definite MD and also patients may not have uniform disease as various pathophysiologic

states are assumed for it. In addition, differences in test setup may be another explanation, including higher accepted threshold for ECoG abnormality in this study or similar differences in VEMP rest.

Wu et al.[8] evaluated vestibular test battery and ECoG in patients with MD and showed that ECoG and VEMP test was positive in 76.9% and 58.0% respectively. The current study showed similar results in ECoG (75.8%) but the cVEMP test results were more positive (71.0%). In addition to technical differences between the two centers where cVEMP tests were performed, which can affect the abnormal result of tests, other factors may be responsible for this difference. Variation in population in the study may be an important factor. Test results can vary in different stages of MD (possible, probable, or definite). In the current study, only definite MD was included. Age of patients can affect the cVEMP results. This test may show false positive in patients older than 60 years, however, specific analysis in the study rejected this effect.

Selection bias may be a potential limitation in this study. As patients included were from a tertiary center, they may have different severity and extension of disease compared to other patients with MD. This point may affect the results and a larger group study in various centers will clarify this vague deduction.

Applying ECoG and cVEMP tests in possible or probable MD patients may be a good topic for future research. Their results could be helpful to predict progression to definite disease or contralateral involvement and also may have the potency to include in the diagnostic criteria of MD with further research on various vestibulocochlear tests. Furthermore, these tests may be assessed for use in follow-up especially after interventions like intratympanic injection or endolymphatic sac surgery.

Despite of some beneficial help for diagnosing MD, neither ECoG nor cVEMP can be used on itself as a diagnostic modality. A combination of both tests for diagnosis may be helpful, as they did not have significant correlation.

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PREDICTIVE VALUE OF ECOCHG IN OFFSPRING/SIBLINGS OF INDIVIDUALS WITH MÉNIÈRE'S DISEASE

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1. Introduction/background/purpose

Ménière's disease (MD) is the most common inner ear disorder in adults with a prevalence rate of 20-40 per 100,000 people. Approximately 46,000 new cases of MD are diagnosed each year in the United States alone. The median age of onset for MD is 51 years of age and is found to be distributed between male and female, respectively 40 and 60%.[1] Familial aspects or genetics of MD have been studied several times since 1941.[2] Researchers have determined the mode of inheritance for identified genes as autosomal dominant. Arweilier-Harbeck states that the literature reports the frequency of familial MD is at 20% and her own retrospective and prospective family survey research revealed 19%.[2] Thus, approximately one fifth of MD patients have a close relative with this condition.

Electrocochleography (ECochG) has emerged as an effective tool in the diagnosis and monitoring of MD/Endolymphatic Hydrops (ELH) (*e.g*., Refs 3, 4). In the early days of ECochG, recordings were most useful when performed during symptomatic episodes. The symptoms most correlated with a positive ECochG were hearing loss combined with aural fullness/pressure.[5] More recent advances in recording parameters and protocols have proven effective even when the patient is asymptomatic. These advancements include performing recordings from the tympanic membrane (versus ear canal) and measuring both the summating potential and action potential (SP/AP) amplitude and area ratios.[3,4,6,7] When using the SP/AP amplitude ratio alone, the sensitivity and specificity of ECochG for diagnosing MD/ELH are approximately 60% and 90%, respectively. When

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both amplitude and area ratios are included, the sensitivity factor improves to 92%, while specificity remains high at 84%.[7]

Since the cause(s) and trigger(s) for MD are so variable (genetic, dietary, allergies, infections, etc.), it is essential that researchers investigate potential preventive measures that can be applied to individuals who are either predisposed or are in route to developing this disorder. One question in need of study is whether ECochG could be used as a potential screening tool for MD in addition to a diagnostic one. Another question raised is whether the initiation of ELH can be detected prior to the onset of symptoms. The clinical implications for these findings could lead to the implementation of preventive measures (*e.g.*, lifestyle changes, pharmaceutical intervention; allergy control) that individuals could take to detour themselves from developing MD and/or mitigating its symptoms.

One option to studying the familial attributes related to MD is to perform ECochG on the offspring and siblings of patients with a definitive diagnosis to assess whether recordings from this population display abnormalities associated with MD prior to the onset of symptoms. In other words, can ECochG be used to determine if the offspring/siblings of patients with MD are predisposed to developing the condition themselves?

The current study is designed to further examine the prevalence of MD among family members and the use of ECochG in helping to diagnose and possibly screen for this disorder. We hypothesize that the offspring/siblings of patients with MD may have a higher incidence positive electrocochleograms than normal/non-MD populations. If so, this finding will facilitate further investigations on the use of ECochG as a screening tool for MD in populations genetically predisposed to this disorder.

2. Methods

2.1. Subjects

All components of this study were done in accordance to the rules and regulations of the University of Kansas Medical Center Human Subject Committee. The current investigation is a pilot study to see if further research using larger sample sizes is warranted.

The control group consisted of a large (greater than 100) data base of normal subjects from whom normative ECochG values have been established. A total of ten individuals served as the experimental group that comprised individuals who are without symptoms, but have a parent/sibling with a definitive/confirmed diagnosis of MD/ELH made by an otolaryngologist. Their ages ranged from 18-58 years and all were from different families. Subjects were recruited from patient approved medical records from physicians of the ENT clinic at University of Kansas Medical Center. All participant data and information was kept confidential and protected. All data collected for the study was stored on

a university approved and secured laptop. To provide further protection this laptop was kept in a locked university clinic room.

Each participant reviewed and signed a human subjects committee consent form prior to testing. Each participant was asked if they have any history for the following symptoms: aural pressure/fullness, vertigo, tinnitus, and hearing loss. Further questioning was done to make sure each patient was fully educated on the manifestation of true symptoms. Otoscopy, tympanometry, and a hearing screening at 20 dB HL between 125-8000 Hz were performed to rule out hearing loss and outer/middle ear pathology.

2.2. ECochG

Bilateral ECochG was performed in the manner described by us in several publications (*e.g.*, Refs 3, 4, 6-9). Briefly, our recordings are made from the tympanic membrane (TM), using a commercially available TM electrode and electrode cable distributed by Sanibel, and either the Interacoustics Eclipse or Natus Nav-Pro AEP unit. The components of interest included the cochlear summating potential (SP) and the whole nerve action potential (AP) of CN VIII to broadband click stimuli, from which the SP/AP amplitude and area ratios were derived (also as described by us numerous times[3,4,6-9]). Amplitude ratios greater than 0.40 and area ratios greater than 2.0 to 90 nHL clicks were considered as positive findings for endolymphatic hydrops.

A potential benefit of this study is the possibility of aiding offspring, siblings, and their physicians to select preventative/management strategies for MD. However, a negative consequence could be the addition of undue stress placed on a participant whose ECochG results are positive, as such a finding may cause them to worry about developing MD. In addition, choosing to implement preventive/management strategies might require life altering decisions. For example, choosing to remove nicotine or caffeine from one's diet may be difficult to implement. To help alleviate these concerns, it was stressed to all subjects that our research is still insufficient to verify whether a positive or negative ECochG has any predictive value for the development MD.

Regardless of findings, participants were given an explanation of their ECochG results.

When a subject's ECochG results were positive for MD, it was explained that these findings do not necessarily indicate that they have this disorder, or even that they may develop it as there is no current evidence to support these claims. However, he/she was alerted to seek medical assistance if they begin to experience any of the symptoms of MD.

2.3. Subject recruitment

A database of 105 patients with confirmed diagnoses of MD and positive electrocochleograms was gathered. Recruitment Letters were sent to each patient informing them about the study and that the researchers were recruiting fam-

ily members. The letters were followed-up with phone calls to provide further information about the study and to gather contact information of interested relatives. These efforts led to an enrollment of ten individuals. One of these individuals was found to have unilateral hearing loss and was therefore excluded from the study. The remaining participants were scheduled for a single testing session (one to two hours) and went through the previously mentioned protocol (consent form, case history, hearing screenings, and ECochG). Analyses of the electrocochleograms were conducted independently by both authors before jointly agreeing on the final measurements.

3. Results/discussion

As indicated above, one participant of the study was excluded from the data analysis due to a moderate hearing loss on one side. Thus, 18 electrocochleograms (nine subjects with recordings for both ears) comprised our database. Table 1 indicates the findings for this group. As can be seen from these data, seven of our nine subjects (nearly 80%) displayed positive electrocochleograms for MD, based on enlarged SP/AP amplitude and/or area ratios. This number is considerably higher than the incidence of positive electrocochleograms we observe for normal/non-MD populations in our laboratory/clinic of approximately 10%. Indeed, even when amplitude or area ratio enlargements alone were noted, the incidence of positive findings exceeded our false positive values.

The results of our study have encouraged us to continue this line of research since seven out of nine individuals had positive electrocochleograms without demonstrating MD symptoms. Obviously, our current database is considerably

Table 1. Incidence of positive ECochG findings from nine subjects based on enlarged SP/AP amplitude and/or area ratios.
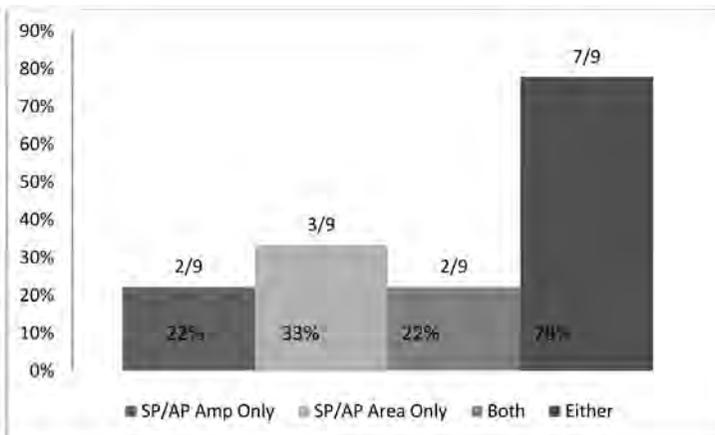
insufficient to make valid comparisons leading to statistical significance. Thus, our results are very preliminary. While a more detailed discussion of our findings is limited by the page restrictions of this manuscript, we do feel that we have sufficient data to warrant the conclusions and recommendations for future research presented below.

4. Conclusions/future research

Our results showed that nearly 80% of asymptomatic siblings/offspring of individuals with a confirmed diagnosis of MD/ELH have electrocochleograms that are positive for this disorder. Among other things it should be noted that both the SP/AP amplitude *and* area ratios were used to derive these outcomes. While the software for making both amplitude area measurements is commercially available on some AEP units (e.g., Interacoustics Eclipse, Natus Nav-Pro), as are TM electrodes (Sanibel), the routine use of ECochG continues to suffer from a lack of standardization across laboratories/clinics. Standardized protocols related to recording approach, recording parameters, and waveform interpretation are currently lacking and in need of definition/further study.

As indicated above, the current study is still in its formative stages. Our database needs to be expanded to include a much larger sample size that also comprises a subset of individuals from the same families. If indeed we are able to expand our sample size enough to validate our preliminary findings, then a longitudinal study that involves periodic, long-term monitoring of our experimental subjects (regardless of ECochG outcome) needs to be conducted. Obviously, a study of this nature would take several years.

Finally, as the science of genetic testing continues to evolve and the use of these procedures becomes more refined/accurate and cost-effective clinically, this technology may eventually be applied to patients with MD. If so, other tests (*e.g.*, ECochG) that may be helpful in predicting MD in both genetically-predisposed and other populations may be overshadowed by a simple blood/saliva test.

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GENETICS/PROTEOMICS

KNOCKDOWN OF HES-1 AND COUP-TFI USING SHRNA GIVES RISE TO NEW HAIR CELLS AND SUPPORTING CELLS IN ORGANOTYPIC CULTURE OF THE ORGAN OF CORTI

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1. Introduction

The auditory sensory epithelium of the inner ear organ of Corti (OC) is composed of two main types of cells that arise from a common progenitor, sensory hair cells (HC) and supporting cells (SC). Sensorineural hearing loss (SNHL) represents one of the major public health concerns worldwide and results from irreversible damage to the sensory HC from the OC or to the spiral ganglia neurons. Auditory sensory cells in mammals lose the capacity for self-renewal 21 days after birth when their re-entry in the cell cycle is blocked by specific cyclins.[1] A variety of mechanisms are responsible for this state, including a complex network of cyclin-dependent kinases and negative cell cycle regulators.[2,3] The *Notch* signaling pathway is an evolutionarily old cell-to-cell communication system, often used to establish interactive patterns of cellular differentiation.

Lateral inhibition through Notch signaling is a very common pathway responsible for specific cell patterns since it rules cell fate and lineage decisions among neighbor cells.[4] Recent evidence suggests that the NOTCH pathway

proteins, including HES1, play a role on basic helix-loop-helix transcriptional factor (*Atoh1* or *Math1*) expression during embryonic development, as well as keeping SC phenotype and preventing them from converting into HC by lateral inhibition mechanism (LIM).[5,6] In other words, HES1 oscillatory expression is important for normal inner ear cell proliferation regulatory mechanism.[7]

It has been shown that the use of gamma secretase inhibitors (GSI) of *Notch* signaling pathway in OC cultures of embryonic and postnatal mice was able to increase *Math1* expression and produce HC, and it also appears to induce mitogenic proliferation of SC.[8] However, results from experiments with deaf adult guinea pigs using the same pharmacological model of *Notch* pathway blockade were less encouraging; cochlear application of GSI resulted in the formation of few ectopic HC suggesting the existence of other regulatory factors, probably related to repression or inhibition of regeneration of damaged cells in the cochlea.[9] Neither is the LIM in OC fully elucidated, nor is it known whether *Notch* signaling remains active in adult OC.

It was observed that toxin-induced damage to adult guinea pig cochlea resulted in increased HES1 and NOTCH1 protein expression, suggesting active inhibition of a regenerative response under this condition.[10] It appears that the *Notch* pathway is altered shortly after cochlear auditory sensorial epithelium injury. Significant up-regulation of cochlear *Hes1* mRNA and protein expression was found after noise-induced hearing loss (NIHL).[11] It was also observed that dexamethasone provides protection against NIHL, possibly by suppressing cochlear *Hes1* expression.[12] Therefore, it may be possible to overcome the inhibition imposed on the regenerative process by knocking down *Notch* signaling pathway. So, in ord0er to evaluate the viability of this regeneration strategy, other interference experiments in the *Notch* signaling pathway need to be conducted.

COUP-TFI (chicken ovalbumin upstream promoter transcription factor I, also known as NR2F1) is an orphan nuclear receptor (ONR), which plays a crucial role as a transcription factor enabling or not progenitor cell proliferation 14[13,14] which regulates many aspects of mammalian development. Tang *et al.* 2006[15] showed that COUP-TFI knockout mice have a significant increase in hair cell number in the mid-to-apical turns. These knockout animals also presented a misregulation of Notch signaling components (Jag1, Hes5, Lfng), consistent with reduced Notch signaling, and correlated with increase in hair cell and increase in support cell differentiation. The authors also showed that when the Notch pathway is inhibited using gamma secretase inhibitor, an increase in hair cell and support cell differentiation was observed in COUP-TF1 knockout mice cochlear cultures, when compared to wild-type cultures. These data where the first to suggest a hypersensitivity to Notch inactivation, in COUP-TFI knockout cochlea; and to show that COUP-TFI plays a crucial role in regulating hair cell and support cell numbers and proliferation, probably by direct modulation of transcription of Notch-related genes (Jag1, Hes5, Lfng).

In the present study, we deliver *Hes1* and *COUP-TFI*-specific shRNA using a lentiviral vector to knockdown these targets in OC organotypic cultures of

three-day postnatal mice, and to compare HC and SC marker expression, by verifying mRNA and protein levels. We selected this shRNA strategy since it promotes a more persistent effect on the tissue. With regards to the vector, the lentivirus was chosen because it is also capable of transducing post-mitotic cells, as this is the case of OC tissue, which is terminally differentiated and incapable of reentering the cell cycle. Our hypothesis is that shRNA-mediated knockdown of *Hes1* and *COUP-TFI* would allow phenotypic changes in OC organotypic culture, leading to increased numbers of HC and SC. This could be a potential therapeutic strategy to be tested *in vivo* in mammals as a treatment for inner ear hearing loss, in the future.

2. Material and methods

2.1. Animals

The experimental protocol has been previously approved by the Internal Review Board on Ethics in Animal Research from the Medical School and the Institute of Biosciences of the University of Sao Paulo (Process Number: 0466/08). All experiments were conducted in accordance with the guidelines for the care and use of laboratory animals established by the American National Research Council according to data previously reported.[16] In this study, we used male and female postnatal day three (P3) BALB/c mice (*Mus musculus*), obtained from specialized breeders (Centro de Bioterismo, University of Sao Paulo School of Medicine, Sao Paulo, Brazil).

2.2. shRNAs

The plasmid pLKO.1-puro-CMV-tGFP (Mouse MISSION® shRNA Plasmid DNA) containing short harpin RNA (shRNA) targeting five different regions of mouse *Hes1* mRNA, named I (CloneID: XM_192801.2-286s1c1); II (CloneID:XM_192801.2-365s1c1); III (CloneID: XM_192801.2-387s1c1); IV (CloneID:XM_192801.2-431s1c1); V (CloneID: XM_192801.2-678s1c1); and five different regions of *COUP-TFI* mRNA named VI (CloneID: NM_010151.1-848s1c1); VII(CloneID: NM_010151.1-1192s1c1); VIII (CloneID: NM_010151.1-1263s1c1);IX (CloneID: NM_010151.1-1530s1c1); X (CloneID: NM_010151.1-1458s1c1), in addition to a control (SHC003), were obtained from Sigma-Aldrich (St. Louis, MO, USA). Each of the five plasmid clones was used to transform bacteria that were further expanded before a maxi-purification of plasmid DNA (QIAGEN, Valencia, CA, USA).

2.3. *Antibodies for immunofluorescence and flow cytometry*

Rabbit polyclonal primary antibodies anti-Hes1 and anti-COUP-TFI were obtained from Abcam (Cambridge, UK), rabbit polyclonal primary antibodies anti-myosinVIIa from Affinity BioReagents (Golden, CO, USA), mouse monoclonal primary antibodies anti-Math1 from DSHB (Iowa City, Iowa, USA), the goat polyclonal anti-Sox2 (Santa Cruz Biotechnologies, Santa Cruz, CA, USA), rabbit monoclonal primary antibodies anti-p27kip1 and rabbit polyclonal primary antibodies anti-Pax2 from Abcam (Cambridge, UK). Secondary antibodies were directed either to mouse, rabbit or goat IgG, and conjugated to AlexaFluor 488 (Jackson Immuno Research, West Grove, PA, USA) or to Alexa Fluor 546 (Invitrogen, Carlsbad, CA, USA). The antibody anti-GFP was mouse monoclonal and conjugated with 488 (Rockland, Limerick, PA, USA).

2.4. *Evaluation of shRNA efficiency*

The efficiency of target knockdown was evaluated in NIH3T3 cells (immortalized embryonic mouse fibroblast, kindly provided by M.C. Sogayar, Biochemistry Department of the Chemistry Institute, University of Sao Paulo). NIH3T3 cells were cultured in high-glucose DMEM culture medium (Invitrogen, Carlsbad, CA, USA), containing 10% fetal bovine serum (Invitrogen, Carlsbad, CA, USA), 1X L-Glutamine, 100 IU/ml penicillin/streptomycin (Invitrogen, Carlsbad, CA, USA), at 37ºC in a humidified atmosphere (5% CO_2). For transient transfection, the cells were cultured in a 24-well plate for 24 hours, then transfected with Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) and 2.5 μg of plasmid DNA according to the manufacturer's instructions. Following transfection, the cells were transferred to a 10 cm-dish with selective medium containing 1 μg/mL puromycin (Invitrogen, Carlsbad, CA, USA). After two weeks of selective culturing, cells were harvested, total RNA extracted, cDNA synthetized, and submitted to polymerase chain reaction (PCR)-based quantitative analysis as described below.

2.5. *Lentiviral subcloning, packaging, and particle production*

The lentiviral pLKO.1-puro-CMV-tGFP vector (Sigma-Aldrich, St. Louis, MO, USA) was used to subclone the oligonucleotide sequences I, II, VII and X yielding respectively the recombinant clones Hes1.I (Species: XM_192801.2-286s1c1/ Alternative species: NM_008235.2.XM_001000689.1 / TRC1trcn0000028927), Hes1.II (Species: XM_192801.2-365s1c1 / Alternative species:NM_00823 5.2.XM_001000689.1 / TRC1 trcn0000028855), COUP-TFI.VII(CloneID: NM_010151.1-1192s1c1 / TRC1 trcn0000026198) and COUP-TFI.X(CloneID: NM_010151.1-1458s1c1 / TRC1 trcn0000026165). The control plasmid (SHC003) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Each clone harbors the TurboGFP (tGFP) cDNA reporter gene. To produce virus particles, each lentiviral plasmids vector was co-transfected with the pCMV-VSV-G and psPAX2

packaging vectors (Addgene, Cambridge, MA, USA) by the calcium phosphate precipitation method in 293T cells (ATCC, Manassas, VA, USA). Viral supernatants were enriched by ultracentrifugation and stored at -80ºC. Vector titers were determined in Mouse fibroblast NIH-3T3 cells (ATCC, Manassas, VA, USA) by flow cytometry analysis of GFP expression and quantified as number of transducing units (TU) per milliliter.

2.6. *Organotypic cochlear sensory epithelium culture and transduction procedure*

After euthanasia, animals were bathed in absolute ethanol, then decapitated and had the temporal bones removed. Cochlear sensory epithelia containing the organ of Corti were surgically isolated using micro-mechanical dissection technique under a stereo-microscope (Discovery V12, Carl Zeiss, Oberkochen, DE); *stria vascularis* and spiral ganglion were removed. The isolated epithelia containing the organ of Corti were transferred to a 48 well plate previously coated with 0.01% poly-L-ornithine (Sigma-Aldrich, St. Louis, MO, USA) and 50 µg/mL laminin (Sigma-Aldrich, St. Louis, MO, USA) and cultured for 24 hours in DMEM-F12(1:1), supplemented with 1X B27, 1X N2, 1X glutamine, 1X insulin, transferrin and selenium (ITS) (all from Invitrogen, Carlsbad, CA, USA), ampicillin at 0.3 µg/mL (Teuto Brazilian Laboratory, Anapolis, GO, Brazil), 20 ng/mL human epidermal growth factor (EGF), and either 10 ng/mL basic fibroblast growth factor (bFGF, Invitrogen, Carlsbad, CA, USA) or 20 ng/mL transforming growth factor alpha (TGFα, Invitrogen, Carlsbad, CA, USA), at 37ºC and 5% CO_2.[17] Viral transduction was performed with $1.6X10^5$ TU per organ in 96 well plate, in the presence of 8 µg/mL polybrene during six hours, the media changed and the isolated epithelia containing the organ of Corti cultured for additional 48 hours (OC explants). Afterwards, RNA extraction and cDNA synthesis were performed as described below, and flow cytometry as presented below.

2.7. *RNA extraction and quantitative real-time PCR analyses*

For either transfected NIH3T3 cells or transduced mouse OC explants, the mRNA levels were determined using PCR of reversely transcribed RNA (RT-PCR) performed under real-time analysis (quantitative RT-PCR, qRT-PCR). For each experiment at least six OCs (three animals) were used in each of the five conditions (two different shRNA vectors for each of the two targets and control vector). We performed three independent experiments to evaluate mRNA levels through qRT-PCR. Total cellular RNA was isolated using QIAGEN RNeasy mini Kit according to the manufacturer's protocol (Hilden, DE). After treatment with DNAse I (Ampgrade, Invitrogen, Carlsbad, CA, USA) for 15 minutes, 2 µg of NIH3T3 cells total RNA or 0.5 µg for OC explants total RNA were employed in cDNA synthesis with oligo-dT according to the manufacturer's recommendations. (SuperScript® III First-Strand Synthesis System, Life Technologies, Carlsbad, CA, USA). Primers were designed specifically

for mouse Hes1, Coup-Tf1, Math1, Myo7a, Sox2, p27kip1 and Pax2 genes
(Table 1). Generally, forward and reverse primers targeted different exons. A
1 μL of cDNA was submitted to qRT-PCR with SYBR green master mix (Life
Technologies, Carlsbad CA), 100 nM of each and RNAse-free water to a final
volume of 15 μL, in StepOnePlus device (Life Technologies, Carlsbad, CA).
Amplification parameters were 50°C for two minutes, 95°C for ten minutes, 40
cycles of 95°C for 15 seconds, 60°C for one minute and a melting curve analysis
(72-95°C, increments of 1°C) was done in each experiment to confirm primer
specificity. Experiments were done in triplicates per each condition. We used
the control plasmid as the reference sample and the Tbp or B2m gene as the
reference gene (Table 1). For each comparison, all triplicate samples for both
groups were assayed in the same run. Samples with no cDNA were negative
controls for all experiments. qRT-PCR efficiencies varied from 1.9 to 2.1. The
threshold cycle (Ct) was normalized to the housekeeping gene Tbp or B2m, and
the $2^{-\Delta\Delta CT}$ method was used to calculate changes in gene expression.[18] All the
data were presented as the mean ± standard error of mean (SEM) and compared
using the One-tailed unpaired t-test with significance of 95%.

Table 1. Primers sequences for the qRT-PCR.

Gene/ Primer	RefSeq	Forward primer Reverse primer	Amplicon
Hes1	NM_008235.2	5'-TCCAAGCTAGAGAAGGCAGAC-3' 5'-GTCACCTCGTTCATGCACTC-3'	149bp
COUP-TFI (NR2F1)	NM_010151.1	5'-AATACTGCCGCCTCAAGAA-3' 5'-ATTGAGAGGATCCCCGTTT-3	125bp
Math1 (Atoh1)	NM_007500.4	5'-CTGTCCCTCCTGGATAGCAC-3' 5'-TCGGGAGAATGCAGCAGATAC-3'	100bp
Myo7a	NM_001256081.1	5'- CAGGCCAGGAGTTTGATGTG-3' 5'-GGTGCATTGGCTTGATGTG-3'	135bp
Sox2	NM_011443.3	5'-CAGGAGTTGTCAAGGCAGAGAAG-3' 5'-CTTAAGCCTCGGGCTCCAAAC-3'	132bp
p27kip1 (Cdkn1b)	NM_009875.4	5'-GGTGGACCAAATGCCTGACTC-3' 5'-TCTGTTCTGTTGGCCCTTTTG-3'	123bp
Pax2	NM_011037.4	5'-CGACAGAACCCGACTATGTTC-3' 5'-GGAAAGGCTGCTGAACTTTGG-3'	133bp
Tbp	NM_013684	5'-CCACACCAGCTTCTGAGAGC-3' 5'-GACTGCAGCAAATCGCTTGGG-3'	145bp
B2m	NM_009735.3	5'-TCGCGGTCGCTTCAGTCGTC-3' 5'-TTCTCCGGTGGGTGGCGTGA-3'	132bp

2.8. *Flow cytometry analyses*

Flow cytometry analyses have been conducted to evaluate the relative number
of OC cells expressing HES1, COUP-TFI, MYO7a and CX26 proteins. One
experiment using 20 animals was conducted but with a pool of at least eight OC
explants for each analyzed condition (Hes1.I shRNA, Hes1.II shRNA, Coup-Tf1.

VII shRNA, Coup-Tf1.X shRNA and control vector). Forty-eight hours after the lentiviral transduction procedure, the mouse OC explants were transferred to a flask containing 100 μL of DPBS (Dulbecco's Phosphate-Buffered Saline – Invitrogen, Carlsbad, CA, USA) and 100 μL of 0.25% trypsin/EDTA (Invitrogen, Carlsbad, CA, USA), and incubated for 15 minutes at 37ºC. Then 50 μL of trypsin inhibitor (Invitrogen, Carlsbad, CA, USA) and 50 μL of DNAse (Invitrogen, Carlsbad, CA, USA) were added and the samples incubated for 15 minutes at 37ºC. The tissues were mechanically dissociated by passage through 300 μL-pipette tips (Eppendorf, Hamburg, DE) and filtered through a 100-μm cell strainer (BD Falcon™, Franklin Lakes, NJ, USA) to remove cell debris. Twenty μL of the supernatant were used for cell morphology observation and counting using an Axiovert 40C microscope (Carl Zeiss, Oberkochen, DE). Cell suspension was centrifuged at 200 x g at 4ºC, for five minutes. For marker analyzed (HES1, COUP-TFI, MYO7a and CX26), the supernatant was discarded and 10,000 cells were re-suspended in 100 μL of phosphate-buffered saline (PBS, Invitrogen, Carlsbad, CA, USA) for each primary antibody analyzed. Cells were fixed in 4% paraformaldehyde (Electron Microscopy Sciences, Hatfield, PA) in PBS for 15 minutes at 4ºC and permeabilized in 0.2% triton X-100 (Sigma-Aldrich, St Louis, MO, USA) for ten minutes at 4ºC. Cells were washed once with PBS, blocked in 2% BSA (bovine serum albumin, Invitrogen, Carlsbad, CA, USA) for 30 minutes at 4ºC, and incubated for 16 hours at 4ºC in the presence of the primary antibody in a 50-fold dilution in PBS, 2% BSA. Cells were washed once with PBS and incubated for one hour at room temperature in the presence of 500 X-diluted Alexa Fluor 488-conjugated anti-rabbit secondary antibodies (Jackson Immuno Research, West Grove, PA, USA) in PBS. Cells were washed once with PBS and resuspended in 300 μL of PBS. A minimum of 5,000 events were analyzed using the 488 LASER channel of FACS Aria II Flow Cytometer (BD Biosciences, Franklin Lakes, NJ, USA) using the FACSDiva software.

2.9. Indirect immunofluorescence analyses

The mouse OC explants were fixed in 4% paraformaldehyde (Electron Microscopy Sciences, Hatfield, PA, USA), for 30 minutes, at 4ºC, and incubated with 30% sucrose, for 16 hours, at 4ºC. OCs were included in Tissue Freezing Medium (JUNG, Nussloch, DE) before freezing and cutting histological sections (10 μm) on a cryostat (CM1850, Leica, Nussloch, Germany). The slides containing the histological sections of mouse OCs were incubated with cold acetone for ten minutes, rinsed in PBS, and permeabilized in 0.3% triton X-100 for 20 minutes at room temperature. Then they were blocked in 10% goat serum (Santa Cruz Biotechnologies, Santa Cruz CA, USA) and incubated for 16 hours at 4ºC with100-fold diluted primary antibodies (or with anti-GFP antibody conjugated with 488) in PBS, 3% BSA (Invitrogen, Carlsbad, CA, USA). The slides were rinsed in PBS and incubated with secondary antibodies (1:400) for two hours at room temperature. The slides were mounted in ProLong Gold Antifade reagent containing 4′,6-diamidine-2-phenyl indol (DAPI, Invitrogen, Carlsbad, CA,
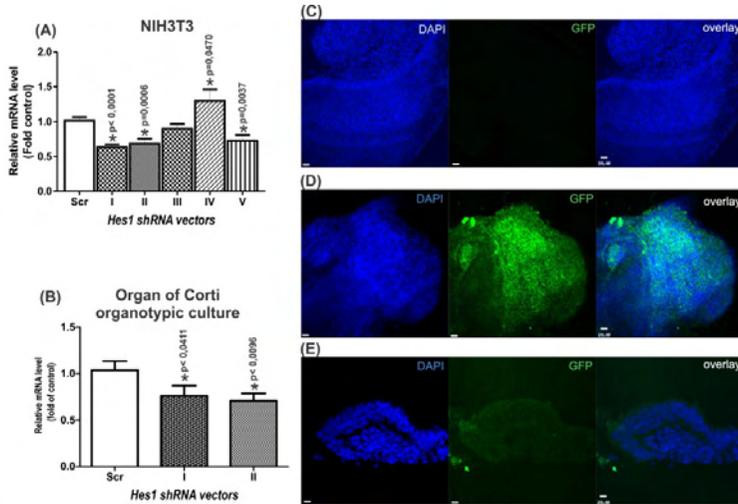
Fig. 1. Efficient transfection of the plasmid vector and transduction of the lentiviral vector with significant downregulation of *Hes1* mRNA levels by shRNA. **A**. qRT-PCR data showing the average fold change of *Hes1* mRNA expression level in NIH3T3 cells after *Hes1silencing* with each of the five (I-V) *Hes1* shRNA expression vectors or control vector. **B**. qRT-PCR results are presented for OC organotypic shRNA assays with two *Hes1* shRNA expression vectors (I and II) and the control vector. Data represents the mean ± standard deviation. Asterisks label indicates significant differences ($p \leq 0.05$) in each shRNA condition group compared to control. **C-E**. Immunofluorescence analysis of OC sections stained with anti-GFP (green) antibody indicates the lentiviral vector reporter gene expression in many cells. **C**. Negative control (without vector transduction); **D**. Transduction with control vector; and **E** transduction with the *Hes1shRNA* expression vector. The nuclei were counter-stained with DAPI (blue). The analysis was performed under a confocal microscope (LSM510, Carl Zeiss, Oberkochen, DE). Scale bar: 50 μm.

USA) for nuclear identification. Images were acquired either by fluorescence microscopy (Axioplan, Carl Zeiss, Oberkochen, DE) using the Isis Fish Imaging Meta System software to collect digital images, or at the LSM510 confocal microscope (Carl Zeiss, Oberkochen, DE), as indicated.

3. Results

We designed five different shRNA plasmid vectors to silence *Hes1* gene and five different shRNA plasmid vectors to silence *COUP-TFI* gene expression. To select the vector with the highest silencing efficiency, we transfected NIH3T3 cultured cells with each of the five shRNA constructs of each gene (*Hes1* or *COUP-TF1*) and a control plasmid. Puromycin treated cells were selected and total RNA extracted for analysis. qRT-PCR was conducted to verify the best plasmid vector in terms of silencing (Figs. 1A and 3A). In NIH3T3 cells transfected with shRNA vectors I or II, *Hes1* mRNA levels were significantly lower than the
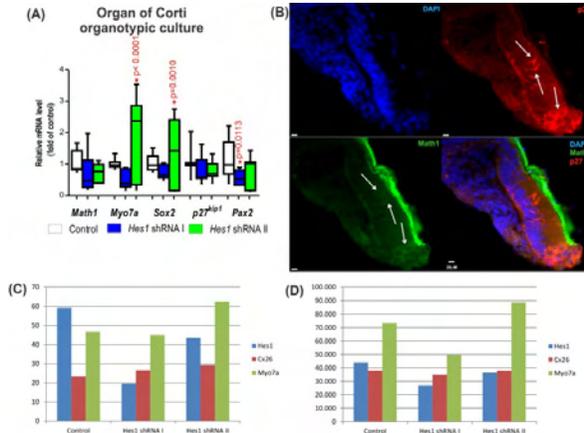
Fig. 2. Phenotypic effects of the downregulation of *Hes1* by shRNA knockdown showing in-creased Myo7a and Sox2 mRNA levels (A) and Myo7a and Cx26 protein levels (C-D) and the presence of HCs emerging together with SCs (B) in OC organotypic cultures. **A**. qRT-PCR data showing the average fold-change of *Math1*, *Myo7a*, *Sox2*, *p27* and *Pax2* mRNA expression levels in OC cells after *Hes1* shRNA knockdown with *Hes1.I* and *Hes1.II* shRNA expression vector or control vector. Error bars represent the standard deviations. Asterisks label significant differences ($p \leq 0.05$) in each group compared to the control vector. **B**. Immunofluorescence results of OC organotypic cultures submitted to *Hes1* shRNA knockdown. The nuclei were counter-stained with DAPI (in blue). The OC mouse pups were stained with anti-Math1 (in green) and anti-p27 (in red) antibodies. Arrows show the presence of emerging new HCs co-expressing HC markers together with SCs markers, after shRNA treatment to knocking down Hes1 mRNA expression. The analysis was performed by confocal microscopy (LSM510, Carl Zeiss, Oberkochen, DE). **C-D.** Results of flow cytometry analysis of OC cells submitted to Hes1shRNA knockdown. The percentage number of cells (C) and fluorescence (D) labeled by the anti-Hes1, anti-Myo7a and anti-Cx26 antibodies is presented for both control vector and *Hes1* groups. Scale bar 50 μm.

control (t-test, N = 3, Hes1.I *Vs*. Control and Hes1.II *Vs*. Control, P < 0.0001 and P = 0.0006), displaying 37% and 32% reduction, respectively (Fig. 1A). In NIH3T3 cells transfected with shRNA vectors VII or X, *COUP-TFI* mRNA levels were significantly lower than the control (t-test, N =3, COUP-TFI.VII *Vs*. Control and COUP-TFI.X *Vs*. Control, P = 0.0247 and P = 0.0255), displaying 59% and 53% reduction, respectively (Fig. 3A). Comparative analyses between Hes1 vectors I and II were not statistically different (t-test, Hes1.I *Vs*. Hes1.II, N = 3, P = 0.2650). Comparative analyses between COUP-TFI vectors VII and X were not statistically different (t-test, COUP-TFI.VII *Vs*. COUP-TFI.X, N = 3, P = 0.5938). Each insert from plasmidial vectors I, II, VII and X were sub-cloned into the lentiviral vector pLKO.1-puro-CMV-tGFP in order to transduce the OC in culture (Fig. 1B and 3B). Both tested constructs (I and II) decreased *Hes1* transcript level in organ of Corti to a similar and significantly reduced level when compared to the control vector, in rates similar to that previously observed in NIH3T3 cells (I = 24% and II = 30%) (Fig. 1B).
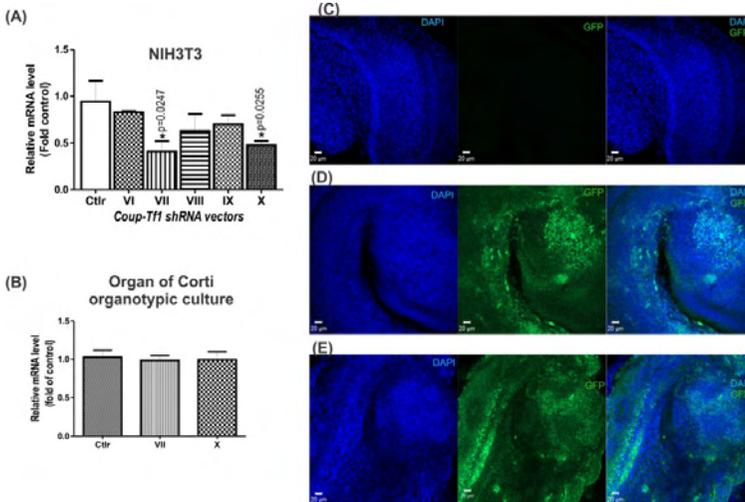
Fig. 3. Efficient transduction of the lentiviral vector and significant downregulation of *COUPTFI* mRNA levels by shRNA. **A**. qRT-PCR data showing the average fold change of *COUP-TFI* mRNA expression level in NIH3T3 cells after *COUP-TFI* shRNA knockdown transfection with each of the five (I-V)*COUP-TFI* shRNA expression vectors or control vector. **B**. qRT-PCR results are presented for OC organotypic shRNA knockdown assays with two *COUP-TFI* vectors (VII and X) and the control vector. Data represents the mean ± standard deviation. Asterisks label indicates significant differences ($p \leq 0.05$) in each shRNA condition group compared to the control vector. **C-E**. Immunofluorescence analysis of OC sections stained with anti-GFP (green) antibody indicates the lentiviral vector reporter gene expression in many cells. **C**. Negative control (without vector transduction); **D**. Transduction with control vector; **E**. transduction with the *COUP-TFI* shRNA expression vector. The nuclei were counter-stained with DAPI (blue). The analysis was performed under a confocal microscope (LSM510, Carl Zeiss, Oberkochen, DE). Scale bar: 50 μm.

Paradoxically, for constructs VII and X, no significant changes were observed in COUP-TFI transcript level (VII = 1% and X = 1%) in comparison to control vector in the OC *in vitro* (Fig. 3B), in opposite to previously observed in NIH3T3 cells. As demonstrated in Figures 1C-E and 3C-E, either for Hes1 or COUP-TF1 shRNA, the transduction with lentivirus was successful and appeared to occur throughout the OC, as most cells were positive for the reporter Turbo Green Fluorescent Protein (tGFP). Flow cytometry data revealed that the Hes1 fluorescence in cells under Hes1.I and Hes1.II shRNA knockdown were reduced to 39% and 18%, respectively, when compared to control vector; and that the number of cells expressing COUP-TFI protein under COUP-TFI.VII and COUP-TFI.X shRNA knockdown were only 1% and 10%, respectively, when compared to the control vector (Figs. 2C and 2D).

Taking into account that the GFP-positive cells express the *Hes1* shRNA leading to approximately 30% reduction in the *Hes1* mRNA and Hes1 protein levels (Fig. 2C-D), we next investigated if this change is sufficient to modify HC and SC numbers and their specific protein marker expression in transduced OC. Knockdown of *Hes1* with *Hes1.II* vector led to robust and significantly
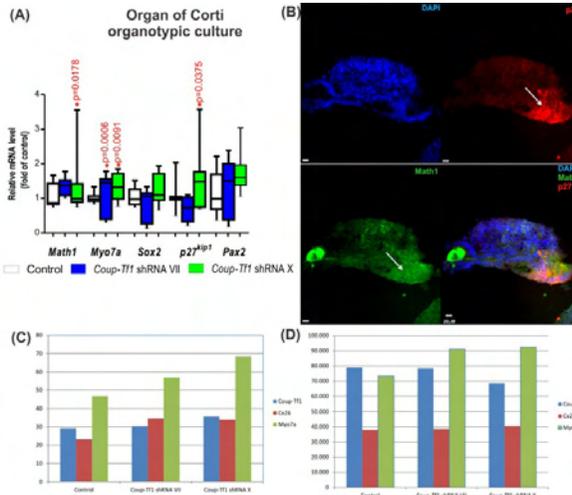
Fig. 4. Phenotypic effects of the downregulation of *COUP-TFI* by shRNA knockdown showing increased Math1, Myo7a and p27kip1 mRNA (A), Myo7a and Cx26 protein levels (C-D), and the presence of HCs emerging together with SCs (B) in OC organotypic cultures. **A**. qRT-PCR data showing the average fold-change of *Math1*, *Myo7a*, *Sox2*, *p27* and *Pax2* mRNA expression levels in OC cells after *COUP-TFI* shRNA knockdown with *COUP-TFI.VII* and *COUP-TFI.X* shRNA expression vector or the control vector. Error bars represent the standard deviations. Asterisks label significant differences ($p \leq 0.05$) in each group compared to the control vector. **C-D**. Results of flow cytometry analysis of OC cells submitted to COUP-TFI shRNA knockdown. The percentage number of cells (C) and fluorescence (D) labeled by the anti-Hes1, anti-Myo7a and anti-Cx26 antibodies is presented for both control vector and *COUP-TFI* groups. **B**. Immunofluorescence results of OC organotypic cultures submitted to *COUP-TFI* shRNA knockdown. The nuclei were counter-stained with DAPI (in blue). The OC mouse pups was stained with anti-Math1 (in green) and anti-p27 (in red) antibodies. Arrows show the presence of new HCs emerging co-expressing together with the SCs, after shRNA treatment to knocking down COUP-TF1 mRNA expression. The analysis was performed by confocal microscopy (LSM510, Carl Zeiss, Oberkochen, DE). Scale bar 50 µm.

increase in *Myo7a* mRNA levels (1.9-fold), and in *Sox2* mRNA levels (1.4-fold) (Fig. 2A). This data was corroborated by augmented immunofluorescence signal and number of cells expressing the protein Myo7a (data not shown) and by the increased number of Myo7a and Cx26 marked cells in the flow cytometry analyses (Fig. 2C-D). On the other hand, qRT-PCR showed that knockdown of *Hes1* with *Hes1.I* vector significantly decreased mRNA levels of *Pax2* (0.59-fold) (Fig. 2A). Taking into account that the tGFP-positive cells express the *COUP-TFI* shRNA leading to 1 to 13% reduction in the *COUP-TFI* mRNA and COUP-TFI protein levels (Fig. 4C-D), we next investigated if this change is sufficient to modify HC and SC numbers and their specific protein marker expression in the transduced OC. Interestingly knockdown of *COUP-TFI with COUP-TFI.X* vector led to robust and significantly increase in *Myo7a* mRNA levels (1.3-fold), *Math1* mRNA levels (1.3-fold) and *p27kip1* mRNA levels (1.5-fold) (Fig. 4A). The knockdown of *COUP-TFI* with *COUP-TFI.VII* vec-

tor led a significantly increase in *Myo7a* mRNA levels (1.1-fold) (Fig. 4A). This was corroborated by augmented immunofluorescence signal and number of cells expressing the protein Myo7a (data not shown) and by the increased number of Myo7a and Cx26 marked cells in the flow cytometry analyses (Fig. 4C-D). Results observed for the *COUP-TFI.X* vector was surprising given the low levels of silencing obtained.

To determine whether Hes1 shRNA and COUP-TFI shRNA treatment induces the transdifferentiation of SCs into HCs, the shRNA-treated OC organotypic cultures were probed for the presence of HC co-expressing with SC markers and/or SC topography. Emerging new HCs co-expressing the SC markers, *Math1* for HCs and *p27kip1* for SCs, were indeed detected after Hes-1 shRNA and COUP-TFI shRNA treatments, shRNA (Figs. 2B and 4B).

4. Discussion

In mammals, expression of *Math1* is critical for the formation of new HC during normal sensory epithelium embryogenic development.[19] After birth, *Math1* expression is progressively attenuated at early postnatal stages and remains low throughout adulthood in outer HC and inner HC. In contrast, *Hes1* expression becomes elevated at late embryonic and early postnatal stages and is maintained at a relatively high level throughout adulthood in SC, which may be one of the mechanisms that maintain the mosaic pattern arrangement of HC and SC in OC auditory sensory epithelium.5,20. Our data suggest that the rise in *Myo7a* and *Sox2* mRNA after shRNA *Hes1* knockdown in OC organotypic cultures of three-day postnatal mice could be attributed mainly to an increase in the number of HC and SC. The same seems to occur after shRNA COUP-TFI knockdown in OC organotypic cultures, since there is a rise in *Math1*, *Myo7a* and *p27kip1* mRNA levels 48 hours after viral vector transfection. These findings are aligned and in agreement with protein levels data observed though immunofluorescence staining for flow cytometry analyses, showing an increase in the number of cells expressing Myo7a and Cx26 markers. So, in both experiments with shRNA viral vectors, Hes1 and COUP-TFI showed similar phenotypic effect in the OC organotypic culture of three-day postnatal mice. The most unexpected of our findings is that, even a slight silencing and incomplete knockdown of the target *Hes1* gene expression (30% by qRT-PCR and 18% by flow cytometry) and *COUP-TFI* gene expression (1% by qRT-PCR and 10% by flow cytometry) in the OC organotypic culture of three-day postnatal mice was enough to change the patterns of HC and SC mRNAs expression and the protein levels for HC and SC markers. Of course these are preliminary data that need to be double-checked in further experiments, but they suggest that a minimum modulation effect on mRNA expression could be sufficient to result in phenotypic change. The phenotypic change was corroborated by new HC arising from transdifferentiation of SC at the immunofluorescence assay after knockdown of *Hes1* and

COUP-TFI using shRNA. We also do not know if these HC and SC increase will result in a functional change in terms of auditory thresholds, but this hypothesis will be tested in the next steps, using an *in-vivo* model. We observed a substantial phenotypic change on OC auditory sensory epithelial and a clear increase on the number of HC and SC, which suggests proliferation of those cells. These new data are important for the development of future strategies of gene therapy in SNHL, but clearly further experiments are needed, since it remains to be demonstrated that these phenotypic changes could induce the production of functional cells.

In vivo studies will be performed, using audiology electrophysiological tests in order to verify the efficacy of our knocking-down strategy to improve hearing in deaf animal models.

Moreover, through further analysis we expect to demonstrate the co-localization of target tissues and virus and to perform additional immunofluorescence staining for flow cytometry analyses in order to improve statistical analysis.

5. Conclusion

We observed phenotypic and molecular changes on OC auditory sensory epithelia and an increase in the number of HC and SC, which suggests proliferation of those cells after knockdown of *Hes1* and *COUP-TFI* using shRNA in the OC organotypic culture of three-day postnatal mice.

6. Funding support

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A PROTEOMICS-BASED APPROACH IN MÉNIÈRE'S DISEASE

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Abstract

Diagnosis of Ménière's disease (MD) is still widely based on clinical basis. Consequences are on inaccuracy in terms of epidemiology, diagnosis and treatment. We have used a proteomics-driven approach to identify potential biomarkers of MD. To this end, plasma was obtained from whole blood of 20 individuals previously diagnosed as suffering from MD, and compared to plasma from healthy donors. A depletion of the highly abundant proteins was performed in order to enhance the chance of detection of the less represented ones, therefore reducing the noise-background. Two-dimensional gel electrophoresis, followed by in-gel tryptic digestion of the selected spots and LC-MS/MS analysis, allowed us to identify a set of proteins whose expression appears to be differentially modulated in patients vs controls. In particular: complement factor H and B, fibrinogen alpha and gamma chains, beta-actin and pigment epithelium derived factor are over expressed; on the other hand, the levels of beta-2 glycoprotein-1, vitamin D binding protein and apolipoprotein-1 are significantly decreased in the plasma of MD-affected individuals. Even though preliminary and not necessarily linked directly to the molecular pathogenesis of the disease, our original findings suggest that a molecular signature, represented by the plasma protein profile previously described, might represent a potentially powerful, innovative and not invasive tool for early diagnosis and clinical management of MD patients. Moreover, our findings uncover a potential starring role for some proteins in the development and fate of this frustrating disease, whose pathogenesis still remains unclear.

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1. Introduction

The etiology and pathophysiology of Ménière's disease (MD) are still under heavy debate. Even though there are published guidelines for diagnosis of MD,[1] there is still a lack of consensus about diagnostic criteria: in fact there is no single feature or subset of features from the history, clinical examination or instrumental tests that are able to establish a certain diagnosis. The aim of this study was to identify potential biomarkers of MD using a proteomics-driven approach.

2. Subjects and methods

2.1. Subjects

The study was performed at the Department of Experimental and Clinical Medicine, Audiology and Phoniatrics Unit and Laboratory of Proteomics and Mass Spectrometry, Magna Graecia University of Catanzaro (Italy). Twelve patients (six men, six women, mean age 51 years) affected by definite, unilateral, MD[1] and eight healthy anonymous donors (pair-matched by sex and age) were enrolled. To confirm the affected side and to exclude other pathologies, all subjects underwent complete otoneurological assessment including caloric and kinetic tests, positional maneuvers, electrophysiological tests (auditory brainstem responses, vestibular evoked miogenic potentials (VEMPs and ocular VEMPs) and imaging studies (gadolinium enhanced Cerebral Magnetic Resonance and Cerebral MR venography, as in Chiarella *et al.*[2]

2.2. Sample collection

Blood plasma was obtained according with Human Proteome Organization (HUPO) plasma proteome guidelines.[3] Four distinct plasma pools were prepared as described elsewhere. Each sample was subjected to the Proteome-LabTM IgY-12 Proteome Partitioning Kit (Beckman Coulter, Crea, CA) to remove the 12 most abundant proteins. This immunoaffinity procedure provides an enriched pool of lower abundant proteins for downstream proteomic analysis. To identify which proteins whose expression appears to be differentially modulated in patients vs controls, samples underwent to Two-dimensional gel electrophoresis, followed by in-gel tryptic digestion of the selected spots and LC-MS/MS analysis.

2.3. Statistical analysis

Student's t-test was used to identify differences in mean values between the two groups. $P < 0.05$ (two-sided) was considered statistically significant.

Table 1. List of proteins differentially expressed in plasma from MD patients vs controls.

Protein	Mass	Score	No. of peptides	MD vs controls
Complement factor H	143,654	673	29	Upregulated
Complement factor B	86,847	660	30	Upregulated
Fibrinogen alpha chain	95,656	320	18	Upregulated
Beta-2-glycoprotein I	39,584	53	1	Downregulated
Vitamin D-binding protein	54,526	88	9	Downregulated
Vitamin D-binding protein	54,526	45	2	Downregulated
No identification	–	–	–	Downregulated
Beta-actin	42,052	210	11	Upregulated
No identification	–	–	–	Downregulated
No identification	–	–	–	Downregulated
Pigment epithelium-derived factor	46,484	71	4	Upregulated
Fibrinogen gamma chain	52,106	189	9	Downregulated
Apolipoprotein A-I	30,759	215	10	Downregulated

3. Results

3.1. Two-dimensional PAGE protein separation and image analysis

Image analysis of the gels, using the Image Master 2D-Platinum software, high-lighted the presence of several spots differentially expressed in the two subgroups (MD patients vs healthy donors); no differences were detected among pools 1, 2 and 3, all belonging to the MD-affected individuals. In-gel digestion and subsequent LC-MS/MS analysis of the excised spots revealed, in MD patients, an increased expression of the following proteins: complement factor H and B (CFH, CFB), fibrinogen alpha and gamma chains, beta-actin and pigment epithelium derived factor. On the other side, the plasma proteome of MD-affected individuals showed decreased levels of: beta-2 glycoprotein-1 (beta-2GP1), vitamin D binding protein (VDBP) and apolipoprotein-1 (Table 1). All differences among the proteins showing either up- or downregulation among the two groups were statistically significant. Three additional protein spots, differentially expressed within the two subgroups, did not correspond to any of the known proteins in the available databases. LC-MS/MS findings on pooled samples were confirmed by performing the analysis on a single patient basis. Further confirmation with an independent assay was obtained by challenging plasma from each of the individuals recruited in the present study with antibodies specifically recognizing the proteins differentially expressed in MD patients by means of Western blotting analysis.

4. Discussion and conclusions

MD is a complex disease with clinical heterogeneity. The diagnosis based on clinical criteria exposes to high risk of bias in study of epidemiology and generally in the identification of affected patients. As a consequence, MD pathophysiology is poorly understood. There are many proposed theories, several intrinsic (genetic, anatomic, metabolic, endocrine, autoimmune, or vascular) other extrinsic (allergic, viral, or traumatic). However, none of these hypotheses has really been accepted.[4]

It is still unclear, for example, whether the presence of endolymphatic hydrops is the trigger of cochleovestibular dysfunction or whether hydrops is an epiphenomenon of a more subtle biochemical perturbation that underlies the disease state.[5] A familial predisposition has been reported in about 3-12% of patients with MD but the genetics of the disease is extremely heterogeneous.[4] A review on the basic science of MD[6] has recently stated that familial MD is transmitted, at least in a subset of patients, in an autosomal dominant fashion, with a variable penetrance and evidence suggestive of anticipation. Linkage analysis has not been successful so far in identifying potential candidate genes in familial MD;[7] on the other hand, the observation of a close association of this disease with certain HLA antigens (specifically HLA-A3, B7, CW7 and DR2),[8,9] raises the possibility of an autoimmune pathogenetic basis. Other evidence supports this hypothesis including the finding of elevated levels of autoantibodies or circulating immune complexes in the serum of some patients,[10,11] the association with a functional variant of a lymphoid protein phosphatase, which inhibits T-cell receptors response in patients with bilateral ear involvement.[12] Moreover, different autoantibodies such as antinuclear antibodies, antiphospholipid antibodies and antibodies against a 68 kD protein have been studied in small series of patients with MD, showing conflicting results.[13-15] In a study aimed at defining the biochemical composition of the 'homogeneous substance' that normally occupies the endolymphatic sac lumen but rapidly disappears in response to expansion of the endolymph volume, Thalmann *et al.*[16] have found that mid-molecular weight, acidic proteins, are the majority of detectable proteins, and that most of these are degraded by deglycosylating enzymes. Overall, the availability of reliable, reproducible and easy-to-obtain diagnostic markers is urgently needed.

In this study, we have identified a proteomic profile from plasma of MD-affected individuals that can be potentially used as a diagnostic tool for large screening studies in this specific population. Among the identified proteins, we would like to focus on few of them because of their involvement in ear-related disorders. CFH is a serum glycoprotein that plays an important role in the alternative complement pathway in fluid phase and on cellular surfaces. CFH, and its related proteins, are strongly activated in otitis media with effusion,[17] underscoring the role played by the alternative complement pathway in the development of inflammation in this particular disease. Beta-2GP1 is a 50-kDa glycoprotein that binds to negatively charged substances (heparin, dextran sulfate) preventing activation of the intrinsic blood coagulation cascade by

interacting with phospholipids on the surface of damaged cells. The presence of beta-2GP1 antibodies has been recently detected in patients with idiopathic sudden sensorineural hearing loss.[18] VDBP is primarily involved in the actin-scavenging system, thus protecting cells from the toxic effect of intravascular actin polymerization.[19] Beta-actin is an ubiquitous protein involved in the formation of filaments that are a major component of the cytoskeleton. This protein has been identified as a potential candidate autoantigen in autoimmune inner ear disease[20]. Moreover, beta-actin mutations altering depolymerization dynamics are associated with autosomal dominant deafness, and dystonia and with non-syndromic hearing loss.[21,22]

As already stated, early diagnosis of MD is not an easy task, our data, derived from the analysis of plasma proteome, provide a novel tool, not invasive and easily reproducible, for early diagnosis and follow-up of MD patients.

Finally, we postulate that the protein profile obtained from plasma analysis, might help, in the near future, the development of innovative, rational and tailored therapeutic schemes for MD in a targeted, nondestructive way.

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ROLE OF OXIDATIVE STRESS IN THE COCHLEAR DAMAGE IN ACQUIRED SENSORINEURAL HEARING LOSS

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Cochlear damage induced by exogenous factors depends on the progressive increase of reactive oxygen species and lipoperoxidative damage in conjunction with the imbalance of antioxidant defenses.[1] Under physiological conditions, the balance between ROS generation and ROS scavenging is highly controlled. On the other hand, unregulated oxidative and nitrosative stresses can result in severe cellular damage, cell death, and consequently whole organ and organism failure.[2] Indeed, the auditory function is bound to undergo oxidative stress during several environmental insults such as exposure to loud sounds, ototoxic drug administration, aging, depletions of dietary antioxidants (*e.g*., vitamins E, C, and D, flavonoids and carotenoids) and micro-nutrients (*e.g*., iron, copper, zinc, selenium) that are needed for proper functioning of antioxidant enzymes (catalase, copper, SODs, or glutathione peroxidase).[3] We have demonstrated that the redox-sensitive transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) plays a critical role in the regulation of cellular defense against oxidative stress including heme oxygenase-1 (HO-1) activation. In this work we describe a link between cochlear oxidative stress damage, induced by noise exposure and cisplatin ototoxicity, and the activation of Nrf2/HO-1 pathway. We also observed the activation of the Nrf2/HO-1 pathway after noise exposure (Fig. 1). In doing so, Nrf2 appears to promote the maintenance of cellular homeostasis under stress conditions. However, in our model the endogenous antioxidant system fails to counteract noise-induced cell damage and its activation is not enough effective in preventing the cochlear damage.[4] Administering antioxidant therapy might be beneficial under these conditions and natural compounds and their abilities to

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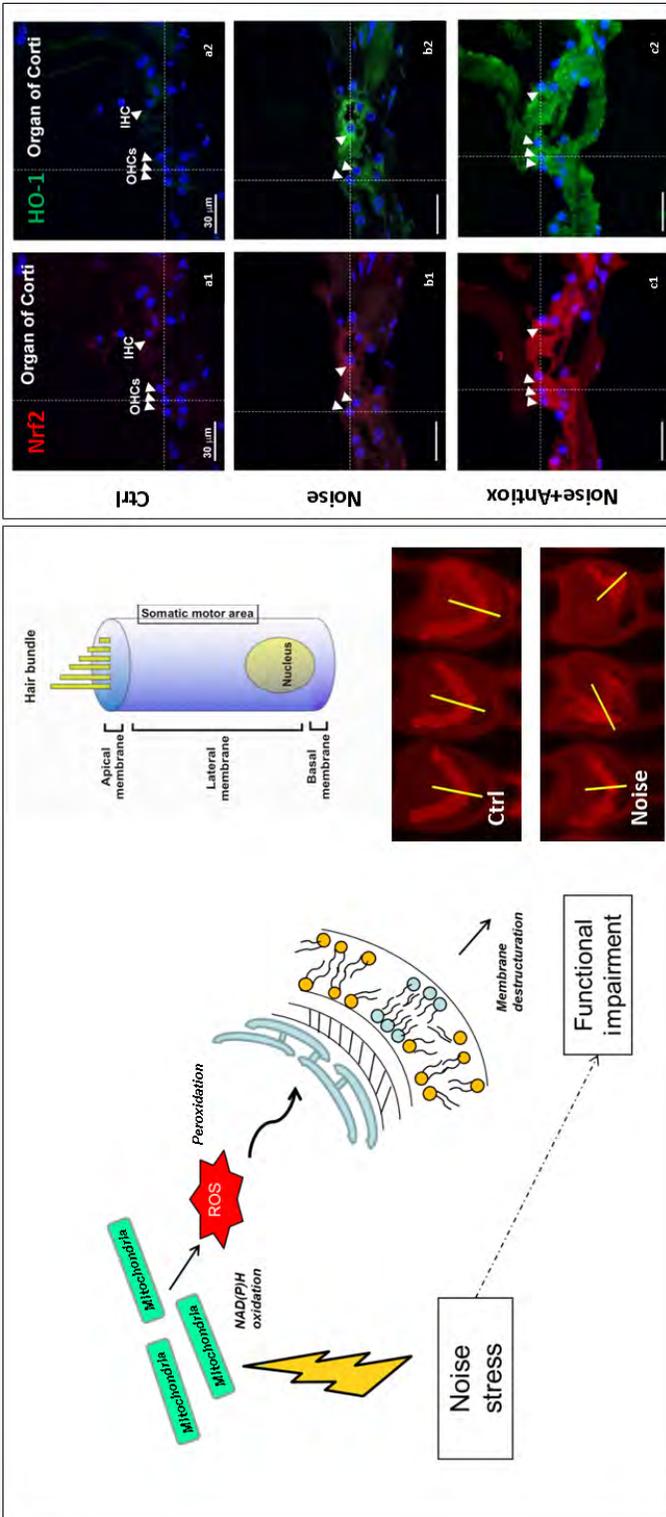
Fig. 1. Model of OHC's functional impairment. Left panel: Noise stress induces NAD(P)H drop, which is followed by free radical accumulation and a consequent rise of plasma membrane peroxidation. Peroxidation leads to a membrane destructuration consisting in a cytoplasmic membrane loss of fluidity. Rhodamine-Phalloidine staining shows the arrangement of OHC hair bundle in the control and Noise groups. Right panel: In the Noise group there is a weak increase in Nrf2 fluorescence (b1) and HO-1 expression (b2). Antioxidant treatment shows a further increase in Nrf2 expression (c1) paralleled by HO-1 overexpression (c2).

act as antioxidants and cell protectants in neuronal systems can provide novel and safe therapeutic options. The knowledge of the time course in the apoptotic events after injury suggest that there is a 'therapeutic time window' in which the properties of each drug can effectively prevent further damage and repair injury. Considering the time course of apoptotic events, the best protection with antioxidants appear to be achieved using higher doses of drugs in a short time from pre-exposure to peri-trauma period. Therefore the therapeutic strategy that interfere with the rapid onset of redox imbalance may represent a rational approach to treat sensorineural hearing loss induced by exogenous factors.

Acknowledgment

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VESTIBULAR ISSUES

OCULAR (oVEMP) AND CERVICAL (cVEMP) VEMPS IN PATIENTS WITH 'CLINICALLY CERTAIN' MÉNIÈRE'S DISEASE

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1. Introduction

The diagnosis of Ménière's disease (MD) has for the last twenty years been based on the American Academy of Otolaryngology – Head and Neck Surgery Equilibrium Committee guidelines.[1] Another validated Ménière's scoring system is the Gibson 10-point score.[2]

The AAO-HNS guidelines and the new two-category classification from the Barany Society[3] do not recognize the validity, let alone the existence of any *in-vivo* test that can confirm endolymphatic hydrops. Gibson[4] and Hornibrook *et al.*[5] have demonstrated the high sensitivity of transtympnanic tone-burst electrocochleography (EcochG) for confirming cochlear hydrops.

Others have measured vestibular myogenic potentials (VEMPs) in MD patients in the hope that they would provide a meaningful diagnostic test, with abnormalities in latency, threshold and amplitude claimed for cervical (cVEMP) VEMPs.[6] It was decided to test cervical VEMPs (cVEMP) and ocular VEMPs (oVEMP) in patients with 'clinically-certain' MD (with *independent* proof of the presence of cochlear hydrops) and in normal non-MD ears.

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2. Method

There were two groups of participants: A control group of 22 with no known vestibular pathologies (nine male, 13 female aged 22 to 63 years; mean age 34 years); a group with 'clinically-certain' MD with EcochG proof of hydrops (14 male, four female; mean age 60 years). All had a pure tone audiogram and were tested with (1) transtympanic EcochG; and (2) cVEMP and oVEMP.

2.1. Transtympanic EcochG

On Amplaid MK 15 with ground electrode on the forehead, reference electrode on the ipsilateral earlobe and active transtympanic electrode in the round window niche (Fig. 1). Air conduction 90 dB clicks and 100 dB tone-burst stimuli were delivered via headphone in a ring over the tested ear.[5] All had a Gibson score of > 7. Endolymphatic hydrops was confirmed by a click SP/AP ratio of > 0.5 and/or a 1 kHz or 2 kHz tone-burst SP in microvolts according to the Gibson criteria.[7]

2.2. VEMPs

By custom-written evoked potential averaging and analysis software. cVEMP: Active electrode over mid sternomastoid muscle, indifferent on upper sternum, ground on forehead. oVEMP: active electrode on orbital margin below eye, indifferent on cheek, ground on forehead.

oVEMPs and cVEMPS can be recorded simultaneously, but were recorded separately. Visual feedback of EMG targeted muscles presented to subjects via LED indicators.

The measured variables were for cVEMP: threshold, and P1N1 and N1P2 peak-to-peak.

Amplitudes (Fig. 2), and for oVEMP: threshold, and N1P1, P1N2 and N2P2 peak-to-peak amplitudes. Significance level was 0.05.

3. Results

- *cVEMP threshold*: No significant differences between MD ears and controls.
- *cVEMP latency*: N1 peak for Meniere's ears significantly prolonged compared to both right and left ears of controls.
- *cVEMP P1-N1 amplitude*: Significant reduction for Meniere's ears compared to right ears of controls.
- *cVEMP N1-P2 amplitude*: Significant reduction for Meniere's ears compared to right ears of control group.
- *oVEMP threshold*: Significantly elevated in Meniere's ears compared to unaffected ears, BUT not from controls.

- *oVEMP latencies*: No statistically significant differences of oVEMP latencies in both groups.
- *oVEMP N2-P2 amplitude*: Significant reduction for Meniere's ears compared to left ears of control group.

4. Discussion

This study has demonstrated cVEMP and oVEMP abnormalities in 'clinically-certain' MD ears in regard to latency and amplitude. However, there is such great overlap between measurements in MD ears and control ears, that VEMP measurements in isolation are not a reliable indication of MD.

The uniqueness of this study is that all the MD ears had an independent confirmation of cochlear hydrops. MD is assumed to start in the cochlea and then move to the vestibule.[8] The jeopardy of any VEMP study done based on symptoms and without this confirmation is that the patient may not actually have MD. Therefore, all studies using only VEMPs for diagnosing MD and vestibular migraine may be flawed without this evidence.

The elucidation of the cause of MD and distinguishing it from other common causes of recurrent vertigo attacks signals an urgent need for agreement on validated tests for confirming cochlear hydrops first, with a new diagnostic category of 'clinically-certain' MD. There is current interest in gadolinium MRI imaging, but it is difficult, expensive and time-consuming and not yet reliable. The transtympanic tone-burst EcochG remains the simplest, cheapest and most sensitive test for diagnosing MD.[4,5]

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PREVALENCE, ASSOCIATED SYMPTOMS AND PROPHYLACTIC MEDICATION EFFECTIVENESS OF VESTIBULAR MIGRAINE IN AN OTOLARYNGOLOGY CLINIC

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1. Introduction

The association between dizziness and migraine was already identified in 1873 by Living,[1] but it was only supported by several clinical studies in the last 30 years. Dizziness and migraine are both common disorders among the general population with a lifetime prevalence of 7%[2] and 13-16%[3] respectively. Certain vestibular disorders occur more frequently in migraineurs and therefore, the comorbidity between migraine and dizziness is higher, *i.e*., 3.2%. In light of this, there is rising recognition of an entity called vestibular migraine (VM), affecting approximately 1% of the general population.[4] VM is considered one of the most common causes of dizziness and clinical features typically include attacks of spontaneous or positional vertigo lasting seconds to days, accompanied by characteristic migraine symptoms such as headache, auras and photo- and phonophobia.[4] Important to add is the fact that the headache does not necessarily have to occur concurrently with the vestibular symptoms. Making an accurate diagnosis in VM patients is often difficult as there are no biological markers, laboratory studies or imaging findings that can prove it. A detailed and systematic history-taking is, therefore, crucial. Similar to migraine, treatment options often involve lifestyle modifications and pharmacological, prophylactic medication is available, but the exact choice is often based on expert opinion

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and preference rather than randomized placebo-controlled trails. We carried out a retrospective analysis to assess the prevalence of VM in the patient population consulting in a specialized otolaryngology clinic, based on the diagnostic criteria as published in 2012.[5] Secondly, we assessed the associated symptoms that present with VM, as well as the effectiveness of prophylactic treatment of two of the most frequently described anti-migrainous drugs in our clinic, *i.e.*, flunarizine and propranolol.

2. Materials and methods

All included patients received routine ear, nose, throat and neuro-otological examinations, augmented with specific otovestibular tests and imaging if required. We used the diagnostic criteria for VM as formulated in 2012.[5] For the complete classification, readers are referred to the original article. These criteria distinct within two groups: a VM group and a probable VM group (PVM). In addition, we created a third group of patients who could not be classified according to criteria (atypical vestibular migraine = AVM) but did show suggestive VM symptoms. We chose to only evaluate flunarizine and propranolol and excluded other medical treatment. Our treatment policy includes propranolol (80 mg) as first choice and flunarizine (10 mg) when there is a history of beta-blocker intolerance, cardiac arrhythmia, asthma and hypotension. Flunarizine is not given when there is a history of depression.

3. Results

Of all 407 patients consulting (between January 2012 and January 2014), 65 (16%) were included and classified in one of the three groups: 17 (4.3%) in the VM group, 23 (5.6%) in the PVM group and 25 (6.1%) in the AVM group. Forty-three females (66.2%) and 22 males (33.8%) were included. The demographics of the patient population is listed in Table 1. We assessed the prevalence of the associated symptoms and a summary can be found in Figure 1.

In total, 31 patients were treated with flunarizine and 68% showed improvement, 10% had no benefit and 22% of the patients reported worsening of their symptoms. Improvement was significantly higher than no benefit or worsening of symptoms ($p < 0.001$), but there was no difference among the three subgroups. Thirty patients were treated with propranolol and symptoms improved for 74% of the patients, 3% showed no benefit and 23% reported symptom worsening. We did not find a significant difference between the three groups for propranolol but we did find a significant percentage of improvement ($p < 0.001$).

Table 1. The demographic profile of the study population.

	Patients (n)	Patients (%)	Mean age (SD)	Female (%)	Male (%)
Total group	65	16.0	46.5y (11.5y)	66.2	33.8
VM	17	4.2	51.0y (13.9y)	64.7	35.3
PVM	23	5.7	46.4y (9.6y)	73.9	26.1
AVM	25	6.1	43.5y (10.9y)	64.0	36.0

VM, vestibular migraine; PVM, probable vestibular migraine; AVM, atypical vestibular migraine; SD, standard deviation; y, years.
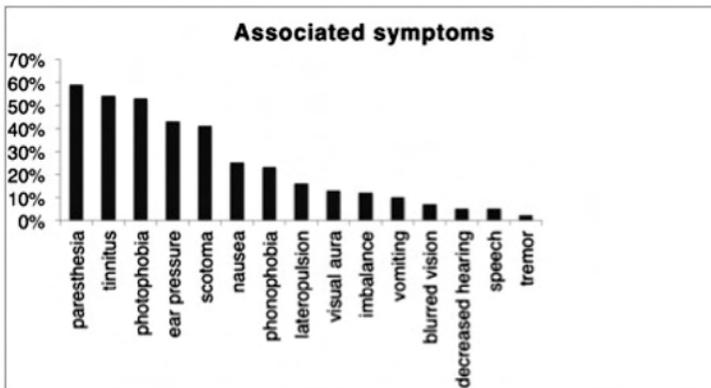
Fig. 1. Prevalence of associated symptoms.

4. Discussion

We found that VM is a frequently occurring entity with a prevalence of 16% (including all three subgroups) or 9.2% (without the AVM group). This is in accordance to previous studies reporting a prevalence of VM between 7%[20] and 11% for dizziness clinics. We found that the majority of patients experience associated symptoms. Surprising was the high prevalence of auditory symptoms such as tinnitus (54%) and aural pressure in one or both ears (43%). When we evaluated the effectiveness of both flunarizine and propranolol as prophylactic treatment, we found a significant higher proportion of symptom improvement when compared to no effect on symptoms and deterioration. So far, few studies focused on the pharmacological treatment options for VM patients and existing studies suffered from small sample sizes or they were not based on controlled, randomized trials. Recently, a randomized controlled trial reported significant improvement of the frequency and severity of dizziness when flunarizine was administered. Propranolol, a non-selective beta adrenergic blocker, is one of the most commonly prescribed drugs for migraine prophylaxis, but its role in

VM treatment has not been fully established yet. We found a beneficial effect on VM symptoms for both drugs, however, our study only has limited power to prove the effectiveness of these medications due to its retrospective character.

Remarkably, we did not find significant differences in the effectiveness of prophylactic medication for the three different VM subgroups. In any case, there are several confounding factors such as spontaneous improvement and the fact that anti-migrainous medication also has as an anxiolytic and antidepressant effect. The presence of a placebo effect should also be considered when evaluating the therapeutic effect since a recent meta-analysis has shown that placebo response rate can be as high as 21%. In addition, there is a high comorbidity of VM and psychiatric disorders such as anxiety and depression. Both mood disorders may also cause dizziness-like symptoms, making a differentiation between the entities sometimes complicated and tricky.

An important limitation is the retrospective nature of this study. Future prospective studies should set up randomized, placebo-controlled protocols in large sample sizes to investigate this further.

In conclusion, VM is a very common cause of dizziness and a variety of neurological and otovestibular symptoms can be associated with it. Lastly, it seems that prophylactic pharmacological intervention can alleviate symptoms for the majority of patients.

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THE MYSTERY OF VERTIGO SOLVED! THE INTERACTIVE GPS SYSTEM

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Diagnosis of dizziness still presents a dilemma to the physician and patient. The importance of history cannot be overemphasized.

The classical classification of central and peripheral vertigo is not clinically useful. Some physicians diagnose everything as Ménière's disease unless proven otherwise. Others do all the diagnostic tests in every case. To save the physician's time and patient's money, the answer is clinical categorization.
Belal (2002) published a roadmap for diagnosis of vertigo. The roadmap makes diagnosis simple and logical in two steps.[1]

- In step 1, you ask five questions in the right order: Onset of vertigo: Acute or chronic?; neurological symptoms ±?; auditory symptoms ±?; positional vertigo ±?; and episodic vertigo ±?

This leads to ten destinations (presentations): four acute vertigo, and six chronic dizziness (Fig. 1).

- In step 2, for each presentation there is an anatomical diagnosis and an etiological diagnosis that require a specific set of diagnostic tests.

In 2012, Belal introduced the GPS (Global Positioning System) for diagnosis of vertigo, *i.e.*, diagnose while driving![2]

In 2015, Belal introduced an interactive GPS system in which the clinician or patient can fallow the track of five questions to lead him into a diagnosis or destination of ten choices. Each diagnosis leads to a set of diagnostic tests to reach the final diagnosis.[3] The system comes in Arabic and English languages.

In conclusion, good history is crucial for diagnosis of vertigo. You need a roadmap so you are not lost in the maze. Clinical categorization is much better

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Fig. 1. The global positioning system (GPS) for diagnosis of vertigo: Diagnose while driving.

Fig. 2. The interactive GPS system for diagnosis of vertigo.

than the classical peripheral/central classification. The GPS interactive system saves the physician's time and the patient's money. Note that times change. You can have multiple pathologies in one patient! A Ménière's patient is not necessarily a Ménière's patient forever! No acoustic tumor does not mean no tumor ever! Run the GPS system every time!

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ROLE OF PERINEURONAL NETS IN VESTIBULAR COMPENSATION

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1. Introduction

The perineuronal net (PNN) is a layer of extracellular matrix enwrapping the soma and proximal dendrites of several types of neuron in the adult central nervous system (CNS), with holes at sites of synaptic contacts.[1]

PNNs are formed at the end of developmental critical periods for experience-dependent plasticity and contribute to the stabilization of specific connection patterns.[2,3] Pharmacological degradation of PNNs in the adult CNS restores juvenile levels of plasticity, improves functional recovery after damage and enhances cognitive functions.[4-11] Vestibular compensation is an attractive model of deafferentation-induced plasticity in the adult brain.

After unilateral damage of the vestibular system, the static syndrome (*i.e.*, symptoms continuously present even in totally stationary subjects) largely disappears in a few days[12] and is followed by a slower process in which dynamic deficits (which manifest as a result of head motion in space) recover, although in many cases never completely.[13,14] Vestibular compensation is attributed to functional/structural plasticity in the VN, cerebellum and related structures.[15]

Previous reports showed reactive neurogenesis and gliogenesis in the ipsilateral VN.[16-20] Significant changes in the expression of brain-derived neurotrophic factor, choline acetyltransferase, GABA, neuroprotective factors, markers of inflammation and extracellular matrix molecules have been detected in the VN after peripheral damage.[21-26]

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In the present study, we investigated whether: (1) the expression of PNNs in the VN of the adult mouse is altered during compensation of postural vestibular deficits, in parallel with enhanced structural plasticity; (2) vestibular compensation is affected in mice with genetically reduced PNNs.

2. Materials and methods

The experiments were performed on three-month-old female FVB, C57BL/6 mice (Harlan, San Pietro al Natisone, Italy) and homozygous Bral2 knockout (KO) mice.[27] Pax2-GFP transgenic mice were used to visualize GABAergic interneurons (28). All procedures were in accordance with the current Italian and European guidelines and directives.

Mice were anaesthetized by intraperitoneal administration of ketamine (100 mg/kg; Ketavet, Bayer) supplemented by xylazine (5 mg/kg; Rompun, Bayer). Unilateral labyrinthectomy was performed as in Gacek and Khetarpal.[29]

2.1. Behavioral tests

Support surface is a very sensitive parameter used for evaluating static posture deficit and recovery after a vestibular lesion.[26,30] Mice were placed in a box equipped with a transparent bottom and filmed from below. The support surface was measured by Image J software (Research Service Branch). Dynamic postural deficits and recovery were evaluated in FVB mice (n = 6) by assessing the air-righting and the landing reflex.[26]

2.2. Histological procedures

Once processed, we evaluated the density of glutamatergic and GABA/glycinergic terminals, the density and size of LVN neurons and the number and staining intensity of PNNs. Morphological evaluations were performed in the lateral VN (LVN), mainly involved in the control of posture and balance through its connections to the spinal cord.[31,32] GABAergic/glycinergic terminals were stained by anti-VGAT antibodies. Glutamatergic terminals, namely vestibular fiber endings[33] as well as several other types of VN afferents were revealed by anti- VGLUT1 or VGLUT2 antibodies.

3. Results

The support surface of the mice was strongly increased at 1 dpo (pre-op: 7.00 ± 0.17 cm^2; 1 dpo: 11.92 ± 1.04 cm^2; ANOVA with repeated measures $F(11,5) = 8.27$, $P = 0.001$). During the following days, the support surface progressively decreased, reaching values approaching pre-operative conditions at 13 dpo (8.59 ± 0.65 cm^2; $P > 0.05$).

After UL, the mice showed profound impairments in dynamic tests, reaching the maximum global score at 1 dpo (3.50 ± 0.22; ANOVA with repeated measures $F(12,5) = 15.47$, $P = 0.001$). A progressive recovery of dynamic reflexes occurred with time, thus the global score gradually decreased, reaching values close to pre-op conditions at 24 dpo (0.92 ± 0.45; $P > 0.05$).

When examining plasticity of glutamatergic axons in the ipsilateral LVN, we found a progressive decrease in the density of terminals, particularly for VGLUT1-positive. In the first two weeks following UL, VGLUT1 terminals density decreased from 1164.02 ± 53.80 to 224.33 ± 20.28 at 12 dpo (81% decrease; $P < 0.001$). No significant difference in the density of GABA/glycinergic terminals was detected at any time point post-lesion. Neither the density (number/mm²) (CTR = 264.68 ± 13.21, 12 dpo = 286.28 ± 13.97, 24 dpo = 246.34 ± 11.14, one-way ANOVA F $= 1.15$, $P = 0.33$) nor the size of neurons (small neurons: CTR = 260.26 ± 6.44[224]lm2, 12 dpo = 277.92 ± 12.55 lm2, 24 dpo = 251.60 ± 9.08 lm2, one-way ANOVA $F(2,527) = 1.42$, $P = 0.24$; large neurons: CTR = 690.97 ± 16.12 lm2, 12 dpo = 681.86 ± 36.78 lm2, 24 dpo = 724.30 ± 24.42 lm2, one-way ANOVA $F(2,158) = 0.87$, $P = 0.42$) were affected by UL. At the contralateral side while the number of VGLUT2 terminals did not change at any time point after UL (Dunnett's post hoc $F(4,64) = 2.45$, P [0.05; Fig. 4f–j, q), the number of VGLUT1 terminals showed a 75% increase at 24 dpo (from 1164.02 ± 53.80 to 2038.09 ± 109.62; Dunnett's post hoc $F(4,86) = 23.94$, $P < 0.001$). Moreover, we found that VGAT-positive terminals were substantially increased in number at 6, 12 and 24 dpo (approximately 65% increase, from 3456.33 ± 283.41 to 5671.42 ± 258.78 at 6 dpo; Dunnett's post hoc $F(4,93) = 12.44$, $P < 0.001$).

Notably, in the ipsilateral LVN, the number of PNN-bearing neurons dropped from 65.76 ± 2.20 to $20.04 \% \pm 3.30$ at 3 dpo (Dunnett's post hoc $F(4,39) = 38.40$, $P < 0.001$). The percentage of neurons with nets then increased progressively with time, becoming $39.89 \pm 3.10\%$ at 6 dpo ($P < 0.001$ when compared to intact LVN), and returning to control levels from 12 dpo ($P > 0.05$). We observed PNN changes also in the contralateral LVN, although to a lesser extent than in the ipsilateral LVN. The percentage of weakly stained nets significantly increased when compared to intact conditions, being 56% at 3 dpo, 83% at 6 dpo and 78% at 12 dpo, while the frequency of strongly stained PNNs decreased (14% at 3 dpo,1% at 6 dpo, 0% at 12 dpo, $p < 0.001$). Intriguingly, after 24 days, the number of weak, medium and strong PNNs was comparable to the intact condition. In the contralateral LVN, we also observed a significant progressive reduction in PNN staining intensity until 6 dpo. Interestingly, Bral2 KO mice showed a faster vestibular compensation, as they returned to pre-operative values at 7 dpo (Dunnett's post hoc $F(7,14) = 13.25$, $P > 0.05$ between pre-op and 7 dpo).

4. Discussion

Our main findings were the following: (1) recovery of static reflexes is accompanied by an increased number of GABA/glycinergic boutons in the contralateral LVN; (2) compensation of dynamic reflexes is additionally accompanied by a dramatic increase in the number of glutamatergic terminals in the ipsilateral LVN and, to a lesser extent, in the contralateral LVN; (3) PNNs are strongly reduced in number and staining intensity during the course of vestibular compensation and are restored when dynamic vestibular deficits are resolved; (4) in mice with genetically defective PNNs (Bral2 KO mice) recovery of postural functions after UL is accelerated. Importantly, for the first time, here we show that in the absence of Bral2, adult CNS plasticity is enhanced. These results may have important implications in view of clinical strategies to improve vestibular compensation in patients who do not recover completely after a unilateral vestibular damage, or to enhance functional recovery after other types of nervous system injury.

In conclusion, our data strongly suggest that UL-induced PNN reduction in the LVN may contribute to the re-opening of a 'critical period' for plasticity, during which substantial axonal remodeling takes place leading to the recovery of vestibular functions.

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IMPROVING DIAGNOSTICS OF PATIENTS WITH VESTIBULAR PAROXYSMIA

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1. Introduction

Vestibular paroxysmia (VP) is defined as short vertiginous spells frequently dependent on head position with an underlying hypothesis that there is a neurovascular conflict (NVC) with the VIII cranial nerve. Although the syndrome was first described more than 30 years ago by Jannetta[1,2] as disabling positional vertigo, there is still a need for more specific diagnostic criteria.

VP is characterized by symptoms such as episodic positional vertigo lasting for seconds of minutes, tinnitus and hearing loss.[3] So far there is no diagnostic golden standard for VP leading to many diagnoses made by exclusion of other episodic vestibular pathologies such as benign paroxysmal positional vertigo (BPPV), vestibular migraine (VM), Ménière's disease and posterior canal dehiscence. This therefore leads to long periods of invalidity, incorrect diagnoses and high costs for the health system. Although analysis of audiovestibular function showed abnormal results, past studies showed no correlation between audiovestibular findings and MRI results.[4,5] Also electronystagmography (ENG) and MRI results have been investigated in patients with VP, however, specific results that may help to diagnose patients with VP have not been found.

Both non-invasive medical treatment with carbamazepine/oxcarbazepine and invasive surgical treatment to replace the vascular compression have been used with success in the past[6].

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2. Materials and methods

The aim of this study was to identify similarities among our patient group with VP, by looking retrospectively at history taking and clinical results. Therefore a study was performed on 21 patients with complaints of episodic vertigo, a NVC on MRI and improvement of symptoms by treatment with carbamazepine or oxcarbazepine.

3. Results

In this study, 57% of the VP patients complained of lightheadedness, while 52% experienced dizziness and 38% were instable. A group of 43% experienced rotational sensations as well as a drunken feeling 43%.

The most common accompanied symptoms were cervical pain (71%), headache (57%), hearing loss (48%), tinnitus (48%), and aural fullness (38%). Symptoms occurred daily in 81% of the patients lasting seconds or minutes (67%) often described by the patient as a continuous burden with short episodes of vertiginous spells.

Symptoms were triggered by positional changes (71%) specifically by head movement in 52% and only 29% by rolling over in bed. In 33% of our patients symptoms occurred spontaneously at rest. Most of the patients experienced symptoms getting worse daily (62%).

Clinical examination showed a persistent non-BPPV type nystagmus (80%). Audiometry revealed normal hearing in eight patients (40%) and abnormal in 12 patients (60%). Among these patients, 42% had a unilateral and 58% a bilateral sensorineural hearing loss (SNHL).

The Chavda Classification was used to specify the anatomy of the NVC. There was no correlation found between audiometry, ENG and the type of loop.

Several other diagnoses were made which led to a mean duration of 19 months, a median of eight months and a maximum of 60 months before efficient treatment for VP. More than 50% of the patients were misdiagnosed with vestibular migraine. Also Ménière's disease was a frequent misdiagnosis in almost 50% of the patients.

4. Discussion

First of all, the comparability of different studies on VP is quite difficult due to differences in inclusion criteria.

Out of 21 patients from our study, eight were assigned to the definite VP groups, eight to the probable VP group and five to the atypical VP group. The atypical VP group consisted of patients who did not fit the definite or probable group defined by the latest diagnostic criteria by Hufner, but still experienced symptom improvement or decrease after treatment with carbamazepine/oxcar-

bazepine. Therefore, five patients out of 21 (24%) would have been missed and not treated correctly if included by the latest criteria by Hufner.[3] Therefore the question rises if there is a need for more specific criteria to diagnose VP.

Moreover, VP may also be associated with headache, visual disturbance or nausea which leads to a difficult differential diagnosis with VM[3]. Many patients in our study also had accompanying neurological symptoms such as headache, photophobia and phonophobia, resulting in initial misdiagnosis and unsuccessful treatment with flunarizine/propranolol for VM. Still some specific results in clinical examination such as a persistent non-BPPV type nystagmus may suggest more arguments for treatment for a VP.

Incidence of sensorineural hearing loss has been reported in 50-100% of patients with VP.[4,5] This was confirmed in our study where audiometry revealed normal hearing in 40% of patients and hearing loss in 60% of patients. Still there are no specific audiometry results found that can make VP diagnosis easier.

Although all of our patients did have a NVC on MRI, no associations were found between the location of the loop and symtomatology or any other clinical examination results. So can the symptomatology of a patient with vestibular paroxysmia presenting with episodic vertigo, left-sided unilateral hearing loss be due to a right-sided NVC? Thus is a NVC a coincidental finding in these patients or is it pathognomonic for VP?

Still audiological results, ENG and MRI results are most helpful in excluding other vestibular pathologies with episodic symptoms such as Ménière's disease.

5. Conclusion

The major results from our study show that patients with VP suffer from vertiginous spells occurring daily and lasting for seconds/minutes triggered by positional changes, mainly head movement but less spontaneously in rest. Clinical examination shows a persistent non-BPPV type nystagmus. Audiometry and ENG do not reveal specific results for VP, but are still needed to exclude other pathologies. Therefore a combination of history taking, clinical examination and imaging is necessary to diagnose VP and more importantly to differentiate it easily from other vestibular pathologies with episodic vertiginous spells such as BPPV, vestibular migraine, Ménière's disease and SCCD.

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VESTIBULAR NEURITIS ACCORDING TO vHIT TESTINGS. CLINICAL ENTITIES AND PROGNOSTIC FACTORS

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1. Introduction

Vestibular neuritis is usually considered to involve the superior portion of the vestibular nerve. Total or superior vestibular neuritis (TVN and SVN) are commonly diagnosed by acute peripheral vestibulopathy with horizontal rotatory nystagmus, nausea or vomiting, postural imbalance and areflexia after caloric tests. Head impulse test (HIT) is positive on the superior and lateral semicircular canal (SCC) during SVN and also affect the posterior SCC in TVN.

Diagnosis of inferior vestibular neuritis (IVN), first described in 1996,[1] can account for a pathology involving the inferior vestibular labyrinth consisting in the posterior SCC and the saccule. People suffering from IVN have a downbeating and torsional nystagmus, normal caloric tests and cVEMP. Some authors add positive HIT on the ipsilateral posterior SCC.[2,3] IVN can be misdiagnosed to a central pathology.[2,4] Thanks to the development of videoHIT (vHIT)[5] which can separately test the six SCCs, an abnormal SCC is possibly identified thus indicating that symptoms can be attributed to a peripheral vestibulopathy. This seems of particular interest in emergency wards to differentiate peripheral and central pathologies.

The aim of this study was to evaluate the impact of vHIT testings on various clinical entities of vestibular neuritis including some associated with cochlear symptoms. The main criterion was to evaluate the incidence of each anatomical type of neuritis at initial clinical presentation. Secondary aims were to evaluate the recovery at three months and to identify specific risks for each type of deficit.

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2. Methods

2.1. Patients

From October 2010 to June 2013, a retrospective study was conducted at the otolaryngology department in a tertiary referral center. The design of the study included all patients over 18 with a first vestibular or cochleovestibular syndrome mimicking vestibular neuritis and to classify clinical features according to the SCC involved on vHIT. Criteria of inclusion were: (1) symptoms of acute unilateral vestibulopathy including vertigo, nausea, vomiting and postural imbalance for more than 24 hours; (2) nystagmus under videonystagmoscopy; (3) abnormal vHIT; (4) normal neurological and oculomotor examination; (5) normal middle ear examination. Patient with cochlear symptoms were also included. We excluded of the study, patients with history of vestibular and cochlear symptoms, petrous bone traumatism, ear surgery or neurological pathology.

2.2. Clinical presentation

The following variables were collected: age, gender, cardio-vascular risks factor. Oculomotricity was tested. Nystagmus was assessed under videonystagmoscopy (Synapsys®, Marseille, France). Provoked nystagmus was elicited by HST and Hallpike manipulation. Variation of nystagmus in gaze position and deviation after Romberg and Fukuda tests were always tested.

2.3. vHIT

Each patient underwent a vHIT during the first 24 hours of admission. Each SCC was independently studied with a high-frequency camera (Synapsys Ulmer ®, France).[6-8] The answer given by was normal or abnormal but there was no gain result.

Three groups were determined according to the number of semicircular canals involved. Group I (one SCC involved), group II (two SCC involved) and Group III (three SCC involved).

2.4. Other investigations

Patients underwent caloric testings, cVEMPs and audiometry within three days after the onset of the symptoms.

MRI was performed to rule out any abnormality on the auditory and vestibular pathways or of the posterior fossa.

2.5. Treatment

Every patient underwent the same treatment with intravenous corticosteroids and anti-emetic. The patient was discharged as soon as they were able to eat,

drink and walk unaided, with a prescription of vestibular rehabilitation and oral corticosteroids.

2.6. Follow-up

All patients had a follow-up at least three months after the beginning of the vertigo. Clinical examination, vHIT, caloric tests and cVEMP were tested.

2.7. Statistical analysis

Statistical analysis included a X2 test that was performed to compare the distributions in age and gender between the groups. Repeated analysis of variance (ANOVA) and unpaired t-test were used to compare groups. Differences and distribution of frequencies were considered statistically significant at $p < 0.05$.

3. Results

Sixty-two patients with acute vertigo and abnormal results on vHIT were analyzed. Subjects were included in the groups even if they had cochlear symptoms. In each group, subgroups were labeled 'a' if there was no auditory symptoms and 'b' if they had auditory symptoms. There was no statistic difference between the three groups in terms of age and gender.

3.1. Initial clinical presentation

In group I, the pathological SCC (n = 28) was always the posterior. Clinical presentation included nausea, postural imbalance in the antero-posterior direction a torsional and down-beating nystagmus. Half of the patients suffered from auditory symptoms. While caloric tests were normal for subjects without hearing loss (group Ia), a hypovalence > 50% was notified for more than 60% of group Ib ($p < 0,05$).

Group II (n = 24) included patients with two deficitary SCC, and, it was always the superior and the lateral SCC. They all had a horizontal rotatory nystagmus pointing toward the healthy ear. Deviation on Romberg and Fukuda tests were oriented on the side of the pathologic vestibule. Caloric tests always showed an ipsilateral areflexia and cVEMPs were normal among 89% of the tests realized. Patients had no auditory symptoms.

Group III (n = 10) had a deficit on the three SCC. Eight of them had no auditory symptoms (group IIIa) and the clinical presentation was close from that of the group II.

When comparing the three groups, caloric tests deficit were normal in group I and pathologic in group II and III. For cVEMP, our data did not show any difference between the three groups.

Concerning CVRF, 39% of patients without cochlear symptoms had none, whereas all patients with auditory symptoms had at least one (p < 0.05).

3.2. Recovery at three months (Fig. 1)

Within three months, all patients without auditory symptoms had a significant improvement of their VOR after vHIT compared with patients with auditory symptoms (p < 0,05). The recovery rate on VHIT was 100% (group Ia), 74% (group II) and 71% (group IIIa). Recovery rates on vHIT were significantly inferior for patients with cochleovestibular symptoms (p < 0.05): 46% for group Ib and 0% for group IIIb.

For all patients without cochlear impairment caloric tests improved, whereas those with cochlear symptoms got worse. Concerning cVEMPs, they improved in patients without auditory symptoms whereas no improvement was observed in deaf patient groups.

Concerning audiometric results, patients with cochlear symptoms did not show any significant improvement, and most of the deafness stayed severe to profound. No auditory symptoms appeared in patients without initial symptoms.
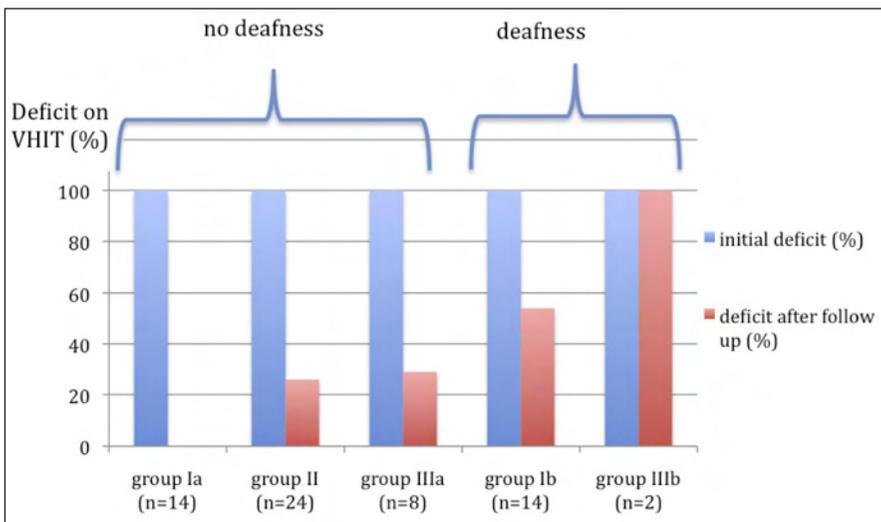
Fig. 1. Recovery at 3 months followed by video head impulse test.

4. Discussion

The main results of this study showed that among patients with deficit of the posterior SCC, two different clinical presentations with or without auditory symptoms exhibited two different patterns of recovery. On one hand, patients without auditory symptoms (group Ia) had all good outcomes with complete
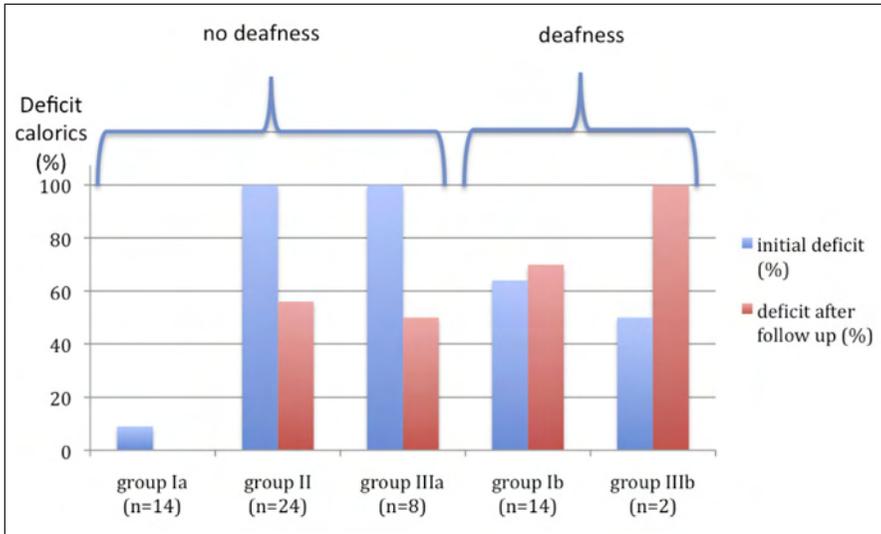
Fig. 2. Recovery followed by calorics.

recovery of their vHIT. On the other hand, patients with auditory symptoms (group Ib) ended up without recovery both from their vHIT results and deafness.

Among group Ia patients, recovery at three months was 100% on vHIT and no auditory symptoms appeared. Associated with normal caloric tests, group Ia seemed to have IVN. Critical analysis of literature shows a prevalence of IVN from 1,2%[2] to 45%.[9] IVN is diagnosed by torsional nystagmus, normal caloric tests, abnormal cVEMPs ± abnormal HIT on the posterior SCC. Some authors found 100% of recovery on cVEMPs at three months,[9] and we had the same results on vHIT. We thought that vHIT is more likely to diagnose IVN than cVEMPs because, unlike the saccule, the innervation of each SCC is made by one specific nerve so that vHIT deficit reflect the abnormal nerve. In 2012, very selective deficits on the lateral SCC have been described.[10] In IVN, the attempt could affect selectively the inferior nerve in the foramen singulare, causing an isolated deficit on the posterior SCC. IVN could be diagnosed more precisely by clinical history, torsional down-beating nystagmus, normal caloric test and an isolated deficit on the posterior SCC using vHIT. Deficits which follow the anatomy of the inferior VN and the quick recovery at three months after anti-edema treatment suggest a neurological peripheral deficit like neurapraxia.

Fifty percent of patients with deficit of the posterior SCC on vHIT had auditory symptoms and, contrary to group Ia, recovery at three months was poor on vHIT (46%), zero on caloric tests and deafness. While the group Ia deficit follows the inferior vestibular nerve, the association of deafness and deficit of the posterior SCC does not follow the topography of the cochlear or vestibular nerve. Nonetheless, the topography of the cochleovestibular artery may give some understanding. This entity has been first described in 1993.[11] They

found five subjects with deafness and normal calorics and suggest the deficit of the posterior SCC by the direction of the nystagmus. All of the subjects had at least one CVRF. This hypothesis was supported by anatomical description of fibrosis in the territory of the commune cochlear artery two months after a cochleovestibular syndrome in women with cardiovascular history.[12] Other authors described deafness with supposed or confirmed deficit of the posterior SCC,[2,11,13,14] but none of them analyzed the recovery.

Vertigo associated to deafness is considered to have poor prognosis on recovery.[14,15] Deafness was often profound and showed no recovery.

Our results showed that CVRF appeared to be significant risk factors associated with cochlear symptoms and outcomes. Almost 40% of patients with IVN had no CVRF whereas all patients with cochlea-vestibular syndrome had at least one. Description of increase of relative risk of myocardial infarction and stroke after sudden deafness and vertigo again suggest a link between CVRF and cochleovestibular symptoms.[16,17] These findings may lead to consider anti-agregant therapy in some selected patients with cochlea-vestibular symptoms.

5. Conclusion

In conclusion, vHIT may help in differentiating two types of posterior SCC deficits. When found isolated, it follows the topography of the inferior vestibular nerve like inferior vestibular neuritis and has a very good prognosis. When the vestibular deficit is associated with deafness, the prognosis is worse for both cochlear and vestibular systems and may account for a microvascular disease of the cochleovestibular artery. This could lead us to consider new treatments for cochleovestibular syndromes focused on a vascular aetiology.

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IMAGING

IMAGING OF ENDOLYMPHATIC SPACE IN PATIENTS WITH MÉNIÈRE'S DISEASE AND NON-OTOLOGICAL DISEASES

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1. Introduction

Visualization of endolymphatic hydrops (EH) in Ménière's disease (MD) has been performed using magnetic resonance imaging (MRI) after intratympanic or intravenous gadolinium (Gd) injection.[1-7] Hallpike and Yamakawa independently but almost simultaneously reported the presence of an enlarged endolymphatic space in the temporal bones of patients with MD, demonstrating that EH is its principal underlying pathology. In fact, EH were confirmed in the cochlea, saccule and utricle on temporal bone specimens.[8,9] After the era of EH confirmation based on temporal bone histopathologic specimens, imaging diagnosis of EH provided useful and dynamic information about inner ear diseases.[1] EH have been detected by MRI in classic or typical MD, moreover, in atypical forms of MD that lack the symptoms of either hear-ing loss or vertigo.[10-12] Recently, It has been reported that pathological endolymphatic hydrops is often observed in asymptomatic ears or in the contralateral ears in patients with unilateral MD.[13] Moreover, some reports showed the size of endolymphatic space had changed in repeated MRI examinations.[14-18] In this study, we investigated the difference of endolymphatic space between MD and the asymptomatic ears of non-otological diseases.

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2. Objective

To explore the existence of endolymphatic hydrops (EH) among patients with clinical Meniere's disease (MD) and asymptomatic ears using magnetic resonance imaging (MRI).

3. Subjects and methods

3.1. Patients

All 804 patients with symptoms of tinnitus, hearing loss and/or vertigo were measured with Gd enhancement of the inner ear MRI in a tertiary referral center in Japan. The 303 patients included in the study had symptoms attributable to MD. All patients seen in the clinic who met the Committee on Hearing and Equilibrium of the American Academy of Otolaryngology – Head and Neck Surgery (AAO–HNS) criteria in 1985 were invited to participate in the study. The mean age of the individuals was 52.9 years (range 13-86 years); 177 were women and 126 were men.

3.2. Control

A total of 19 patients with non-otological diseases were tested with informed consent: 11 male, eight female; age range 24-79 years, average age 56.6 years. None of the normal subjects reported any audiology, vestibular, neurological problems (apart from standard refractive errors). Thirteen patients with sinusitis, three patients with vocal cord polyp, one patient with epiglottic cyst, and two patients with benign parotid grand tumor were evaluated.

All patients gave informed consent to participate in the study. The study protocol was approved by the Ethics Review Committee of Nagoya University, Japan (approval number 2012-0144).

MRI was performed 24 hours after intratympanic injection of gadolinium or 4 hours after intravenous gadolinium administration (Fig. 1).

4. Results

EH in cochlea were present in 297 out of 402 MD ears (73.9%) and 15 out of 38 asymptomatic ears (39.5%). Significant cochlear hydrops was present 212 out of 402 MD ears (52.7%) and four out of 38 asymptomatic ears (10.5%). EH in the vestibule was present in 340 out of 401 MD ears (84.8%) and three out of 38 asymptomatic ears (7.9%). Significant vestibular hydrops was present 230 out of 401 MD ears (57.4%) and none of 38 asymptomatic ears (0%), respectively.
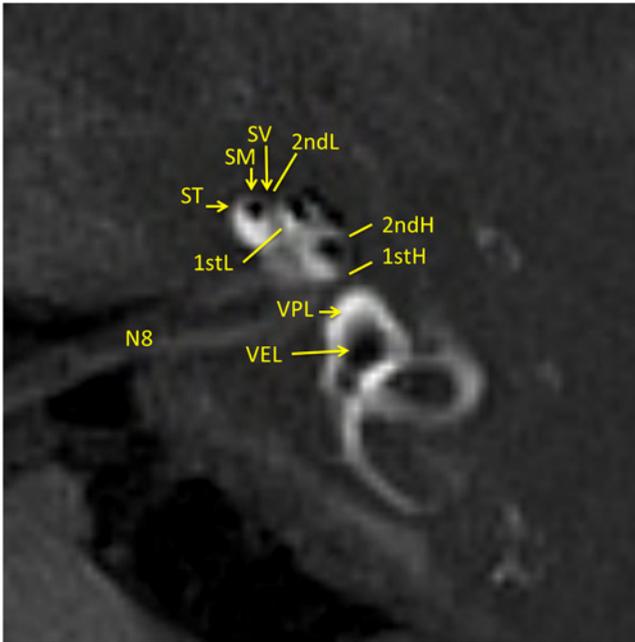
Fig. 1. Separate visualization of endolymphatic and perilymphatic spaces and bone in human using 3D real IR TSE on 3 T MRI after intratympanic administration of Gd-DTPA. Images were obtained 24 h after transtympanic injection of 50 mM Gd-DTPA at 3T using a 32 channel head coil. N8: 8th cranial Nerve; SM: Scala Media; ST: Scala Tympani; SV: Scalavestibuli; VEL: Vestibular Endolymph; VPL: Vestibular Perilymph; 1stL: basal Lower turn; 1stH: basal Higher turn; 2ndL: Second Lower turn; 2ndH: Second Higher turn.

5. Conclusion

All of definite MD patients had EH in the cochlea and/or vestibule in our study. The main cause of the high rate of cochlear EH was that our criteria assessed the largest EH in the cochlea or physiological EH as seen in temporal bone study. Further study is needed for silent cochlear EH may contribute to the pathogenesis of MD or not. On the other hand, vestibular EH with asymptomatic ears was obviously lesser than with disease ears, this showed strong association with inner ear symptoms or disease.

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ENDOLYMPHATIC HYDROPS IN PATIENTS WITH UNILATERAL AND BILATERAL MÉNIÈRE'S DISEASE

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1. Introduction

For patients with unilateral Ménière's disease (MD), progression of the disease to the non-affected side is a major concern. It has been reported that the frequency of progression from unilateral to bilateral MD increases with an increase in the duration of MD. Recently, it was reported that evaluation of the endolymphatic space size is possible after intravenous injection of an ordinary dose of gadolinium (Gd) contrast agents, using 3-Tesla (3T) magnetic resonance imaging (MRI).[1,2] We attempted to evaluate the endolymphatic space size on both sides in patients with unilateral and bilateral MD, and to investigate factors influencing progression to bilateral MD from the aspect of endolymphatic hydrops (EH) formation.

2. Materials and methods

The subjects for this investigation included 29 patients with definite unilateral MD and 12 patients with definite bilateral MD, according to the criteria established by the American Academy of Otolaryngology – Head and Neck Foundation in 1995.[3] To select typical patients with unilateral MD, we selected patients whose average hearing levels on the non-affected side at 500 Hz, 1000 Hz, and 2000 Hz were 20 dB or less. Three-dimensional fluid-attenuated inversion recovery (3D-FLAIR) MRI was performed using a 3-T MRI scanner after administration

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of Gd contrast agents to visualize the EH space. Among the 29 patients with unilateral MD, six patients received both intratympanic and intravenous Gd injections, and 23 patients received only intravenous Gd injection, as previously described.[1,2,4,5] Among the 12 patients with bilateral MD, one patient received a bilateral intratympanic Gd injection, while 11 patients received only intravenous Gd injections. The hybrid of reversed image of positive endolymph signal and native image of positive perilymph signal (HYDROPS) and 3D FLAIR were used to recognize EH.[6]

3. Results

Among the 29 affected ears of unilateral MD, zero, five, and 24 ears had no, mild, and significant EH respectively, in the cochlea; three, six, and 20 ears had no, mild, and significant EH respectively, in the vestibule. Some degree of EH was observed in either the cochlea or the vestibule. On the non-affected side, 15, 10, and four ears had no, mild, and significant EH, respectively, in the cochlea; 13, 10, and six ears had no, mild, and significant EH, respectively, in the vestibule. In eight of the 29 non-affected ears, neither the cochlea nor the vestibule showed EH. All 12 patients with bilateral MD had significant EH at least in the cochlea or the vestibule.

The EH scores in the cochlea and the vestibule for each ear were added together. The relationship between the affected and the non-affected ears is shown in Figure 1. Statistical analysis indicated that there was a relationship between the degree of EH in the affected and the non-affected ears (Cochran-Mantel-Haenszel test; $P = 0.0420$).

Figure 2 shows the effects of the duration of MD in groups with no, mild, and significant EH in the cochlea and the vestibule, for the non-affected ears. There was no relationship between the degree of EH on the non-affected side and the duration of MD .
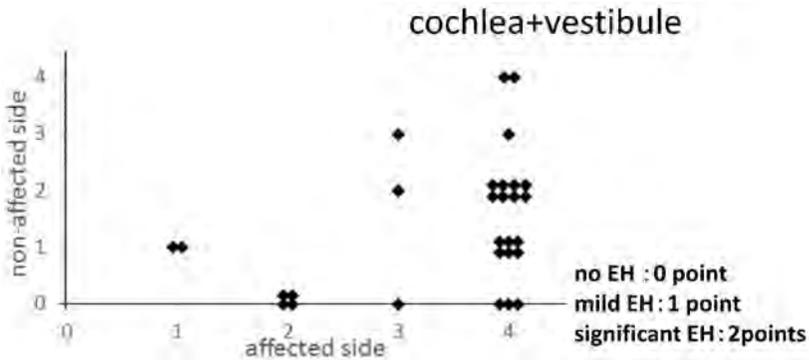
Fig. 1. EH in the affected and the non-affected side. (Cochran-Mantel-Haenszel test; $P = 0.0420$.)
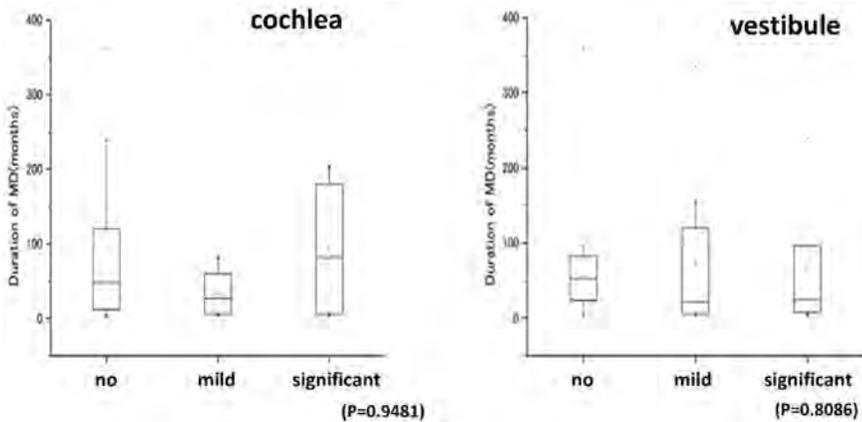
Fig. 2. EH of the non-affected side and the duration of MD. (General linear model trend test.)

4. Discussion

In this study, all patients with definite MD had EH. In patients with definite unilateral MD, 100% had EH in the cochlea and 90% had EH in the vestibule on the affected side; 48% had EH in the cochlea and 55% had EH in the vestibule on the non-affected side. Thus, EH appeared more frequently in the non-affected ears of patients with unilateral MD than in the ears of patients without MD; hence, the significance of EH in MD was apparent.[7] Gu *et al.*[8] showed that EH was observed on both sides after intratympanic Gd injection in all patients with bilateral MD.

Kariya *et al.*[9] reported that the number of spiral ganglion cells was significantly lower and the loss of inner and outer hair cells was significantly greater in the contralateral temporal bones in patients with unilateral MD than that in normal controls. They also reported that all subjects with bilateral MD had bilateral cochlear EH. Merchant *et al.*[10] described that all patients with classical symptoms of MD showed EH in at least one ear.

Most studies have reported that the frequency of progression to bilateral MD increased with the duration of the disease.[1,11-13] However, the present study did not find an association between the duration of MD and the endolymphatic space size on the non-affected side. There were cases without EH on the non-affected side, although the patients had had MD for a long time. It is assumed that the probability of progression to bilateral MD from unilateral MD is extremely low in case of absence of EH on the non-affected side. However, a longitudinal study is required to confirm this assumption.

5. Conclusion

MRI revealed that all ears with definite MD had EH either in the cochlea or in the vestibule. We assume that chances of progression to bilateral MD from unilateral MD are low when there is no EH on the non-affected side. MRI may provide useful information regarding progression from unilateral MD to bilateral MD.

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MRI EVALUATION OF ENDOLYMPHATIC HYDROPS AND CLINICAL APPLICATION FOR SURGICAL MANAGEMENT

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1. Introduction

Visualization of endolymphatic hydrops (EH) has recently become possible using MRI with contrast agents. The relationship between EH on MRI and otological examinations in patients with Meniere's disease has been investigated, and it was found that physiological function was related not only to the degree of EH, but also to the persistence of EH. EH could be found in cases of candidates for middle ear surgery, such as otosclerosis, and preoperative EH could be a risk factor for inner ear disturbances following surgery. We investigated the presence of EH on MRI in ears with clinical otosclerosis or ossicular anomalies, and further studied to compare preoperative MRI findings and postoperative symptoms following surgery to evaluate the efficacy of such MRI evaluation for the management of ears with otosclerosis or ossicular anomalies.

2. Materials and methods

Subjects diagnosed as having otosclerosis or ossicular anomalies and agreed to MRI examination were randomly recruited in the study. Ears were evaluated by MRI performed four hours after intravenous injection of gadolinium. The degree of EH in the vestibule and cochlea was classified into three grades (none,

mild, and significant). Imaging data were compared with clinical findings. In ears operated, imaging data concerning the degree of EH were compared with postoperative clinical findings.

3. Results

Varying degrees of cochlear EH and vestibular EH were observed (Fig. 1). Episodes of acute sensorineural hearing loss with rotatory vertigo occurred in some ears that showed severe EH in the cochleae and vestibules. Severe EH, however, was also observed in ears without such symptoms. The postoperative course in all ears with no EH in the vestibule was uneventful, with successful improvement of hearing levels, but a case with severe EH in the vestibule had postoperative nystagmus and long period of dizziness.
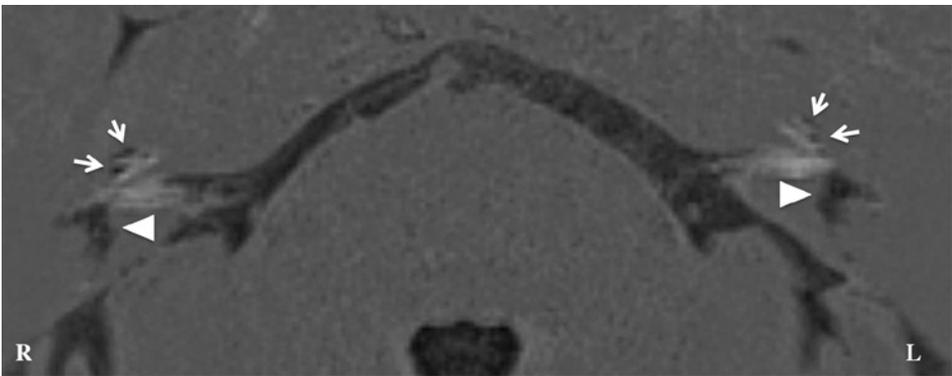
Fig. 1. HYDROPS (hybrid of reversed image of positive endolymph signal and native image of positive perilymph signal) is used to detect endolymphatic hydrops (EH). The black areas represent the endolymphatic space in the labyrinth, and the white areas represent perilymphatic space. The presence of EH can be visualized as black areas surrounded by gadolinium-filled perilymph. Severe cochlear EH (arrows) and vestibular EH (arrowhead) are present in the right (R) and the left (L) ears.

4. Discussion

Our findings suggest that EH should be considered a risk factor for surgical complications after stapes surgery or ossiculoplasty, especially in patients with cochlear and/or vestibular symptoms. Usage of overlong prostheses could damage the membranous labyrinth in the presence of vestibular EH, and this might result in postoperative complications. In addition to the procedure of stapes surgery, the presence of EH in the vestibule might have contributed partially to continued dizziness. Not considering such risk factors in these patients can produce unex-

pected results. Local anesthesia is a better choice in terms of safety because it allows the surgeon to confirm the patient's hearing and the presence of vertigo.

5. Conclusions

The presence of EH in ears with otosclerosis or ossicular anomalies was clearly visualized in the present patient series. Moreover, the presence of EH in the vestibule on MRI might be a high risk factor in ears that are candidates for stapes surgery. Such MRI evaluation could provide useful information for managing symptoms related to EH.

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MRI INNER EAR IMAGING AND TONE BURST ELECTROCOCHLEOGRAPHY IN THE DIAGNOSIS OF MÉNIÈRE'S DISEASE

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1. Introduction

The diagnosis of Ménière's disease (MD) has for the last twenty years been based on the American Academy of Otolaryngology – Head and Neck Surgery Equilibrium Committee guidelines.[1] Another validated Ménière's scoring system is the Gibson 10-point score.[2] The AAO-HNS guidelines and the new two-category classification from the Barany Society[3] do not recognize the validity, let alone the existence of any *in-vivo* test that can confirm endolymphatic hydrops.

The initial promise of transtympanic electrocochleography (EcochG) for diagnosis hydrops with a click stimulus has been dispelled. Gibson[4] and Hornibrook *et al*.[5] have demonstrated vastly improved diagnostic sensitivity using tone bursts (Fig. 1).

In 2007, Nakashima *et al*.[6] used intratympanic gadolinium to produce clear images of endolymphatic hydrops in MD inner ears, raising hopes that this may be the gold standard and the basis of comparison of any competing test.

2. Methods

One hundred and two patients requiring an MRI scan to exclude an acoustic neuroma were studied. Those with recurrent vertigo were assigned according

Table 1. Gibson1 EcochG voltage criteria for hydrops.[7]

Stimulus	Intensity (dB HL)	SP more –ve than: (μv)	SP/AP ratio
1 kHz tone burst	< 25	-6	
	20-35	-6	
	40-55	-6	
	60-75	-3	
2 kHz tone burst	< 25	-9	
	20-35	-7	
	40-55	-5	
	60-75	-5	
Click			0.5

the 1995 AAO-HNS criteria[1] Definite, Possible or Probable Ménière's Disease, with Definite being a Gibson score of > 7.[2] All had a pure-tone audiogram and transtympanic tone-burst EcochG with positivity for hydrops based on Gibson's voltage criteria[7] (Table 1).

After the EcochG, an eight-fold dilution of Multihance gadolinium two mls was placed in the tested ear over 30 minutes. The MRI scan in a General Electric HDX 3-T scanner was 24 hours later.

3. Results

In 10/102 (10%) gadolinium entry to the inner ear was inadequate. In 90% it was good or suboptimal but adequate to diagnose hydrops according to the Nakashima *et al*. grading system[8] (Figs. 1 and 2). In 14/102 (14%) EcochG tone-burst traces were unobtainable due to the magnitude of the hearing loss. See Tables 1 and 2.

Only 21% (21/102), all with MD, had a positive click EcochG (SP/AP ratio > 0.5) and all of these had a positive tone-burst response. In 30 patients with clinically Definite MD, gadolinium MRI was positive in 14 (47%). Tone-burst EcochG diagnosed hydrops in 8/14 (57%) of patients with clinically Probable MD. In clinically Definite MD 25/30 (83%) had a positive tone-burst EcochG, and a positive result for EcochG or MRI was seen in 26/30 (87%) and in 26/28 (93%) if two with profound hearing loss are excluded.

4. Conclusion

This study confirms the insensitivity of the click response for diagnosing hydrops, and it should be abandoned world-wide. It reconfirms the greatly enhanced diagnostic sensitivity of the tone-burst EcochG. Even though adequate MRI imaging was achieved in 90% tone-burst EcochG was a far more sensitive test, and diagnosed hydrops in 57% of patients classified as Probable MD.
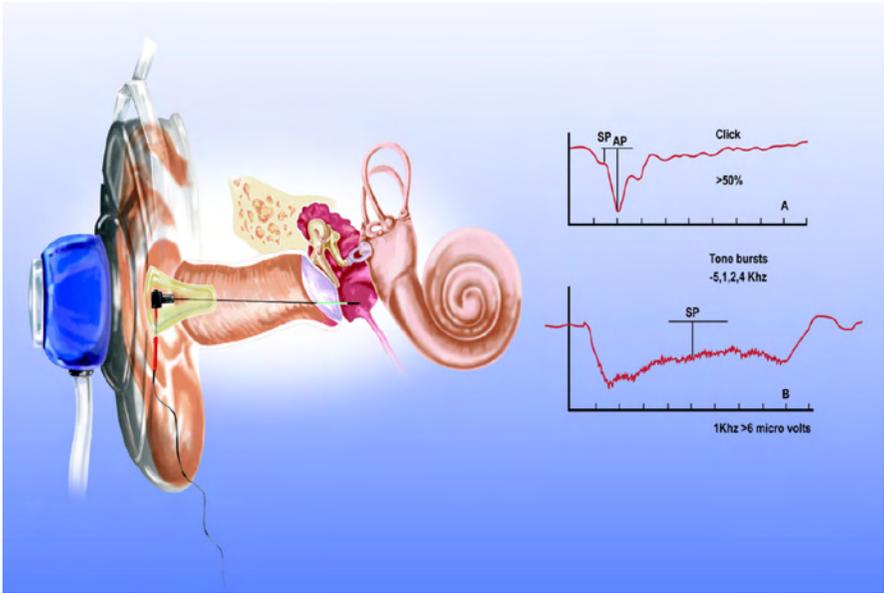
Fig. 1. Transtympanic EcochG in a right ear showing the responses to a 90 dB click and to a 100 dB 1 kHz tone burst. The tone-burst summating potential (SP) is measured from the baseline in microvolts (Gibson). (From: Acta Otolaryngol 2011;131:613-617 with permission from Informa: Taylor & Francis.)
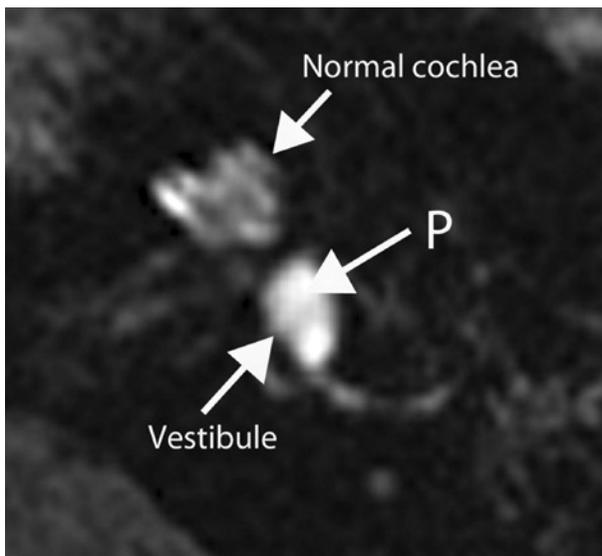
Fig. 2. MRI of a normal (non-hydropic) ear. P = perilymph; E = endolymph.

Table 2. Results of MRI and EcochG in each clinical category.

Clinical category	Number	Gadolinium MRI positive (%)	Tone-burst EcochG (%)	Click EcochG (%)
Definite MD	30	14 (47)	25 (83)	9 (30)
Probable MD	14	4 (29)	8 (57)	4 (29)
Possible MD	13	1 (8)	4 (31)	2 (15)
Asymmetric SNHL	25	2 (8)	7 (28)	4 (16)
Sudden SNHL	18	1 (6)	5 (28)	2 (11)
Asymmetric tinnitus	2	0	0	0

Table 3. Distribution of hydrops as demonstrated by Gadolinium MRI.

Distribution of hydrops	Number in study	EcochG positives
Vestibule only	6	6
Cochlea only	10	7
Vestibule and cochlea	6	4
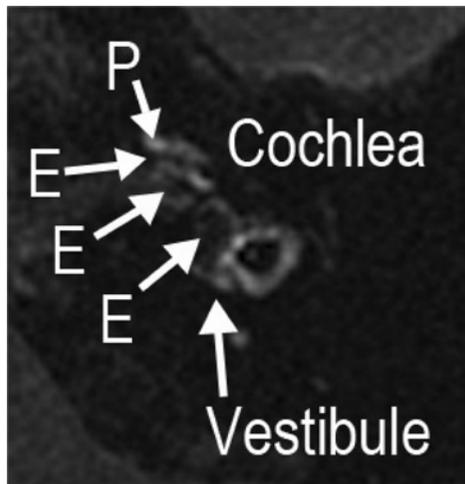
Fig. 3. MRI of the left ear of a MD patient with enlargement of the endolymphatic compartment in the cochlea and endolymph filling the vestibule. P = perilymph; E = endolymph.

In Meniere's disease hydrops begins in the cochlea and then moves into the vestibule9. It seems likely that what is observed on MRI inner imaging depends on brand of scanner, head coil specifications and the possibility that gadolinium entry may favor the vestibule. There is an urgent need for the diagnosis of MD to be based on objective testing and for a new category of 'Clinically Certain'. So far, tone-burst EcochG is the simplest, cheapest and most sensitive test available.[10]

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MR IMAGING OF INNER EAR ENDO-PERI-LYMPHATIC SPACES AT 3 TESLA AFTER INTRATYMPANIC CONTRAST AGENT ADMINISTRATION IN DEFINITE MÉNIÈRE'S DISEASE

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1. Introduction

Ménière's disease (MD) is an inner ear disorder characterized by fluctuating neurosensory hearing loss, aural fullness, tinnitus and recurrent episodes of vertigo.[1] The exact cause of MD is unknown. Factors associated with MD are endolymph excessive production and/or reduced resorption, ions alterations, genes mutations, vascular alterations, allergic reactions, viral infections, and many others [2-5]. In 1938 Hallpike and Cairns [6] examined temporal bone specimens of two patients affected by MD. They observed that the endolymph system was dilated and called this finding 'Hydrops labyrinthi', a term already used in 1929 by Wittmaack for a case of serous labyrintitis.[7] Since then, dilatation of the endolymph system has always been related to MD and till now final diagnosis of MD requires the histopathological confirmation of endolymphatic hydrops[8] that is feasible only *ex vivo*. Consequently, MD researchers have always focused on an objective examination able to demonstrate endolymphatic hydrops also *in vivo*. Magnetic resonance imaging (MRI) has recently found growing applications in this research field. High magnetic fields, 3D high-resolution sequences and contrast agent administration permit to show the structures of the inner ear with greater detail than before. Definite MD patients were already studied by using MRI,[9-12] but typical findings of definite MD at MRI are not yet defined.

Hence, our aim was to determine the main findings of definite MD by using 3-Tesla (3T) MRI after intra-tympanic contrast agent administration.

2. Materials and methods

2.1. Patients

Twenty-four patients (ten men and 14 women, range 38-78 years, mean age 52.8 years) with diagnosis of definite MD[8] were included in the study. In six patients both ears were affected. Exclusion criteria were history of ear surgery, previous intra-tympanic gentamicin injection, acute illness, as well as general contraindications to MRI. All patients were admitted to the Otolaryngology Unit of our Department 24 hours before MRI to perform routine clinical examination, audio-vestibular functional tests and trans-tympanic contrast agent injection. To inject contrast agent the patient was placed in the supine position with his/her head turned 30° toward the healthy (or less affected) ear compared to body midline and a local anesthetic (lidocaine hydrochloride 10%) was topically applied on the lateral surface of the eardrum. A 25 G needle was inserted in the postero-inferior quadrant of the eardrum and 0.4/0.5 ml of contrast agent (Gadobutrol diluted eight-fold with saline) were injected in the middle ear. Following the injection, the patient had to wait in the supine position with his/her head turned 45° toward the unaffected side for 30 minutes, to facilitate the passage of Gadobutrol through the round window membrane into the perilymphatic space.

2.2. Image acquisition

Patients underwent MRI one day after contrast agent administration since it has been shown that a 24-hours latency ensures optimal perilymph enhancement.[9] All acquisitions were performed with a 3T MRI scanner (Philips Achieva X Series, Philips Medical System, Best, Netherlands) equipped with a sense dedicated eight-channel head coil. Imaging protocol included a T2-weighted 3D Turbo Spin Echo (TSE) DRIVE sequence acquired on the axial plane (Fig. 1) producing very high resolution cisternography images [2000 ms TR, 200 ms TE, 212 × 165 acquisition matrix (Recon: 224), voxel size 0.8 mm × 0.8 mm × 0.8 mm, 30 slices, scan time of 1 min and 50 sec], a 3D-Fluid Attenuated Inversion Recovery (3D-FLAIR) sequence acquired on the axial plane (Fig. 2) producing positive perilymph images (PPI) [9000 ms repetition time (TR), 458 ms echo time (TE), 2500 ms inversion time (TI), flip angle 120°, echo-train length 110, acquisition matrix 212 × 165 (Recon: 224), voxel size 0.8 mm × 0.8 mm × 0.8 mm, 30 slices, scan time of 16 min and 21 sec], a T2 weighted TSE sequence on the axial plane [3000 ms TR, 80 ms TE, 416 × 295 acquisition matrix (Recon: 560), voxel size 0.5749 × 0.7187 (AP mm × RL mm), 4 mm slice thickness, 30 slices, scan time of 1 min and 6 sec], and a 3D turbo field echo (TFE) T1-weighted sequence acquired on the sagittal plane [10 ms TR, 4.7 ms TE, acquisition matrix 212 × 148 (Recon: 224), voxel size: 0.8 mm

Fig. 1. Cisternography image – T2 3D TSE DRIVE sequence. A set of additional -90 recovery pulses applied at the end of the echo train on T2 3D TSE DRIVE sequence causes high signal intensity from the fluid-containing spaces (perilymphatic spaces plus endolymphatic spaces) with higher signal-to-noise ratio and lower sensitivity due to *flow voids* than multislice sequences.
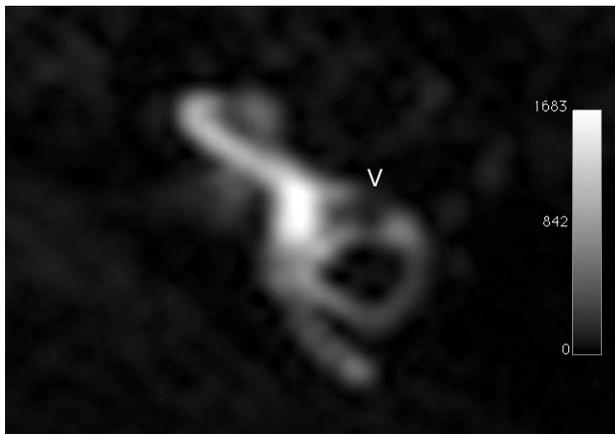
Fig. 2. Positive perilymph image (PPI) – 3D FLAIR sequence. A 2500 ms IT on 3D-FLAIR sequence causes a positive longitudinal magnetization of the perilymphatic fluid filled with contrast agent and a negative longitudinal magnetization of the endolymphatic fluid and surrounding bone. Consequently, 3D-FLAIR sequence produces positive perilymph images. Arrowhead indicates a filling defect of CE in the lateral semicircular canal.

× 0.8 mm × 0.8 mm, slice thickness 4 mm, 30 slices, scan time of 1 min and 52.5 sec]. The overall MRI acquisition time was 21 min and 9 sec.

2.3. Image analysis

For post-processing analysis, DICOM files were transferred to a dedicated work-station and a free open-source software (OsiriX v.3.6.1) was used. Two neuro-

radiologists with respectively five and 20 years of inner ear imaging experience performed manual segmentation of the vestibule by tracing perilymphatic space contours on each PPI slice in joint session; sum of the traced areas gave the perilymphatic space volume. The same procedure was performed on cisternography images to assess total inner ear fluid space volume. Endolymphatic space volume was so calculated as the difference between total inner ear fluid space volume and perilymphatic space volume. Vestibular endolymphatic hydrops was graded considering the ratio between endolymphatic space volume and total inner ear fluid space volume. A ratio of one third or less indicated no vestibular hydrops; a ratio between one-third and a half indicated mild vestibular hydrops; a ratio of more than a half indicated severe vestibular hydrops. For cochlea and semicircular canals, endolymphatic hydrops was assessed as positive if the two neuroradiologists detected a filling defect of contrast enhancement (CE) on PPI.

2.4. Statistical analysis

All data were stored and analyzed in an anonymized manner. IBM SPSS Statistics (release 20.0) was used for descriptive statistical analysis.

3. Results

We had no adverse reaction due to contrast medium injection.

Four patients were excluded from analysis because adequate enhancing of perilymphatic spaces was not obtained. MRI demonstrated endolymphatic hydrops in 19 patients (5% of false negatives). Vestibule was involved by endolymphatic hydrops in 18 patients (90%): the grade of hydrops was severe in 11 cases and mild in seven. Endolymphatic hydrops involved the posterior semicircular canal in five patients (25%), the lateral semicircular canal in 11 patients (55%), the superior semicircular canal in eight patients (40%), and the cochlea in ten patients (50%).

4. Discussion

Although electrocochleography, caloric tests, evoked potentials, and glycerol test allow indirect demonstration of endolymphatic hydrops, the MD diagnosis remains a challenge and is still based on clinical course, duration of symptoms, and hearing tests. Indeed, diagnostic accuracy of audio-vestibular functional tests is low and a diagnostic exam able to demonstrate endolymphatic hydrops *in vivo* is still required. Several studies have showed that MRI is feasible,[10,11] but it is not yet used in clinical practice for many reasons: diagnostic accuracy not yet tested on large sample size, correlation of MRI with histology not feasible, correlation of MRI with functional tests influenced by the low diagnostic

accuracy of the latter, lack of standardized techniques, typical MRI findings of MD not yet defined.

In our series, MRI showed endolymphatic hydrops in 95% of patients. In other series MRI demonstrated endolymphatic hydrops in 100% of selected patients.[11,12] However, it should be noted that MD patients with a histology negative for endolymphatic hydrops have also been reported.[13,14]

We observed a very high prevalence of vestibular involvement (90% of patients). In support of our findings, Nakada *et al*.[15] and Kato *et al*.[16] also reported that the vestibule was more commonly affected by endolymphatic hydrops at MRI than the cochlea. MRI also permitted to grade vestibular endolymphatic hydrops as in our study it was mild in 39% of cases and severe in 61%. However, this finding needs to be correlated with objective functional tests and clinical course.

The cochlea was involved in only ten patients of our series (50%). This finding is even more reliable after considering that false positives for endolymphatic hydrops in the cochlea are more frequent than in the vestibule.

For instance, in Nakada *et al*. series[15] MRI revealed endolymphatic hydrops in the cochlea of normal controls and not in the vestibule. Moreover, endolymphatic hydrops is frequently observed in the upper turn of the cochlea of individuals without ear diseases at autopsy[17] and it may depend on the spontaneous variability of EH in the normal population.[18]

Semicircular canals involvement was not frequent but when present it is easy to detect at MRI (Fig. 2).

Limitations of the study were: no normal subjects as controls, no bilateral gadolinium injection and consequently no intra-patient controls, no images summation or subtraction algorithms, no correlations of MRI findings with audio-vestibular functional tests. Four patients were excluded from analysis because of poor perilymph CE: reduced permeability of the blood-perilymph barrier by diseases not emerged before could be a possible cause. We chose the trans-tympanic injection of contrast media (even if intravenous[19] or trans-Eustachian tube[20] administration is possible) because is simpler, gives higher gadolinium perilymph concentration, and is less frequently followed by complications. Moreover, in few patients the vestibular function was even improved after injection; if confirmed, it could be the effect of changes in the middle ear pressure exerting micro-pressures over the oval window.

5. Conclusion

MRI shows high sensitivity in detection of endolymphatic hydrops. The vestibule seems to be more affected than the cochlea and semicircular canals. MRI grading of vestibular hydrops is feasible but still needs to be correlated with audio-vestibular functional tests and clinical course.

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UNDERSTANDING MÉNIÈRE'S DISEASE (MD): THE CONTRIBUTION OF ENDOLYMPHATIC HYDROPS IMAGING (EHI)

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1. Introduction

Since Nakashima's landmark report in 2007,[1] MRI imaging of endolymphatic hydrops (EHI) has led to a reappraisal of the significance of hydrops in cochlear pathology and Ménière's Disease (MD). Here we review the reliability of EHI in predicting MD and the correlation between EHI and audiovestibular function tests.

2. Reliability of EHI

In definite MD, hydrops is present on EHI in 90 to 100% of cases.[1-4] However, hydrops may be present on imaging without the classical picture of MD. It has been postulated that in the 10% of cases of MD where hydrops is not present on EHI that the disease may be in an early stage. Histopathologically, hydrops has been also demonstrated in 5-25% of patients who did not have MD during their lifetime.

3. Relations between degree and site of EHI and functional tests

3.1. Audiometry

In most studies, there is a significant positive correlation between hearing loss and cochlear EHI.[5-7] This association has been seen in both 2D and 3D volumetric analysis and more significantly in the latter.[6,7] (Sepahdari $\rho = 0.89$; $p = 0.0003$; Gurkov $r = 0.747$, $p = 0.001$).

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Wu[8] found that PTA thresholds in the affected ear at low ($\rho = 0.479$, $p = 0.000$) and middle ($\rho = 0.422$, $p = 0.001$) frequencies were significantly correlated with the extent of EHI in all turns of the cochlea, especially the apical turn. Some studies have also revealed that patients with a longer duration of disease have a greater extent of hydrops on EHI.[9-12]

3.2. Sacculus

The cVEMP is reduced or absent on the affected side in 35-54% of MD.[13-15] Several studies show a relationship between a reduced cVEMP and hydrops on EHI, for example Fiorino[3] who found that hydrops on imaging correlated with a reduced cVEMP ($r = 0.673$, $p = 0.003$). It appears that severe hydrops on EHI is most frequently observed in the sacculus.[16]

3.3. Electrocochleography (ECOG)

ECOG with click stimuli and either transtympanic or extratympanic recording has shown no correlation between an elevated SP/AP ratio and hydrops on EHI.[3,5,17,18] Hornibrook[11] found an elevated SP/AP ratio in 87% of definite MD patients, using transtympanic ECOG with tone-burst stimuli. In his study, on the other hand, EHI revealed hydrops in 47% of cases. The low detection rate of hydrops may in part be attributed to the MRI acquisition sequence used in this study.

3.4. Caloric test

No positive correlation has been found between canal paresis (CP) and hydrops on EHI in the vestibule or the horizontal semicircular canal (hSCC) ampulla.[3,19,20] However, Gurkov[21] has found that hydrops on EHI extending from the vestibulum into the posterior non-ampullated crus of the hSCC was associated with CP.

4. Conclusions

- There is a significant positive correlation between hearing loss and cochlear hydrops.
- There is a correlation between saccules function and vestibular hydrops.
- A longer duration of disease is associated with a higher degree of hydrops.
- Tests of audiovestibular function and EHI are complementary to each other in the diagnosis of MD.

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THE RELATIONSHIP BETWEEN PERILYMPH AND CEREBROSPINAL FLUIDS IN MÉNIÈRE'S DISEASE: NEW FINDINGS IN MRI AFTER INTRATYMPANIC ADMINISTRATION OF GADOLINIUM

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It is well-established that the perilymph (PE) and endolymph (EN) are essential for the hair cells function, while the training sites, the forming mechanisms and the relationship between these liquids with cerebrospinal fluid (CSF) are still not completely understood.

The PE is thought to be derived from both CSF and the vascular supply[1] but its composition is not uniform in the cochlea and differs from the scala vestibuli to the scala tympani.[2] The observations about the distribution of mannitol, sucrose and proteins, and others studies on the spread of radioactive tracers,[3-5] suggest that the origins of the vestibular and the tympanic PE are independent. Kinetic studies have highlighted that the vestibular PE is the main source of EN production[6] and would be formed from the plasma through a hemato-perilymphatic barrier and epithelial secretion.[7]

The origin of the tympanic PE, however, is discussed because the scala tympani communicates with both the scala vestibuli by the helicotrema, and with CSF by the cochlear aqueduct[8] (CA), and is usually considered a transition liquid between the vestibular PE and CSF. The CA function is not yet fully elucidated, but it is thought to play a role in maintaining fluid and pressure balance between the inner ear and the CSF.[9] For sudden variations of CSF pressure it behaves as a low-pass filter with a time constant of about 2 s[10] but in humans it is almost always virtual[11] and its closure is an age-related physiological event.[12]

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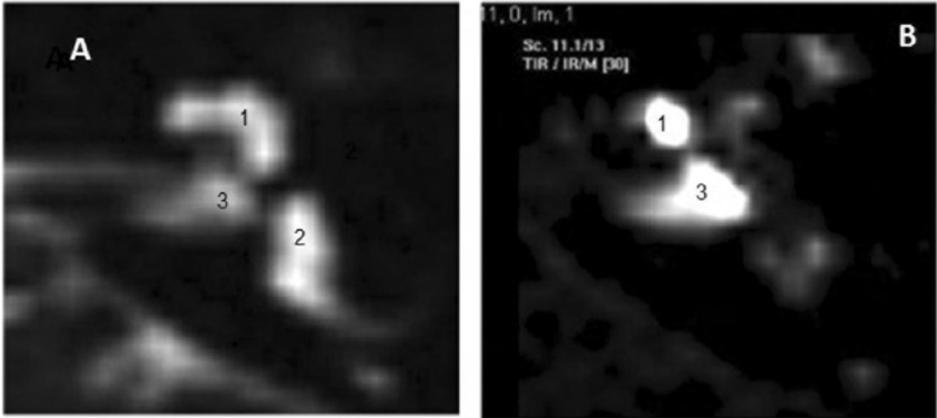
Fig. 1. RMI A: before (A) and after 24 hours after intratympanic GD administration. 1: Cochlea; 2: Vestibular tract; 3: Internal auditory canal. In A, all fluids (PE, EN, CSF) are visible. In B, the perilymph alone is visible, the escape of perilymph in the internal auditory canal.

The anatomical complex of CA and round window membrane (RW) seems to be significant for fluid flow resistance related to the position of the round window membrane.[13]

The CSF from CA proceeds toward the tympanic scale and it is driven with a pulse depending on respiratory oscillations that allow to rapid removal of the substances from the basal turn of the scala tympani.[14] In reality all we know about the physiology of CA has been observed in rodents and not in humans, so our certainties are actually just hypotheses.

Very schematically all we know of the relationship between EN, PE and CSF is that the PE, produced from plasma, is removed by filtration producing EN and through the cochlear duct that drains the PE towards the intracranial space. The EN, however, is removed by the endolymphatic sac. In other words, the circulation of PE would depend on the cochlear duct while the EN depends on endolymphatic sac. With intratympanic injection of gadolinium, used to visualize the endolymphatic hydrops of Ménière's disease (MD), many authors[15,16] showed that CSF in the internal auditory canal (IAC) was enhanced while in the CA or the singular canal this enhancement was absent.[16,17] We have observed with RMI 23 patients, affected by MD, before and 24 hours after intratympanic injection of gadolinium. In all patients the CA was absent in RMI and the gadolinium spread into the IAC (Fig. 1). These findings highlight a new way of PE diffusion into CSF and raise many questions about the relationship between CSF and inner ear fluids in patients with MD. The escape of the gadolinium into the patient with MD could be considered as a labyrinthine fistula, sensitive to changes in pressure of the CSF? Literature showed that the variations of pressure of CSF can produce vestibular disorders. The vestibular aqueduct, if enlarged, can generate traumatic hearing loss through an increase of intracranial pressure.[18] The link between PE and CSF can produce gusher during stapedotomy
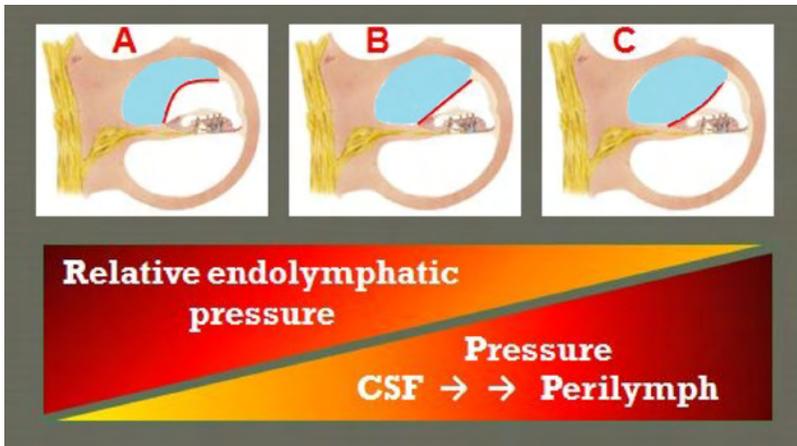
Fig. 2. Model of possible relationship between internal ear fluids and CSF pressure. **A**. When the CSF pressure decreases, the endolymphatic pressure increases relatively and can lead to MD. **B**. when the pressure of CSF is normal, the endolymph has a normal space. **C**. When the CSF pressure increases, the endolymphatic space decreases and can lead to compression of Corti's cells with Ménière-like symptoms.

or cochleostomy.[19] In the minor syndrome vertigo and oscillopsia are produced by changes of intracranial pressure and the natural plugging can cause MD-like symptoms.[20] Oto-emissions are modified after ventriculoperitoneal shunting due to internal ear depressurization.[21] A low pressure of CSF leads to low perilymphatic pressure with relative endolymphatic hydrops.[11,22].

Today we know that the CA in patients with MD is not useful to remove PE and the better way of diffusion is the IAC only. It is possible that variation of pressure in CSF into the cerebellopontine angle can be transmitted to PE which would lead to a high or low perilymphatic pressure.

The attention of the researches on the pathogenesis of MD was frequently focused on EN pressure fluctuation or its electrolytic variations and the PE was considered a fluid 'adaptable' to endolymphatic changes. The escape of GD in the IAC highlights a different pathophysiology, showing how in the MD the IAC is the main place of the perilymphatic dispersion, and confirms the existence of a continuous stream of GD from the round window to the IAC. In our opinion, the changes in pressure of the CSF by changing the perilymphatic pressure, and then the endolymphatic pressure through the Reissner membrane, could play a role in causing or maintaining idiopathic MD (Fig. 2).

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DEHISCENCE SYNDROME

MIGRAINE HEADACHE AND THE MIGRAINE VARIANTS OF HEMIPLEGIC MIGRAINE, OCULAR MIGRAINE AND VESTIBULAR MIGRAINE IN OTIC CAPSULE DEHISCENCE SYNDROME: OUTCOMES AFTER TARGETED REPAIR

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Abstract

Objective: Patients with peripheral vestibular dysfunction due to gravitational receptor asymmetries frequently experience migraine headaches, which resolve or improve after surgical intervention. This was tested with pre- and postoperatively quantitative measurements in three cohort groups with superior semicircular canal dehiscence syndrome (SSCDS) symptoms with: 1) superior canal dehiscence (SCD) repaired via a middle cranial fossa craniotomy and canal plugging only; 2) otic capsule defects not visualized with imaging (no-iOCD) repaired with round window reinforcement (RWR) only; or 3) both SCD plugging and subsequent development of no-iOCD followed by RWR.
Study design: Prospective patient series.
Setting: Tertiary referral center.
Patients: There were 13 adult and 4 pediatric patients with SSCDS (otic capsule dehiscence syndrome). Eight patients had no-iOCD and RWR exclusively, 5 had SCD and plugging exclusively, and 4 had both SCD plugging followed by

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development of no-iOCD with RWR. These cohorts included SSCDS with two different dehiscence locations.

Interventions: The Headache Impact Test (HIT-6) was completed to assess the impact of their disease on their migraine headaches pre- and postoperatively. The presence and outcome of treatment of the three migraine variants: vestibular migraine, ocular migraine and hemiplegic migraine were also studied.

Main outcome measures: Quantitative and statistical analysis of their cognitive and neurobehavioral function.

Results: There was a statistically significant improvement in the HIT-6 scores postoperatively in all groups. For those patients with vestibular migraine, ocular migraine and hemiplegic migraine, resolution of these variants was observed.

Conclusions: There was a marked overall improvement in migraine headaches and the migraine variants postoperatively. Greater longitudinal data and greater subject numbers are necessary to better understand the association of migraine with otic capsule dehiscence syndrome and resolution postoperatively.

1. Introduction

Wackym et al. reported a prospective cohort of 12 patients with long-term follow-up and with SSCDS, six with radiographic evidence of superior canal dehiscence (SCD) treated with a middle cranial fossa approach and plugging; and six with no imaging visible otic capsule dehiscence (no-iOCD) treated with round window reinforcement (RWR).[1] It has been suggested that the term SSCDS be replaced with otic capsule dehiscence syndrome (OCDS) because the same SSCDS symptoms and diagnostic findings can occur with lateral and posterior semicircular canal dehiscence, internal carotid artery-cochlea dehiscence, posterior semicircular canal-jugular bulb dehiscence, posttraumatic hypermobile stapes footplate (personal communication, Dr. Arun Gadre, August 1, 2015) and in patients with no-iOCD.[1-8] By definition, all of these entities represent perilymphatic fistulae at different sites of the otic capsule.

More recently, we published a study focused on the longitudinal cognitive and neurobehavioral outcomes after surgically managing this same cohort of 17 patients.[2] This study used a battery of neuropsychological tests to provide the first quantitative characterization of the preoperative and postoperative cognitive function changes in patients undergoing surgical management of their OCDS. These neuropsychological tests showed distinctive patterns of improvement or delays in improvement that provided greater insight into the nature of the cognitive dysfunction these patients experience and suggest that additional interventions may maximize and/or accelerate their cognitive recovery. Video

recordings of consenting patients before and after intervention help to further document these obvious alterations in ways that complement standardized neuropsychology testing.[3,9-16]

In this report we focus on the migraine prevalence, character and outcomes of surgical management of OCDS resulting from 2 different types of otic capsule dehiscence in this same patient cohort.[2]

2. Methods

2.1. Subjects

The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration. Our Institutional Review Board approved these studies.

Seventeen, healthy subjects with SSCDS/OCDS who had SCD, no-iOCD or both agreed to participate in and completed the study (Table 1). There were 13 adults and 4 children. The entire cohort had a mean age of 39.5 years (range 12.99-60.29 years) at first surgery, with 5 males and 12 females. Group 1 (no-iOCD only) (n=8) had a mean age at first surgery of 39.4 years (range 12.99-60.29 years), with 2 males and 6 females. Group 2 (both SCD and subsequent no-iOCD) (n=4) had a mean age at first surgery of 43.42 years (range 33.46-53.79 years), with 1 male and 3 females. It should be noted that in Group 2 (SCD and no-iOCD) all RWR operations occurred subsequent to SCD plugging of a radiographically identified SCD. Group 3 (SCD only) (n=5) had a mean age at first surgery of 36.36 years (range 14.48-56.30 years), with 2 males and 3 females. On 1 October 2015, the entire cohort had a mean age of 42.08 years (range 15.32-63.17 years). Group 1 (no-iOCD only) had a mean age on 1 October 2015 of 42.15 years (range 15.32-63.17 years, n=8). Group 2 (SCD and subsequent no-iOCD) had a mean age on 1 October 2015 of 46.61 years (range 37.43-57.60 years, n=4). Group 3 (SCD only) had a mean age on 1 October 2015 of 38.36 years (range 16.13-59.22 years, n=5). The patient demographics, clinical features at referral, preoperative migraine characteristics, history of trauma and surgical procedures performed for each subject are summarized in Table 1.

The SCD patients were all surgically treated with superior semicircular canal plugging via the middle cranial fossa and all no-iOCD and SCD patients already treated with plugging who later developed a no-iOCD were treated with RWR. The detailed methods have been published previously.[2]

2.2. Headache Impact Test

All 17 patients were also asked to complete the Headache Impact Test (HIT-6). The patients were studied retrospectively on one occasion following their surgical procedures. They were contacted by email and sent two copies of the HIT-6,

with standard instructions on how to complete it; patients were assured of their privacy and of data confidentiality in the study. They were asked to recall and complete one copy, to the best of their abilities, as they would have responded before they underwent the surgery for their otic capsule dehiscence(s). A second copy was to be completed to reflect their status at the present time. They were instructed to return the questionnaire electronically or via facsimile. For the three groups, 6 (75%) no-iOCD patients, 4 both SCD and no-iOCD patients, and 4 (80%) SCD only patients returned their HIT-6 questionnaires. The HIT-6 questionnaires were scored by a neutral observer who was not involved in patient care, who used the scoring system validated for this instrument ("Never" = 6 points; "Rarely" = 9 points; "Sometimes" = 10 points; "Very often" = 11 points; "Always" = 13 points).[17,18] The final HIT-6 score was obtained from simple summation of the six items and ranges between 36 and 78, with larger scores reflecting greater impact. Headache impact severity level was categorized using score ranges based on the HIT-6 interpretation guide.[17,18] The four headache impact severity categories are little or no impact (49 or less, [Class I]), some impact (50–55, [Class II]), substantial impact (56–59, [Class III]), and severe impact (60–78, [Class IV]). The pre- and post-treatment scores were examined with standard descriptive statistics (mean, SD, range). When comparisons between the pre- and post-treatment scores were made, the data were analyzed using repeated-measures analysis of variance and least significant differences tests for paired comparisons, establishing 0.05 as the criterion level of significance.

3. Results

While not the focus of the present study, once each patient completed their final surgical procedure and medical management resolved any of the factors complicating their postoperative recovery, their presenting symptoms and signs were returned near their baseline before developing SSCDS/OCDS.[2]

Migraine headache was present in 88% (7/8) of subjects with no-iOCD only, 100% (4/4) of subjects with SCD and subsequent no-iOCD, and 80% (4/5) of subjects with SCD only (Table 1). Interestingly for these patients the migraine headaches by clinical report resolved in all patients,[3,9,10] including those with vestibular migraine, ocular migraine and hemiplegic migraine (Table 1); however, the HIT-6 data revealed that there was a highly statistically significant improvement pre- versus postoperatively ($p<0.001$) overall and between groups (Fig. 1), yet there were 2 patients who quantitatively became Class II and one patient remained a Class IV. The remaining 11 patients became Class I. For the no-iOCD patients, the mean HIT-6 score was 74 (range 68-78 [all Class IV], SD±4 preoperatively and 45.7 (range 42-49 [all Class I], SD±3.14) postoperatively. This improvement was statistically significant ($p<0.001$). For the both SCD and subsequent no-iOCD patients, the mean HIT-6 score was 69.3 (range 57-78 [1 Class III, 3 Class IV], SD±9.7 preoperatively and 46.8 (range 36-53 [2 Class II

and 2 Class I], SD±8.10) postoperatively. This improvement was statistically significant (p<0.001). For the SCD only patients, the mean HIT-6 score was 69.8 (range 61-76 [all Class IV], SD±6.34 preoperatively and 44.5 (range 36-61 [1 Class IV and 3 Class I], SD±11.27) postoperatively. This improvement was statistically significant (p<0.001).

As shown in Table 1, 15 of 17 patients (88.2%) were diagnosed with migraine and/or migraine variants and managed medically using drugs to prevent migraine from occurring (e.g., topamax, zonegran, verapamil, or tricyclic antidepressants) before undertaking surgical intervention. For the no-iOCD patients (n=7), the mean duration of treatment was 11.3 months preoperatively (range 2-19 month, SD±6.8 months). For the both SCD and subsequent no-iOCD patients (n=4), the mean duration of treatment was 19.8 months (range 4-62 months, SD ± 28.2 months). For the SCD only patients (n=4), the mean duration of treatment was 36.5 months (range 14-60 months, SD±21.2 months). All of the migraine variants resolved postoperatively.

4. Discussion

The focus of this study was to detail the prevalence, character and improvement in migraine headache and the migraine variants of hemiplegic migraine, ocular migraine and vestibular migraine after intervention in a cohort of 17 patients in more detail than we have previously.[2]

4.1. Migraine headache

Vestibular migraine (VM), also termed migraine-associated dizziness, has become recognized as a distinct clinical entity that accounts for a high proportion of patients with vestibular symptoms (for review see Furman et al.). A temporal overlap between vestibular symptoms, such as vertigo and head-movement intolerance, and migraine symptoms, such as headache, photophobia, and phonophobia, is a requisite diagnostic criterion. Physical examination and laboratory testing are usually normal in VM but can be used to rule out other vestibular disorders with overlapping symptoms such as with OCDS no-iOCD or SCD. Vestibular migraine patients do not have sound-induced dizziness and nausea or autophony; however, when these patients have endolymphatic hydrops, they can have sound sensitivity that borders on a Tullio phenomenon. For this reason, when a high-resolution temporal bone CT with color 3D volume rendering shows no evidence of SCD, all patients suspected as having no-iOCD should be treated as a VM patient, as were the patients in this study cohort, since medical management, if successful, avoids unnecessary surgery.[1,2]

Vestibular migraine is an example of the integral overlap between vestibular pathways and migraine circuit triggers and central mechanisms for premonitory symptom generation. Information transmitted by peripheral vestibular sensory

Table 1. Preoperative patient demographics, migraine characteristics, trauma history and surgical procedures performed.

Patient	Sex	Age at first surgery	Current age*	Diagnosis at initial referral	Preoperative migraine character	Trauma history	Surgery 1	Surgery 2	Surgery 3
Group 1. Patients with otic capsule dehiscence syndrome and no imaging visible otic capsule dehiscence only									
1**	M	12.99	15.32	TBI, migraine	24/7, light sensitivity	Snowboarding accident, LOC	R RWR	L RWR	
2**	F	17.19	19.36	Conversion disorder, migraine	24/7, severe	Concussion after falling down stairs, later influenza and vomiting	R RWR		
3	F	35.32	37.81	Migraine	24/7, light sensitive, vestibular migraine with episodic rotational vertigo	No	R RWR		
4	F	43.03	45.93	Migraine, ELH	Frequent, light sensitivity	No	L RWR		
5**	F	46.82	49.46	Migraine, hemiplegic migraine	24/7, light sensitive, left hemiplegic migraine, ocular migraine, rare vestibular migraine	Not before 1st surgery, recurrence after intractable vomiting and appendicitis	L RWR	L RWR	
6	F	49.29	53.10	Migraine, ELH	Frequent	Airplane flight descent	R RWR		
7	F	50.32	53.01	Migraine, MD	Frequent, light sensitive	No	R RWR		
8	M	60.29	63.17	Autophony	No	No	L RWR		

Patient	Sex	Age at first surgery	Current age*	Diagnosis at initial referral	Preoperative migraine character	Trauma history	Surgery 1	Surgery 2	Surgery 3
Group 2. Patients with otic capsule dehiscence syndrome and having superior canal dehiscence and subsequently another otic capsule dehiscence not visualized with imaging									
9	F	33.46	37.43	Migraine	Migraine	No	R SCD	R RWR	
10	F	35.47	37.95	Migraine, fall while rock climbing	Migraine, ocular migraine	Rock climbing fall	R SCD	L SCD	L RWR
11	M	50.94	53.45	SCD, migraine	Daily 'sinus HA' with normal CT	No	R SCD	R RWR	
12	F	53.79	57.60	MVA, concussion, migraine	Daily migraine	MVA, concussion	R SCD	R RWR	
Group 3. Patients with otic capsule dehiscence syndrome and having superior canal dehiscence only									
13	M	14.48	16.13	Migraine, concussion	Daily migraine, light sensitivity	Multiple concussions	R SCD		
14	F	16.38	17.78	Migraine	24/7, light sensitivity	No	R SCD		
15	F	39.98	41.88	Migraine	Daily migraine, light sensitivity	No	R SCD		
16	M	54.66	56.90	Otolithic crisis of Tumarkin, ELH	No	Sudden hearing loss with rotational vertigo, left	L SCD		
17	F	56.30	59.22	SCD, migraine	Daily migraine, light sensitivity	Symptomatic with flying and driving over mountains	L SCD	R SCD	

*As of 1 October 2015 (age in years); **See video links in references 3,9,10; 24/7 = migraine headache present constantly, 24 hours per day and 7 days per week; ELH = endolymphatic hydrops; LOC = loss of consciousness; MVA = motor vehicle accident; RWR = round window reinforcement; SCD = superior canal dehiscence; TBI = traumatic brain injury.

Fig. 1. Headache Impact Test (HIT-6). The HIT-6 data revealed that there was a highly statistically significant improvement pre- versus postoperatively (p < 0.001) overall and between groups, yet there were two patients who quantitatively became Class II and one patient remained a Class IV postoperatively. The remaining 11 patients became Class I. For the no-iOCD patients, the mean HIT-6 score was 74 (range 68-78 [all Class IV], SD ± 4 preoperatively and 45.7 (range 42-49 [all Class I], SD ± 3.14) postoperatively. This improvement was statistically significant (p < 0.001). For the both SCD and subsequent no-iOCD patients, the mean HIT-6 score was 69.3 (range 57-78 [1 Class III, 3 Class IV], SD ± 9.7 preoperatively and 46.8 (range 36-53 [2 Class II and 2 Class I], SD ± 8.10) postoperatively. This improvement was statistically significant (p < 0.001). For the SCD only patients, the mean HIT-6 score was 69.8 (range 61-76 [all Class IV], SD ± 6.34 preoperatively and 44.5 (range 36-61 [1 Class IV and 3 Class I], SD ± 11.27) postoperatively. This improvement was statistically significant (p < 0.001). Both = SCD plugging, subsequent development of no-iOCD managed with RWR; no-iOCD = no imaging visible otic capsule dehiscence only managed with RWR; SCD = superior semicircular canal dehiscence only managed with middle cranial fossa approach and plugging. (Copyright © Ear and Skull Base Center, used with permission.)

organs and the vestibular nerve to the medulla and pons is an external trigger within the migraine circuit construct proposed by Ho and coworkers.[20] This model is based upon the distribution of the neuropeptide CGRP, which has a complex distribution within the vestibular periphery.[21] Migraine headache is nearly always present in patients with gravitational receptor dysfunction type of vertigo caused by no-iOCD or SCD, but infrequently with rotational receptor dysfunction type of true rotational vertigo.[1-3,13-16] This is an important concept as no-iOCD and SCD can induce migraine and the three variants of migraine – hemiplegic migraine, ocular migraine and vestibular migraine.[1-3] As shown in Table 1, 33% (2/6) patients in each cohort had migraine variants; two no-iOCD patients had intermittent ocular migraines and one SCD had intermittent ocular migraines while the other had intermittent vestibular migraines. The latter explains why some patients with no-iOCD or SCD, who normally only have

gravitational receptor dysfunction type of vertigo (disequilibrium) can have episodes of vestibular migraine and infrequent true rotational vertigo attacks. It should also be noted that the character of the migraine headaches was different between our two cohorts. The migraine headaches were characterized as "24/7" with exacerbation of the intensity of the headache in the no-iOCD group. These patients also had a greater degree of light sensitivity with many of the patients wearing sunglasses much of their waking day and physicians finding the room lights off when entering the examination room. In this series, as is the case in clinical practice, surgical management of no-iOCD and/or SCD resolves the migraine headaches; however, sometimes there is only a marked decrease of the frequency and intensity of the migraines, as migraine has a high incidence overall.[1-3,9-11,13-15,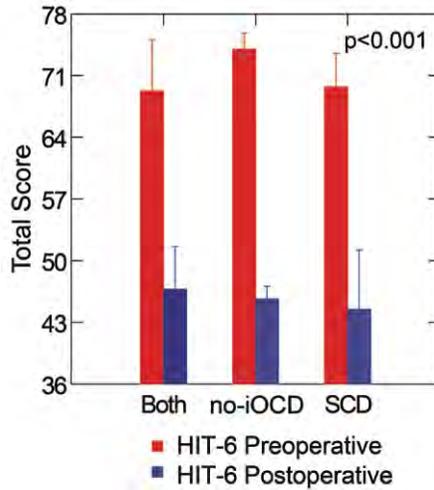19] The HIT-6 data revealed that there was a highly statistically significant improvement pre- versus postoperatively (p<0.001) overall and between groups (Fig. 1), yet there were 2 patients who quantitatively became Class II and one patient remained a Class IV. The remaining 11 patients became Class I.

5. Conclusion

These data demonstrate that migraine headaches and the migraine variants of hemiplegic migraine, ocular migraine and vestibular migraine in patients with otic capsule dehiscence resulting in OCDS, regardless of etiology, exist, can be measured and that improvements in these migraines can be accomplished with appropriate, targeted, vestibular surgery.

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SUPERIOR CANAL DEHISCENCE AND 'NEAR-DEHISCENCE' SYNDROME: CLINICAL AND INSTRUMENTAL ASPECTS

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Abstract

Even though superior canal dehiscence syndrome (SCDS) has already been widely studied from a clinical, etiopathogenetic and therapeutic point of view, some diagnostic aspects have yet to be clarified. It is well-known that high-resolution CT (HRCT) scan tends to overestimate the prevalence of the bony defect requiring the detection of lowered thresholds of air-conducted (AC) VEMPs to confirm the diagnostic suspect. The most recent definition of the so-called near-dehiscence syndrome (NDS), in which an extremely thinned bony roof of the superior canal results in the onset of a symptomatological scenario overlapping SCDS, has allowed to explain most cases of incongruence between imaging analyses and electrophysiological data. Moreover, no univocal explanation to the wide symptomatological and semeiological variability of SCDS has been offered yet. The aim of this paper is to face the diagnostic dilemma offered by the complexity of this two-fold syndrome (SCDS vs NDS) reviewing the clinical and instrumental data and submitting to statistical analyses a subsample of 100 patients (193 ears) selected from a group of 242 patients (114 M, 128 F, mean age 56.8 y, range 8-88 y) showing a dehiscence or a an extreme thinning of the superior canal at least from one side at HRCT scans. Firstly, we verified the effectiveness and the diagnostic accuracy of imaging in confirming electrophysiological data, considering the threshold lowering of AC cervical VEMPs as the gold standard in diagnosing an increased inner-ear admittance due to SCD and we offered physiopathogenetic explanation for those cases of

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incongruence between results. Secondly, we sought among instrumental set (AC and BC cervical and ocular VEMPs, video head impulse test) the test allowing the best diagnostic criteria for 'near dehiscence' and the parameter correlating most significantly with the size of dehiscence in case of SCDS. While mostly all data collected can reliably differentiate SCDS from NDS, from this study emerges that ocular VEMPs represent an effective method to detect the 'near-dehiscent' condition among the normal cases.

1. Introduction

Superior canal dehiscence (SCD) was first described in 1998 as an independent clinical disorder.[1] It is considered a possible explanation for sound- (Tullio phenomenon) and pressure-induced vertigo (Hennebert sign) and for hyperacusis to bone-conducted sounds.[1,2] This alteration, *i.e.*, a bony dehiscence overlying the superior semicircular canal (SSC), acts as a 'third mobile window' on the inner ear similarly to other labyrinthine capsule abnormalities inducing comparable clinical-instrumental signs to SCD.[3] Therefore, mechanical stimuli activating cochlear compartment are able to induce endolymphatic flows even inside the vestibule, causing the onset of peculiar symptoms.[3] The typical clinical picture, characterized by pulsatile tinnitus, hyperacusis, sounds and/or strains-induced nystagmus, mild-low frequency air-bone gap (ABG) in spite of normal middle ear function and abnormal vestibular evoked myogenic potentials (VEMPs) both to air and bone conducted sounds, depends on a sort of 'open-system hypersensibility' of the inner ear caused by a reduction of intralabyrinthine impedance induced by the bony defect.[3] It is possible to observe exclusively vestibular symptoms, only cochlear symptoms, or a combination of the both of them.[2,4,5] An analysis of the literature reveals a certain clinical polymorphism. Hypothesis and evidences attributing to SCD the onset of a wider clinical scenario including the so-called atypical manifestations of the syndrome have recently emerged.[6-8] This aspect undoubtedly represents a fascinating challenge for dizzy patients' management and it opens new perspectives in the understanding of physiopathogenetic mechanisms and in the diagnostic work-up of this alteration. Even though more than fifteen years have been passed since the first description of SCDS, it still presents several 'dark sides' offering new sparks for interesting interpretations, especially concerning some physiopathogenetic and diagnostic aspects. Firstly, although there is a remarkable number of studies of clinical-morphological correlations attributing precise clinical pattern to particular anatomic features of the dehiscent SSC,[9-11] no unanimous explanations for the SCD-induced symptomatological variability have been offered yet. Secondly, the role of high-resolution CT (HRCT) of temporal bones as gold standard for SCD diagnosis has already been re-dimensioned. In fact, the conspicuous discrepancy between the prevalence of the dehiscence among temporal bones specimens[12-13] and its radiological appearence[14-17] suggests a tendency to overestimate

the bony defect when using CT scans alone as diagnostic criteria. Moreover, a convincing evidence of a remarkable overlap of symptoms among patients with dehiscence and those with an extreme thinned bone overlying the SSC has been progressively coming up through the years. These aspects assume unavoidably importance for those patients requiring 'etiologic' therapeutic solutions due to disabling symptoms. Fortunately it has been widely demonstrated that, in addition to the presence of peculiar symptoms and findings, radiological evidence of dehiscent SSC should always be confirmed by electrophysiological data of threshold lowering of air-conducted (AC) vestibular evoked myogenic potentials (VEMPs).[3,18,19] Nevertheless, if the absence of abnormal potentials could explain the reason why some patient with radiologic evidence of dehiscence does not experience peculiar symptoms and signs (so-called false positive), it has already been initially hypothesized[20] and later demonstrated[21] that an extreme thinning of the roof of the SSC could lead patients to develop the same typical symptoms of SCDS (i.e. Near-Dehiscence Syndrome, NDS). Nowadays, the introduction of further electrophysiological criteria[22-25] and new techniques offering quantitative measurements of angular vestibulo-ocular reflexes (AVOR) of canals in high frequencies[26-27] has allowed a more exhaustive insight in the intralabyrinthine micromechanical modifications in case of SCD. The aim of this paper is to face the diagnostic dilemma offered by the complexity of this two-fold syndrome (SCDS vs NDS) by reviewing clinical-instrumental data of a considerable sample of patients diagnosed with a dehiscence or a an extreme thinned SSC at least from one side at HRCT scans. Considering the threshold lowering of AC-cervical VEMPs as gold standard in diagnosing an increased inner-ear admittance due to SCD, we verified the effectiveness and the diagnostic accuracy of imaging in confirming electrophysiological data, and we offered physiopathogenetic explanations for those cases of incongruence among results. Then, starting from radiological data, we sought among instrumental set (AC and BC cervical and ocular VEMPs and video head impulse test) the test allowing the best diagnostic criteria for 'near dehiscence' and the parameter correlating most significantly with the size of dehiscence in case of SCDS, by submitting to statistical analysis a significant sample of patients.

2. Methods

The study was conducted performing a retrospective review of clinical-instrumental data of a cohort of 242 patients (114 males, 128 females, mean age 56.8 years, range age 8-88 years) submitted to temporal bones HRCT scans competed by reformatted images along vertical canal axes at our institution. Radiological analysis showed a dehiscence or an extremely thinned bony roof of SSC at least from one side in each patient. All of them referred at our ENT Unit from January 2013 to April 2014 for different clinical issues. In 51 patients (21%) HRCT was performed as a diagnostic follow-up for recurrent BPPV attacks or

refractory to repositioning maneuvers or with atypical features (*i.e.*, positioning nystagmus without paroxysmal feature or due to suspected SSC involvement); 22 subjects (9%) developed a clinical picture consistent with Ménière's Disease or with Delayed Endolymphatic Hydrops. Sixteen patients (7%) affected by chronic middle ear infections required temporal bones imaging to verify ossicular integrity and exclude labyrinthine erosions, while a postoperative radiological assessment was suggested for nine patients (4%) who underwent ineffective stapedotomies (n 4) or uneventful middle ear exploratorations for suspected post-traumatic perilymphatic fistula (n 5). Imaging completed the diagnostic work up in six patients (2%) presenting with unilateral sudden hearing loss, in 36 patients (15%) with unilateral/bilateral progressive conducted or sensorineural hearing loss and in nine patients (4%) complaining of unilateral long-standing tinnitus. Finally, remainders 93 (38%) showed typical symptoms consistent with a 'third mobile window' mechanism as sound/pressure evoked vertigo, hyperacusis, autophony and pulsatile tinnitus. Only people with complete clinical data were considered in the final analysis. Those subjects who underwent radiologic examinations with low resolution techniques (X-ray beam collimation higher than 0.625 mm) or without suitable DICOM-files were excluded from the study in order to obtain a pool of temporal bones scans based on images uniformity. Similarly, people with middle ear pathologies and/or previous ear surgery were excluded, since middle ear integrity is required for electrophysiological testing. Moreover, three additional patients with concurrent small vestibular schwannoma in radiological follow-up were not admitted in the study because of possible interference in data collection due to the cranial nerve tumor.[28] Thus the final study group included 100 patients (193 ears), 49 males and 51 females (mean age 57 years, range 26-81 years). All of them underwent the same detailed otoneurologic assessment including pure-tone audiometry, impedance audiometry, air- (AC) and bone-conducted (BC) cervical (c) and ocular (o) VEMPs and high-frequency VOR study for all six semicircular canals with the video Head Impulse Test (vHIT).

2.1. CT scanning

All subjects of the study group underwent Multi Detector CT scans with a 16-channel (Lightspeed Multislice Scanner, General Electric Medical System) or 64-channel helical CT system (Lightspeed VCT LS Advantage 64 slices, General Electric Medical System) according to temporal bone protocol parameters: 120 peak kV, 225 mAs, 0.5 pitch, 1-s rotation time, 0.625-mm slice thickness, 0.625-mm X-ray beam collimation, 512×512 acquisition matrix, 16 cm acquisition field of view, bone reconstruction algorithm. An isotropic voxel measuring $0.6 \times 0.6 \times 0.6$ mm was thus obtained at this collimation. In pediatric patients radiation dose was reduced to 120 mAs for safety reasons. The images were acquired parallel to the orbito-meatal axial plane and reconstructed in the coronal plane. Min-IP reconstructions of the posterior (along Stenver plane) and superior semicircular canals (along Pöschl plane) for each temporal bone were also obtained. This way, the SSC could be seen as a ring, facilitating examin-
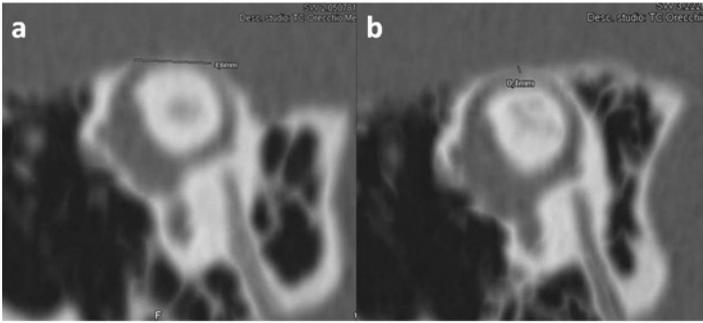
Fig. 1. Temporal bones HRCT scans with reformatted images along Pöschl plane showing a dehiscent (a) and a normal (b) SSC. **1.a.** In case of SCD, dehiscence size was measured. **1.b.** In case of intact SSC, thickness of the bony roof was measured.

ers' task in assessing the integrity of the bone overlying the canal. All scans were evaluated by the same team of expert radiologists from our institution with a special interest in head-and-neck imaging. All of them were blinded to the patient's clinical history. For each scan, radiologists were asked to specify if SSC roof was clearly dehiscent (SCD) or intact (not SCD). In cases of SCD, the size of the dehiscence was evaluated measuring the distance between bony remnants of the canal defect (Fig. 1a). Among not SCDs, in cases of bony roof thickness < 0.3 mm (*i.e.*, mean thickness of SSC roof – 1 standard deviation (SD) according to histopathological findings[12]), canal was considered thinned (thin). In cases of thickness > 0.3 mm, it was considered normally protected (norm) (Fig. 1b).

2.2. Audiometry

Pure-tone audiometry was performed over the frequency range of 125 to 8000 Hz for air conduction (AC) and 250 to 4000 Hz for bone conduction (BC) in a soundproof room using standard clinical procedures. Appropriate masking was used for BC testing and, when needed, for AC. Pure tone average (PTA) for AC was calculated across 500-4000 Hz, while mean BC threshold (BCT) was calculated across 500-2000 Hz. The average mild-low frequency ABG was derived by subtracting the BC threshold from the AC threshold for each individual frequency including 250, 500 and 1000 Hz; only ABG ≥ 20 dB was considered significant. Tympanometry and ipsi/contralateral acoustic reflexes were also tested. In case of type-A tympanogram, peak compliance value (C) was calculated for each ear.

2.3. Vestibular Evoked Myogenic Potentials Testing

Potentials were recorded using an Epic Plus evoked potentials system (Labat, Mestre, Italy) with a two-channels averaging capacity. All EMG potentials were

amplified and the signal was bandpass-filtered from 10 Hz to 1 kHz. Potentials were recorded with active surface electrodes placed symmetrically over the most prominent part of each sternocleidomastoid muscle (SCM), reference electrodes were placed on each clavicle and a ground electrode was applied over the upper sternum. During the data collection, as concerning AC cVEMPs, the patient was supine with his head turned on his non-stimulated ear side in order to get a tonic contraction of SCM ipsilateral to the stimulus presentation; as for BC cVEMPs (with stimulus at the forehead), tonic SCM activation on both sides was obtained by instructing the patient to raise his head constantly of about 30 cm from the supine position for at least 15 seconds. oVEMPs were recorded using a differential bipolar montage, with surface electrodes placed 1 cm beneath (positive) and 3 cm (negative) beneath each lower eyelid. A ground electrode was placed over the sternum. Patients were required to direct their gaze upwards for the duration of the recording in order to properly activate the inferior rectus and inferior oblique muscles of both eyes. Ongoing electromyographic (EMG) activity of tested muscles was visually monitored on an oscilloscope to ensure sufficient muscle contraction during the acquisition. As for both AC cVEMPs and oVEMPs, tone bursts (TB) were delivered unilaterally through a pair of insert earphones. Stimulus parameters were frequency 500 Hz, duration 8 ms (raise/fall 2 ms, plateau 4 ms), starting intensity 120 dB SPL and stimulation rate 5 Hz. The threshold was obtained by decreasing in steps of 10 dB or increasing in steps of 5 dB from 120 dB SPL, depending upon the presence or absence of potentials, respectively (maximal released intensity of 125 dB SPL). The lowest stimulus intensity at which a clear and repeatable biphasic wave was detected was considered as threshold.[18,23] As for BC cVEMPs and oVEMPs, 500 Hz TB (8 ms, 1.0 V, 0.6 A) stimuli were delivered to the midline (Fz) by a hand-held minishaker with an attached perspex rod (type 4810, Bruel and Kjaer P/L, Denmark). Vibratory stimulation was varied in intensity and amplification through a power amplifier (type 2718, Bruel and Kjaer). BC oVEMPs were also tested even after impulsive Fz stimuli using 0.5-ms clicks with positive polarity (intensity 0.7 V, 0.6 A).[22,29]

For each trial a total of at least 70 sweeps were averaged for a time analysis of 100 ms, and the responses were reproduced at least twice. As for cVEMPs, the first biphasic response (p13-n23) on the ipsilateral SCM to the stimulated side was analyzed (ipsilateral response) by calculating the peak-to-peak amplitude between p13 and n23 waves. The first negative (n1) and the first positive (p1) waves were analyzed for oVEMPs, calculating the peak-to-peak amplitude; in this case, responses recorded under the patient's right eye were interpreted as the activation of his left maculae and vice versa (crossed response). In case of no reliable response (VEMPs absent), for the purpose of statistical analysis, a threshold value corresponding to 130 dB SPL (*i.e.*, 5 dB higher than the maximal intensity delivered by the instrument) and an amplitude of 0.1 µV were assigned. Seven final values corresponding to as much electrophysiological parameters, namely 2 threshold values (AC cVEMPs and AC oVEMPs) and 5 amplitude

values (AC cVEMPs, BC cVEMPs, AC oVEMPs, BC oVEMPs to TB and BC oVEMPs to click), were obtained for each ear.

Since we aimed to compare radiological data with electrophysiological analysis of threshold of AC cVEMPs (considered as the diagnostic gold standard to detect an increased inner-ear admittance due to SCD[4,19]) to verify the effectiveness and the diagnostic accuracy of imaging in confirming a dehiscence of SCC, we first identify as control group those ears among 193 considered with a 'normal' SSC (*i.e.*, with a bony roof thickness > 0.3 mm) on imaging. We calculated the mean threshold value of AC cVEMPs ± DS (110.9 ± 7.2 dB SPL) among them and we considered as 'lowered' (*i.e.*, indicating increased inner-ear admittance) a threshold measuring the mean threshold value -2 DS (96.5 dB SPL). This value corresponds to the mean AC cVEMPs threshold value of a control group of normal subjects previously assessed in our laboratory. We also considered as 'indicator' of SSCD any repeatable response despite the presence of a significant low-frequency ABG (≥ 20 dB) on the tested ear.[4]

2.4. Video Head Impulse Test

All subjects from the study group were submitted to the assessment of the angular vestibule-ocular reflex (aVOR) gain for each semicircular canal with the video Head Impulse Test (vHIT). aVOR gain for horizontal and vertical canals in response to high-frequency head stimuli was tested by using an ICS video-oculographic system (GN Otometrics A/S, Denmark). Passive, unpredictable 5-20°, 50-250°/s and 750-5000°/s² head impulses were delivered manually on the plane of the horizontal and vertical canals while the patient was asked to keep looking at a earth-fixed target, as reported by MacDougall *et al.*[26,27] At least 15 stimuli were delivered for stimulating each canal and averaged to get the corresponding mean aVOR gain. Only data corresponding to SSC aVOR gains were considered in statistical analyses. SSC aVOR gain was considered as deficient in case of value < 0.66, *i.e.*, mean gain value of 'normal' SSC (0.87 ± 0.11 dB SPL) -2 DS (value similar to 0.68 as indicated for vertical canals in literature[27]).

2.5. Statistical analyses

All statistical analyses were performed using SPSS for Windows version 18.0. Student's T-test was used to compare the results of the instrumental tests (SSC aVOR gain, AC cVEMPs threshold and amplitude, AC oVEMPs threshold and amplitude, and amplitude of BC cVEMPs, BC oVEMPs to TB and BC oVEMPs to click) among groups of 'SCD', 'thin' and 'norm' ears, and between radiological 'false positive' (FP) and 'true negative' (TN). A value of $p < 0.05$ was used to determine if a correlation was statistically significant. Logistic regression models where the Pearson correlation coefficient $r > 0.5$ or < -0.5 defines a strong (significant) relationship were used for the correlation studies among

dehiscence size and clinical-electrophysiological data (PTA, BCT, ABG, C in addition to the abovementioned variables).

3. Results

An amount of 193 temporal bones CT scans (100 patients) with the corresponding qualitative radiologic assessment ('SCD', 'thin' or 'norm') were considered in this study. According to radiologists' opinion, 88 scans (45.6%) evidenced SCD, while 105 (54.4%) scans showed thick (83 'norm') or extremely thinned (22 'thin') intact canal roof. On the contrary, according to electrophysiological data, only 62 ears (32.1%) ears presented with pathologic lowering of AC c-VEMPs threshold or any potential despite a relevant ABG on audiometry evoking an 'open system hypersensitivity' due to SCD, while 131 ears (67,9%) showed normal or absent potentials. Considering electrophysiological criterion as the gold standard for the diagnosis of SCDS causing lowered inner ear impedance,[4,18] a total of 47 true positive (24.4%, TP), 41 false positive (21.2%, FP), 90 true negative (46.6%, TN) and 15 false negative (7.8%, FN) ears were finally derived. Results are shown in Table 1. Sensitivity, specificity, and positive and negative predictive values of 0.625 mm-collimated CT are shown in Table 2. All cases of mismatch between radiologists' assessment and electrophysiological data (FP and FN ears) corresponded to temporal bones with radiological evidence of dehiscent/thinned bone overlying the SSC. In other words, no case of FN showed a thick bone overlying SSC dome or additional causes of 'third mobile window' mechanisms.[3] Instrumental results (SSC aVOR gain, AC cVEMPs threshold and amplitude, AC oVEMPs threshold and amplitude, and amplitude of BC cVEMPs, BC oVEMPs to TB and BC oVEMPs to click) of FP ears (41)

Table 1.

		Radiologic assessment	
		SCD	not SCD
AC cVEMPs threshold	**lowered**	47	15
	normal	41	90

Table 2.

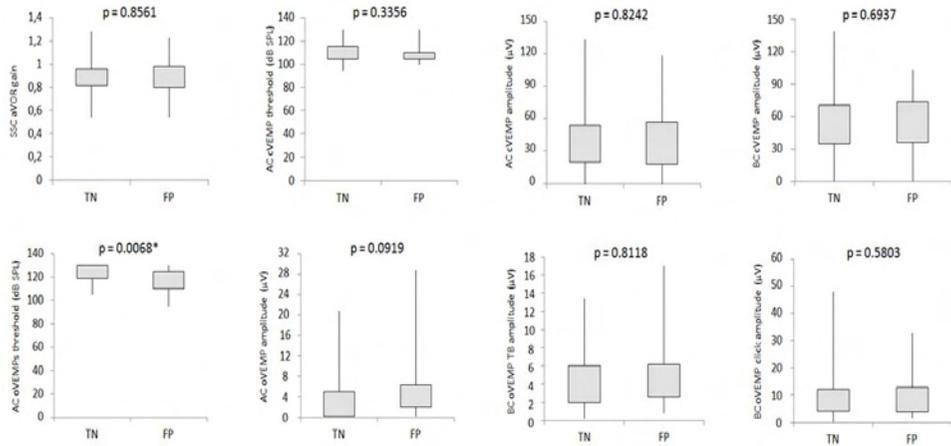| HRCT scans (0.625 mm collimation)	
Sensibility	47/62 (75.8%)
Specificity	90/131 (68.7%)
Positive predictive value	47/88 (53.4%)
Negative predictive value	90/105 (85.7%)

Fig. 2. Box plots showing comparison of instrumental data in true negative (TN) and false positive (FP) canals with corresponding level of significance (p). Significant differences were calculated using the student's T-test; * indicates level of significance (p) < 0.05.

were then collected and correlated to TN ears (90) data, aiming to seek statistically significant differences detecting any possible electrophysiological criterion separating apparently dehiscent (but actually thinned) canals (FP) to normal ones. Box plots showing the eight correlations performed with corresponding significance level (p) are represented in Figure 2. From this analyses it is possible to deduce that AC oVEMPs threshold could be likely able to separate FN form normal ears (TN) (p=0.0068). Then, dividing all 193 SSC in 'SCD' (88), 'thin' (22) and 'norm' (83) canals, according to the radiological assessment, we sought the most reliable instrumental criteria (among SSC aVOR gain, AC cVEMPs threshold and amplitude, AC oVEMPs threshold and amplitude, and amplitude of BC cVEMPs, BC oVEMPs to TB and BC oVEMPs to click) in separate these three different categories. Each correlation between 'SCD' and 'thin' canals, using any of the eight parameters considered except BC oVEMPs to click amplitude, showed statistical significance. Criteria better differentiating (p < 0.0000) these two categories seemed to be AC cervical and ocular VEMPs thresholds and both AC and BC (TB stimuli) oVEMPs amplitudes. Conversely, the only parameter separating 'thin' from 'norm' canals was BC oVEMPs to TB amplitude (p=0.0144) (Fig. 3). Then, 88 'SCD' ears as assessed by radiologists were submitted to a correlation analyses seeking instrumental criteria (among PTA, BCT, ABG, C, SSC aVOR gain, AC cVEMPs threshold and amplitude, AC oVEMPs threshold and amplitude, and amplitude of BC cVEMPs, BC oVEMPs to TB and BC oVEMPs to click) better correlating with dehiscence size. Scatter plots showing the 12 correlations are represented in Figure 4. From these analyses, a strong positive correlation exists between SCD size and both ABG and BC cVEMPs amplitude, while SSC aVOR gain and both AC cVEMPs and oVEMPs thresholds are correlated with dehiscence size with a strong negative relationship.
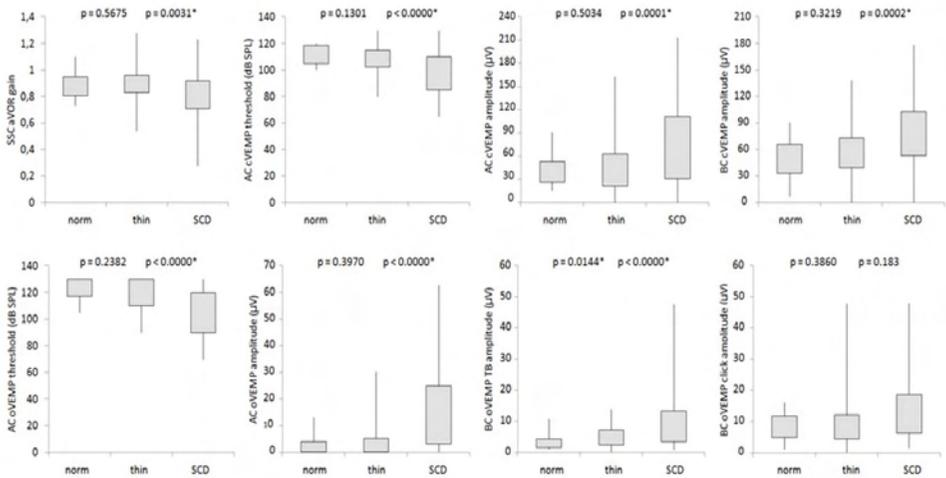
Fig. 3. Box plots showing comparison of instrumental data in normal (norm), thinned (thin) and dehiscent (SCD) canals with corresponding level of significance (p). Significant differences were calculated using the student's T-test; * indicates level of significance (p) < 0.05.

4. Discussion

In general, our data agree with literature where a tendency to overestimate dehiscence among CT scans emerges. Together with other previously published data,[14,15,17,30] our findings suggest that imaging alone cannot be considered as a gold standard test and that symptoms and instrumental signs must be carefully assessed before establishing a SCD diagnosis and recommending surgical treatment. In fact, we found 41 FP ears (21.2%) where positive radiological findings were not confirmed by electrophysiological data. Theoretically, there are several possible causes that could explain this finding:

(a) Technical problems related to HRCT system used, including low resolution algorithms,[15,31] voxel size and partial volume averaging,[14,17,32] field of view width and beam hardening artifact[17] could all impede to detect the presence of a thin bone < 0.1 mm over the canal. There may also be bias related to radiologists' learning curve in the correct interpretation of images for the presence of SSCD[31]. All these conditions represent the so-called 'real false positive'.

(b) Methodological problems associated with electrophysiological study, such as a not optimal SCM contraction, excessive ABG due to an associated conductive impediment (for example an association with otosclerosis or concomitant middle ear pathologies) or concurrent saccular and/or inferior vestibular nerve deficit. These factors provide for the so called 'apparent false positive'. In other words, ears in this category would have been classified as TP if the above-mentioned elements had not occur.

(c) Condition of 'natural plugging' where the dehiscence is wide enough to let dura occupy the entire canal lumen or canal bony remnants collapse within
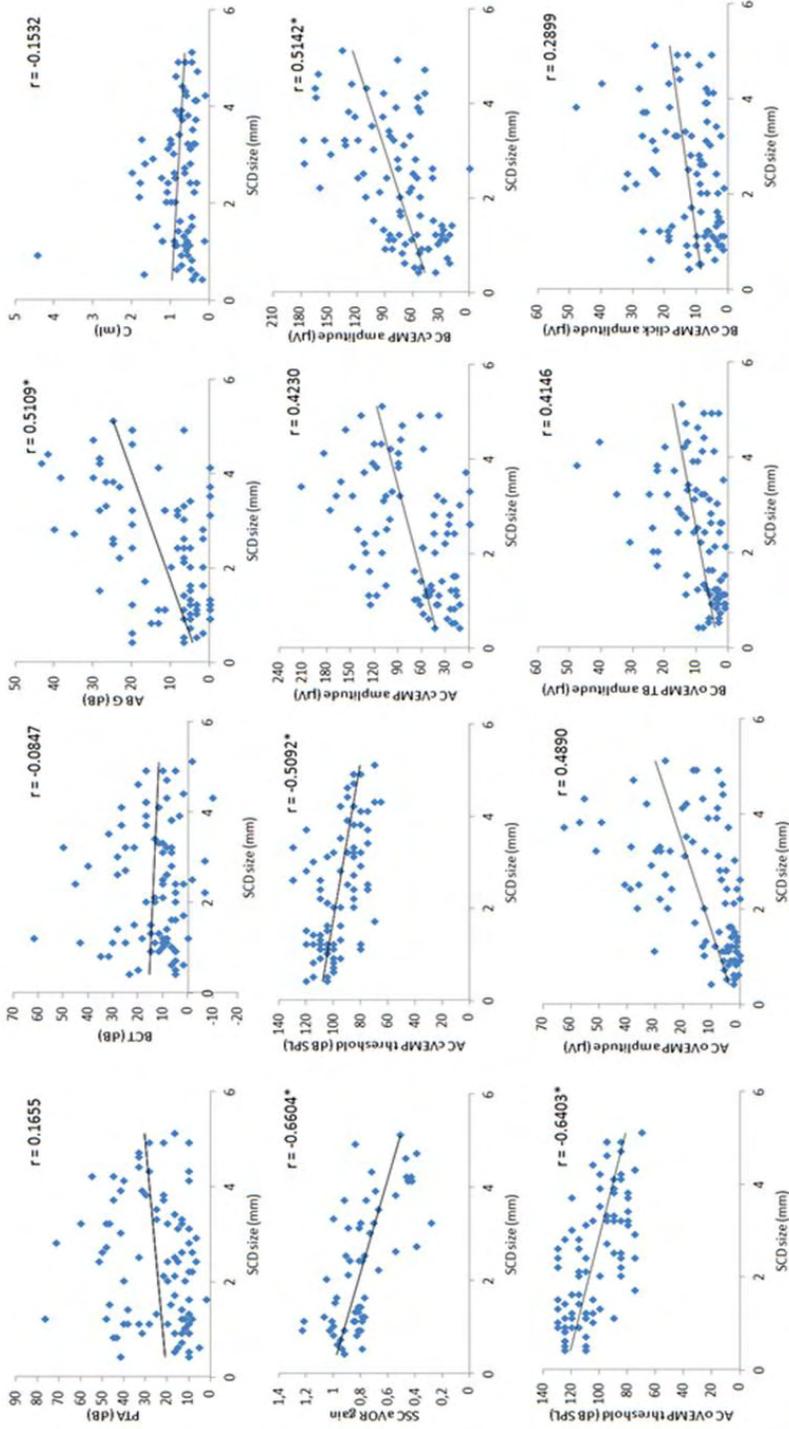
Fig. 4. Scattered plots showing correlations between SCD size and instrumental data with corresponding Pearson correlation coefficient (r). Logistic regression models were used; * indicates strong relationships (r > 0.5 or < -0.5).

the SSC resulting in a transient or permanent occlusion of the canal.[33] This condition does not allow electrophysiological testing to be diagnostic, resulting in normal or increased threshold of AC VEMPs. Theoretically, the application of quantitative measurements of SSC aVOR gain in high frequencies should detect the loss of canal functionality in this condition.[33] It is also possible that in case of a dehiscence due to a deep groove of the Superior Petrosal Sinus (SPS), this venous structure could represent a valid barrier opposing the onset of a decreased inner ear impedance. Even in this case, we are facing the so-called 'apparent false positive' as it actually represents a TP.

Nevertheless, similarly to another study,[6] we found also a significant amount of ears (n 15, 7.8%) with a thinned but apparently intact SSC in which electrophysiological signs of third mobile window occurred (FN). In these conditions we might be in front of technical bias related to HRCT scans accuracy. In such cases the syndrome may arise from sub-millimetric dehiscences or microfractures of thin bone which cannot be detected by conventional HRCT scans, or from rare cases of near-dehiscent bone where the bony roof is so compliant to reduce inner ear impedance. These cases remind to remark the importance of electrophysiology in otoneurologic practice, as SCD diagnosis would have been otherwise missed if VEMPs study had been neglected.

In summary we could face three different clinical scenario corresponding to as many anatomic pictures of dehiscence or extreme thinned SSC roof (Table 3). The first one, probably the most frequent situation in daily clinical practice, is represented by temporal bones with thinned bone overlying SSC appearing as thinned (TN) or dehiscent (FP) canal on CT scans. This condition may result in vague and subsided symptoms (especially cochlear as autophony), mainly mainly due to abnormal trasmission of pressure changes and endogenous sounds through the thin bone, despite unremarkable clinical tests and negative electrophysiology. This anatomic picture could thereafter prefigure an impairment of symptoms (such as vertigo spells, tinnitus and hyperacusis) and even detectable instrumental signs in particular conditions, for example after a head trauma or prolonged physical straining. It could be hypothesized that an extremely thin layer of bone over the canal could be warped by relevant changes of intracranial/intratympanic pressure resulting in perturbation of labyrinthine micromechanics and activation of canal receptors, likewise to what happens in case of dehiscence.[20] It could be possible that this thinned bone, even though frail and compliant, represents a barrier for the labyrinth, preventing from detection of electrophysiological changes in inner-ear impedance. This finding was also reported in four patients affected by NDS.[21] The second scenario is represented by true dehiscence (SCD), with its huge variance of classical symptoms and instrumental signs (such as lowering of AC VEMPs threshold, torsional evoked nystagmus, conductive hearing loss), interpreted on imaging as TP or FN. In these first two conditions SSC function should be perfectly preserved as symptomatology is caused by activation of ampullary receptors by endolymphatic flows. Finally, clinicians could face cases in which dehiscence is large enough to allow the middle fossa dura

Table 3.

	Thinning	SCD	Natural plugging
CT scans assessment	TN / FP	TP / FN	FN
AC cVEMPS threshold	normal	lowered	absent / normal
SSC aVOR gain	normal	normal	deficit

to prolapse into the canal lumen, partially or totally compressing the membranous SSC. Here endolymphatic flows are impeded resulting in inactivation of the canal reflexes and a hypofunctioning aVOR should be detected by vHIT. This condition is called 'natural plugging' as it can be definitely assimilated to a postoperative labyrinthine condition after surgical canal occlusion.[8,33,34] The canal plug may have occurred progressively and be stable, as a final step of a long standing process, or may be unstable, leading to recurrent vertigo spells whenever canal occlusion happens. The former situation should not result in relevant symptoms, mainly because of the slowness of its course, allowing the brain to activate compensating mechanisms. The latter condition could be responsible for recurrent vertigo spells variably associated with cochlear symptoms, resulting in Ménière-like clinical pictures as suggested in a previous study by our group for three patients affected by definite Ménière's disease.[8] In fact, a transitory hydropic state of the inner ear has been already proposed by other authors[35] for explaining the initial spontaneous irritative nystagmus detectable in the first postoperative hours in patients undergoing canal plugging. Once the dura has returned to the initial position, the labyrinthine perturbation recedes and the patient should perceive regression of cochleo-vestibular symptoms. Even if it is not yet possible to trace the natural history of the pathology, in our opinion these three clinical conditions could represent the natural evolution of an anatomically instable bony roof of the SSC (thinning → dehiscence → natural plugging).

Though correlation analyses showed that each instrumental test could help to differentiate dehiscent from intact SSC, only few of them represent useful distinguishing criteria for those 'shadow areas' represented by radiological FP and thinned SSC, *i.e.*, for those cases where AC cVEMPs does not detect signs of lowered inner ear impedance. In these circumstances the utricular macula, assessed by oVEMPs, seems to respond in a significantly different way from saccular receptors. In fact, we found significant difference in both AC oVEMPs thresholds and BC oVEMPs to TB amplitudes comparing normal canals with FP and thinned SSC, respectively. Moreover, a slight increase of AC oVEMPs amplitude has already been detected preoperatively in a group of patient affected by NDS.[21] This finding could represent the starting point for new insights into those still undefined aspects of this syndromic picture. As for correlation with dehiscence size, our study confirms some results already obtained by other authors. In fact, wide-sized dehiscence seems to result in a SSC aVOR gain re-

duction likely due to a plug effect made by dura.[33,34] Similarly, wide dehiscence seems to correlate with an increased dispersion of acoustic energy (evidenced by mild-low frequencies ABG)[10,36] and with reduction of intralabyrinthine impedance detected by AC cervical and ocular VEMPs threshold.[36] Lack of univocal results among different correlation studies performed so far probably reflects the difference among criteria used to select patients. In fact, in most studies morphologic data were based on imaging that, as already remarked, includes unavoidably bias itself.

5. Conclusions

In conclusion, besides threshold analysis of AC-cVEMPs, a multi-modal and multi-parametric study of vestibular receptors should be preferred, collecting all different electrophysiological criteria suggested in literature (Table 4). These different parameters, although representing reliable options for SCD diagnosis, should rather be considered as complementary criteria in the study of labyrinthine function, as they measure different types of vestibular receptors. In fact, it could be possible that a subsample of dehiscences responds preferentially to bone-conducted stimuli rather than air-conducted sounds, making an SCD diagnosis difficult if the complete battery of vestibular testing is neglected. These findings, in addition to all above-mentioned criticalities concerning imaging assessment, could help to explain the frequent incongruence between radiologic and clinical data, thereby justifying the symptomatological polymorphism related to SCD. Nevertheless, these gaps require further physiopathogenetic insights to better define the syndrome and clarify its puzzling aspects.

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Table 4.

Authors (reference)	Tests	No. of patients	SCD diagnosis	Stimuli	Criteria
Brantberg K *et al.* 1999 (18)	AC cVEMPs	3	radiological	Clicks and 500 Hz TB	threshold analysis
Streubel SO *et al.* 2001 (37)	AC cVEMPs	10	radiological	clicks	threshold analysis
Brantberg K *et al.* 2004 (22)	BC cVEMPs	4	radiological	500 Hz TB	amplitude
Rosengreen SM *et al.* 2008 (23)	AC oVEMPs	9	radiological	500 Hz TB	amplitude and threshold analysis
Brantberg K & Verrecchia L 2009 (38)	AC cVEMPs	20	radiological	90 dB Clicks	amplitude
Roditi RE *et al.* 2009 (39)	AC cVEMPs	67	radiological	250 Hz TB	threshold analysis
Manzari L *et al.* 2012 (29)	BC oVEMPs	26	radiological	500 Hz TB	amplitude
Taylor RL *et al.* 2012 (24)	AC c & oVEMPs	14	radiological	TB	tuning curve (amplitude)
Zhang *et al.* 2012 (40)	AC & BC oVEMPs	6	radiological	TB	tuning curve (amplitude)
Janky KL *et al.* 2013 (41)	AC oVEMPs	11	intraoperative	105 dB clicks & 500 Hz TB	amplitude
Zuniga MG *et al.* 2013 (42)	AC oVEMPs	29	intraoperative	105 dB clicks & 500 Hz TB	amplitude
Manzari L *et al.* 2013 (25)	AC & BC oVEMPs	22	radiological	TB	tuning curve (amplitude)

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RETROSPECTIVE COHORT STUDY ON HEARING OUTCOME AFTER TRANSMASTOID PLUGGING IN SUPERIOR SEMICIRCULAR CANAL DEHISCENCE SYNDROME: SAFE AND EFFECTIVE STRATEGY IN SSCD SYNDROME

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1. Introduction

The superior semicircular canal dehiscence (SSCD) syndrome is caused by a dehiscence of the bony capsule overlying the superior semicircular canal (SSC), thereby creating a mobile third window to the inner ear.[1] Symptoms can present isolated or in combination, and include Tullio phenomenon, pressure induced vertigo, hearing loss, autophony and hyperacusis of bone-conducted sounds. The diagnosis can be made by the combination of the typical history, temporal bone CT-scan and cervical vestibular-evoked myogenic potentials (cVEMP) findings.

High-resolution multi-detector CT is crucial to detect any dehiscence in the bony capsule of the inner ear, but should be accompanied by evaluation of cVEMPs to confirm the increased responsiveness of the labyrinth induced by the third mobile window lesion.

In case of incapacitating symptoms, surgical exclusion of the mobile third window can be offered. Plugging and capping of the SSC have been proven more effective surgical techniques in comparison to resurfacing.[2] Both the middle fossa and the transmastoid approach have been described as approaches to reach the SSC. The main disadvantages of the middle fossa approach are its potential complications including epidural hematoma, facial palsy, seizures, leakage of cerebrospinal fluid, etc.[3] Furthermore, literature suggests that plugging through

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the middle fossa approach produces up to 25% of sensorineural hearing loss (SNHL), without affecting speech discrimination.[4]

The primary aim of the study was to gain insight in the effect of transmastoid plugging on postoperative bone and air conduction thresholds as a measure of safety. The secondary aim was to study symptom relief as a measure of effectiveness.

2. Materials and methods

2.1. Ethics

The study was designed and conducted according to the Declaration of Helsinki (1996). Ethic committee approval was obtained to report outcome in this population.

2.2. Study design

We performed a retrospective chart review on all consecutive patients who underwent surgery for the SSC dehiscence syndrome from 2008 to 2015. Plugging through transmastoid approach was performed in every case. No patients were lost to follow-up.

2.3. Setting

Single tertiary referral otology department. The two senior authors (PVDH and VVR) performed all surgeries.

2.4. Participants and study size

All 12 patients that were diagnosed with SSCD syndrome and underwent transmastoid SSC plugging were included. Eligibility criteria for surgery included incapacitating autophony or hyperacusis of bone-conducted sounds typical to the SSCD syndrome, temporal bone CT finding of an ipsilateral dehiscent SSC, increased amplitudes and reduced thresholds at cVEMP. SSCD patients presenting with vertigo as sole symptom were not operated.

2.5. Surgical technique

A retro-auricular incision is used. Corticosteroids and antibiotics are administered intravenously to provide optimal protection of the labyrinth. A standard mastoidectomy is performed identifying the lateral semicircular canal. The anterior crus of the SSC can be found at a right angle to the anterior part of the lateral semicircular canal. The SSC bony capsule has to be skeletonized and blue-lined with a one-mm diamond burr until the endosteum is visualized. The endosteum

is opened with standard otosclerosis instruments. The endolymphatic duct can now be visualized through the fenestration. A one-mm fat plug is inserted gently in order to gradually compress the endolymphatic membrane. The fat plug is slowly pushed downwards towards the ampulla to plug the anterior crus. A second plug with bone pate is put on top to secure the fat plug. Subsequently the posterior crus is identified more medially by skeletonizing the inside of the SSC centered on the subarcuate artery. We take care not to enter the posterior crus over 180° to avoid entering the common crus. After opening the endosteum, we also insert a fat plug and a bone pate plug in the posterior crus and push it towards the location of common crus, without plugging the common crus.

2.6. Quantitative variables

Preoperative and postoperative audiometry data were collected in a dedicated prospective database and reviewed retrospectively. All audiograms were performed by classified personnel according to the ISO-389 (1975) standard. The guidelines of the Committee on Hearing and Equilibrium for the evaluation of conductive hearing loss were applied. Preoperative and postoperative pure-tone averages (PTAs) were calculated of 500, 1,000, 2,000, and 4,000 Hz, as required. The postoperative ABG was calculated as postoperative air conduction (AC) minus postoperative bone conduction (BC). SNHL was defined as a postoperative loss in bone conduction PTA of 1, 2 and 4 kHz exceeding 15 dB hearing level (dBHL).

2.7. Qualitative variables

Preoperative and postoperative symptoms and complications were reviewed in the electronic patient record system and collected in a database.

2.8. Bias and statistical methods

No obvious sources of bias were identified. No missing outcome data. No comparisons were made.

3. Results

3.1. Participants

A total of 12 patients with 13 operations were included in this case series. One patient did not experience enough symptom relief and needed revision. This patient experienced symptom relief after the revision surgery with stable BC thresholds. The population consisted of six males and six females that underwent transmastoid SSC plugging in six left (+ one revision) and six right ears. Autophony was present in 11 out of 12 patients preoperatively, after surgery no

single patient suffered from autophony. Five out of 12 patients suffered from Tullio phenomenon preoperatively, whereas only one patient experienced this symptom after surgery. Four out of 12 patients suffered from pressure induced vertigo before surgery, three patients experienced this symptom postoperatively. Before surgery pulsatile tinnitus was present in nine out of 12 patients, whereas only three patients suffered from this symptom postoperatively. Seven out of 12 patients complained of hyperacusis before surgery, only one patient still suffered from this symptom after surgery

3.2. Outcome data

Median AC PTA amounted to 25 dBHL and 18 dBHL, before and after surgery, respectively. Median BC PTA amounted to 11 dBHL and 16 dBHL, before and after surgery, respectively.

Median ABG PTA before surgery equaled 13 dBHL in comparison to 5 dBHL after surgery.

None of the patients had adverse events such as facial palsy, epidural hematoma, seizures, cerebrospinal fluid leakage. All patients experienced transient nausea due to the sudden obliteration of the SSC. Two patients developed a posterior canal BPPV, which resolved with one Epley maneuver in both patients.

3.3. Main results

Overall, a decrease in AC PTA and an increase in BC PTA was observed, resulting in an overall reduction in ABG PTA. None of our patients experienced postoperative SNHL in this study.

A decrease in all five symptoms was seen in this population. This decrease was most obvious in autophony, 11 out of 12 patients suffered from autophony preoperatively and this symptom disappeared in every patient.

4. Discussion

In SSC dehiscence patients with incapacitating symptoms surgical treatment can be offered to separate the labyrinth from the third mobile window. Initially, the preferred approach to reach the SSC involved the middle fossa craniotomy. However, in 2001 Brantberg described the transmastoid approach, which obviates the need for a middle fossa craniotomy and its potential complications such as epidural hematoma, facial nerve injury, cerebrospinal fluid leak and delayed onset of seizures.[5,6]

Recurrence of symptoms has been observed in a significant portion of patients undergoing resurfacing. Plugging of the SSC through the middle fossa approach has been reported to enable the highest rate of symptom relief, but was also reported to produce a significant amount of postoperative SNHL in up to 25% of

cases. Therefore we studied the hearing outcome and the symptom relief in our group of patients who underwent SSC plugging through transmastoid approach.

We did not observe postoperative SNHL, defined by the Committee guidelines as bone conduction PTA of 1, 2 and 4 kHz exceeding 15 dBHL. When studying hearing outcome reported in earlier transmastoid SSC plugging case series only two cases (total number of patients 59, including our series) were reported to have a SNHL.[5,6]

This study has a rather small sample size, although it is the second largest study on transmastoid SSC plugging. Its retrospective nature is a known disadvantage to studies reporting on surgical techniques. However, hearing outcome data was collected in a dedicated prospective audiometry database and no patients were lost to follow-up.

We can conclude that SSC plugging is a safe technique which not necessarily leads to a detrimental effect on BC or AC thresholds. Even in one revision case, where the SSC was opened again, no SNHL was observed. We can confirm the high rate of symptom relief reported in earlier studies on SSC plugging, which presents a reliable treatment option to the patient that reports incapacitating autophony and hyperacusis of bone-conducted sounds.

5. Conclusion

This study has demonstrated the safety and effectiveness of transmastoid plugging in case of SSCD syndrome producing incapacitating symptoms. It is safe to the cochlea and effective because of its high rate of symptom relief. The potential complications related to the middle fossa approach can be avoided by the transmastoid approach.

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TRANSMASTOID PLUGGING OF SUPERIOR SEMICIRCULAR CANAL DEHISCENCE: THE SUBARCUATE CANAL ON HR-CT IS A USEFUL SURGICAL LANDMARK

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Abstract

Objectives: The aim of this study was to evaluate the reliability of the subarcuate canal as a surgical landmark for plugging of the superior semicircular canal via the transmastoid approach in superior semicircular canal dehiscence syndrome (SSCCDS).

Materials and methods: High-resolution CT scans of ten temporal bones without a SSCCD were compared with ten temporal bone HR-CTs with a SSCCD with special attention on the location of the subarcuate canal through the superior semicircular canal. On images reformatted in the plane of the SSCC a circle was drawn over the canal and its radius was measured. Then the distances from the subarcuate canal to the anterior and posterior arms of the SSCC were measured. The circle was also divided in quadrants and the location of the subarcuate canal was noted.

Results: The average ratio between the radius of the circle and the distance from the subarcuate canal to the posterior arm of the SCC was three to one. In all cases, the subarcuate canal was found in the posterior half of the circle and thus coursed closer to the non-ampullary (posterior) arm than the ampullary (anterior) arm of the SSCC. Furthermore, in 20 out of 38 ears the subarcuate canal was located in the supero-posterior quadrant. These findings were correlated with peroperative observations in 15 operated cases.

Conclusions: The subarcuate artery is a good landmark in the treatment by plugging of the SSCC dehiscence syndrome via the transmastoid approach.

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Pre-operative evaluation of the HR-CT scan images can provide detailed information on the course of the subarcuate artery, except in some cases of severe deformities.

Key words: Subarcuate artery, high-resolution CT scan, superior semicircular canal dehiscence, plugging

1. Introduction

Minor *et al.*[1,2] first postulated the existence of the superior semicircular canal dehiscence syndrome (SSCCDS) where a dehiscence of the bone overlying the SSCC creates a mobile third window to the inner ear. This third window creates abnormal volume displacements within the membranous labyrinth causing dizziness, hearing loss, hyperacousis, and autophony.[3] High-resolution spiral volume CT scanning is essential to establish the diagnosis showing an absent bony roof over the SSCC and the larger the dehiscence the more prominent the symptoms are.[4] The use of 0.5-mm cuts and reformats in the plane of the SSCC have been shown to increase the positive predictive value to 93%.[4] Because of the possibility of a false-positive CT scan,[5] CT examinations should not be used as the sole criterion for establishing a diagnosis of SSCCD syndrome. Thanks to its higher resolution, cone beam CT-scanning allows nowadays even more precise visualization of a dehiscent SSCC, but is not yet available in all centers.

We evaluated the location of the subarcuate canal as a landmark for plugging of the superior semicircular canal via a transmastoid approach which is preferred by many otologists.[6,7] After cortical mastoidectomy, the lateral SSCC is visualized, then the posterior SSCC is identified after exposure of the sino-dural angle and removal of retro-labyrinthine mastoid cells. In order to make two small fenestrations (labyrinthotomies) in the superior SSCC, right below the dehiscence, both arches of the superior SSCC are blue-lined before opening and plugging. First the anterior (ampullary) arch of the superior SSCC is blue-lined. Then the posterior arch of the superior SSCC is located above the common crus. This non-ampular arm is much more medially located than the anterior arch and only reached after deeper drilling into the petrous bone. While searching for this posterior arch, the subarcuate artery will be encountered. The plugging is performed using fascia and covered with bone pate. The purpose of the study was to evaluate whether the subarcuate artery can be used as a landmark during surgery.

2. Material and methods

Ten HRCT scans for middle ear pathology were compared with ten HRCT scans in patients with SSCCD. On double oblique reformats of sagittal images,
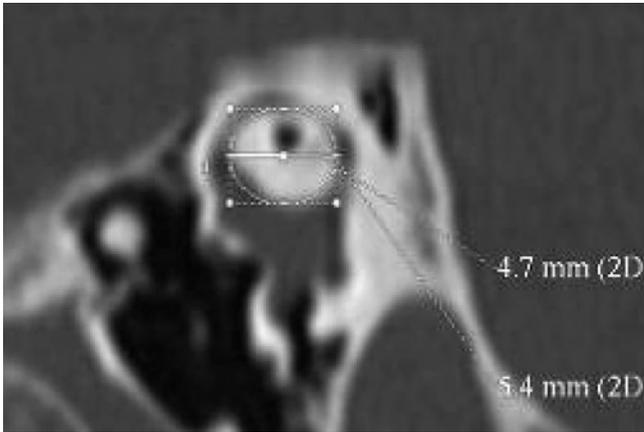
Fig. 1. After reformatting in the plane of the SSCC a circle is drawn (in a normal ear). The radius of the circle and the distance to the subarcuate canal are measured.
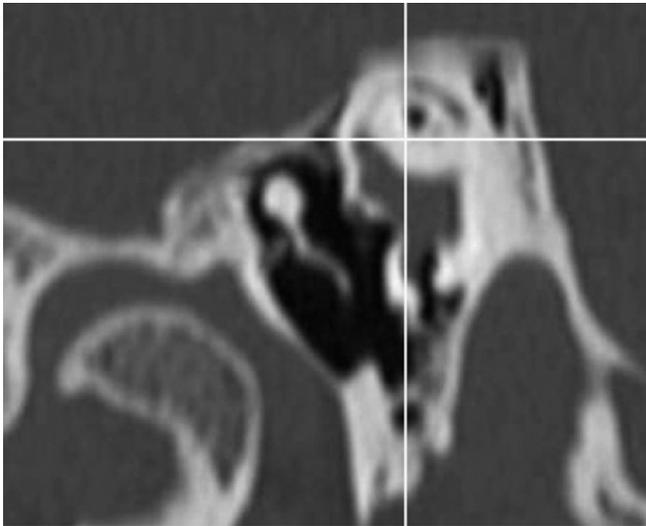

Fig. 2. The normal SSC circle is divided in quadrants by a 0 degree and 90 degree line to evaluate in which quadrants the subarcuate canal could be found. In this scan the center of the SSSC is located in the supero-posterior quadrant.

the plane of the lateral SSC was established and this plane was used to find the plane of the superior SSC (Fig. 1) and a circle along the SSCC was drawn. The radius of the circle was drawn parallel to the roof of the vestibule and the distance to the subarcuate canal (Fig. 2) was measured. Two separate researchers performed the measurements. Then we divided the circle in quadrants by a 0 degree and 90 degree line to evaluate in which quadrant the subarcuate canal could be identified (Fig. 2).

3. Results

Using the above-described reformatting of the CT scans, in all patients with normal inner ear morphology the subarcuate canal could be identified.

The measurements of the radius of the circle in the patients with normal inner ear morphology (n = 20 ears) ranged from 5.1 to 6.5 mm (mean 5.9 mm). The distance of the radius up to the subarcuate canal ranged from 3.7 to 5.3 mm (mean 4.4 mm). Subtracting these distances results in the distance from the subarcuate canal to the posterior end of the circle representing the superior SSC (range 0.6-2.1 mm, mean 1.5 mm).

The measurements of the radius of the circle in the patients with SSCD syndrome (n = 18 ears) ranged from 5.6 to 7.3 mm (mean 6.4 mm). The distance from the ampular end to the subarcuate canal ranged from 4.0 to 5.6 mm (mean 4.7 mm). The mean distance from the subarcuate canal to the posterior end of the circle was 1,7 mm (range between 1.4 and 2.1 mm).

Because of the small numbers and the relative inaccuracy of any manually performed measurements, we did not perform statistical analysis on the distances as measured in mm. Instead, we looked at the ratio between the distances and found an average ratio of three to one in the patients with normal inner ear morphology as well as in the patients with SSCD syndrome (Fig. 3).

In all studied cases, the subarcuate canal was located in the posterior half of the circle representing the SSCC. The subarcuate canal was also located in the supero-posterior quadrant in 20 of 39 ears (49%). In 12 ears it was located just on the line between the supero- and infero-posterior quadrant and in six ears it was located high in the infero-posterior quadrant.

These observations were used during 15 consequent surgeries when searching for the posterior arch of the SSCC and were consistent with preoperative images (Fig. 4).
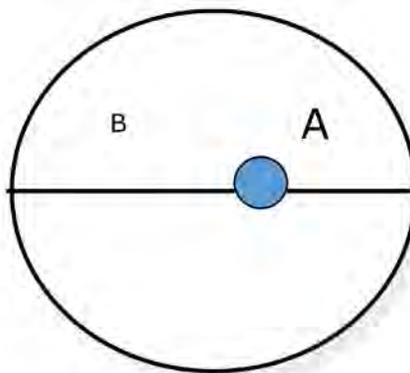

Fig 3. The average ratio between the radius of the circle through the SCC and the distance from the posterior arch to the subarcuate canal was 3 to 1 or the B-distance equals 2 x the A-distance.
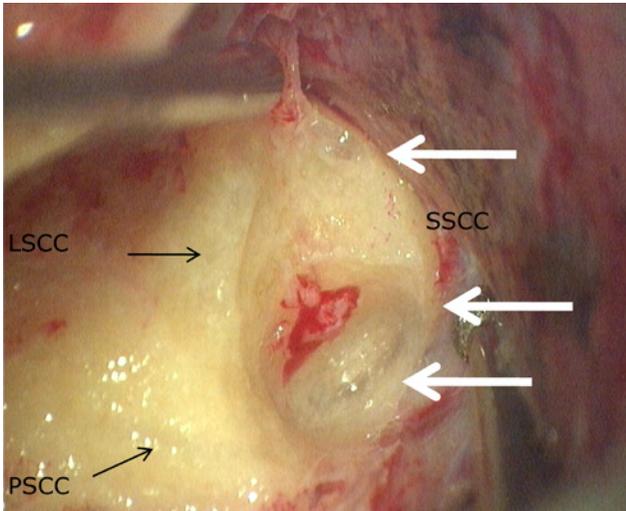
Fig. 4. Per-operative image in a left SSCCD ear with exposed LSCC and PSCC and showing the blue-lined anterior (upper white arrow) and posterior (lower white arrow) arches of the SSCC and the subarcuate artery (middle white arrow) being much closer to the posterior arm than to the anterior arch.

Fig. 5. In one case of SSCCD, the malformation of the labyrinth was so severe that the subarcuate canal could not be identified and the measurements could not be performed.

In one case of SSCD however the malformation of the SCC was so severe that the subarcuate canal could not be identified and the measurements could not be performed (Fig. 5).

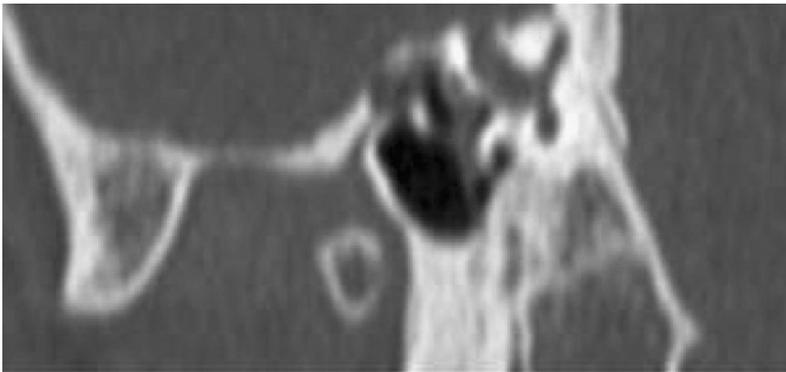Using the above-described reformatting of the CT scans, in all patients with normal inner ear morphology, the subarcuate canal could be identified. In one case of SSCD, however, the malformation of the SCC was so severe that the subarcuate canal could not be identified and the measurements could not be performed (Fig. 4).

In all the other cases the subarcuate artery could be used as a landmark for evaluating the level of the posterior arm of the SSCC. Neuronavigation does not have the required resolution to help us identifying the exact position of the posterior arch of the SSCC.

Up to now this subarcuate canal landmark has been useful during 15 consecutive surgeries for SSCD.[9] All patients except one had a significant improvement of their symptoms and no case of sensorineural hearing loss was deplored. Comparison of preoperative with postoperative MR images shows the efficacy of the plugging (Fig. 6).

Conclusions

The subarcuate artery is a good landmark in the surgery for SSCC syndrome by the transmastoid approach as long as one bears in mind that it is often located in closer proximity to the posterior arch of the SSCC and not in the middle of the SSCC as is often stated in textbooks. Pre-operative evaluation of the HR-CT scan can provide detailed information on the course of this subarcuate canal.

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CLINICAL ISSUES

CEREBRAL VENOUS INSUFFICIENCY IN MÉNIÈRE'S DISEASE

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1. Introduction

Ménière's disease (MD) is an inner ear disease characterized by incapacitating vertigo, often with nausea and vomiting, typically lasting hours, tinnitus, pressure in the head or ear during vertigo spells, audiological evidence of fluctuating low-frequency and/or progressive sensorineural hearing loss. The incidence of MD, estimated 120-190 per 100,000,[1,2] is difficult to determine because of its occasional subtle onset, fluctuating symptoms, and long period of remission. As implied by its definition, MD's true etiology and pathophysiology remains incompletely understood. The underlying mechanism for these symptoms is thought to be an endolymphatic hydrop and several pathological mechanisms have been proposed.[3-4] The venous drainage of the inner ear is carried out by the vein of cochlear aqueduct (anterior and posterior vestibular veins) and the cochlear vein (Axelsson's common modiolar vein)[5] and the vein of vestibular aqueduct. The venous blood empties either directly into the inferior and superior petrosal sinus or internal jugular vein.[6] Based upon both the anatomy of venous inner ear drainage and the pathogenic mechanism suggested by Godlowski, an existing excess of endolymphatic volume could be secondary to a chronic reduced or altered venous drainage of the anterior and posterior vestibular veins and/ or of the cochlear veins into the venous cerebrospinal system (IJVs). In 2006, Zamboni introduced the concept that chronic impaired venous outflow of the central nervous system is associated with multiple sclerosis (MS), coining

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the term of chronic cerebrospinal venous insufficiency (CCSVI). The diagnosis
of CCSV requires the evaluation of five ultrasound parameters that assess both
neck and head venous blood flow and anatomy.[7-9] CCSVI is diagnosed if a
patient has an abnormality in two or more of the five parameters. Is it possible
to hypothesize that CCSVI for congenital anatomical alterations or functional
acquired alterations represents a predisposing factor for developing inner ear
disorders as MD? The aim of this study was to evaluate the venous drainage
of the neck and head in 32 patients affected by definite MD and compare the
results with a healthy control group matched for age and sex.

2. Materials

The study group consisted of 32 patients (13 men and 19 women, mean age 50
years ± 9.1 SD) affected by definite MD admitted to the Department of Sensory
Organs of University 'Sapienza' of Rome. The healthy control group consisted
of 97 patients (42 men and 55 women, mean age 38.9 years ± 14.2 SD) in gen-
eral good health with no history of ear, neurological or vascular diseases. All
subjects underwent an echo-color Doppler (ECD) of the cerebrospinal venous
flow. The morphology of the IJVs was assessed by means of high resolution B-
mode ultrasounds (ECD equipped with 2.5 and 7.5-10 MHz probes and Qualite
Doppler Profile system – QDP) and hemodynamics, adopting the diagnostic
criteria recently approved in a consensus conference hemodynamic parameters
considered in this study are as follows:[10-13] (1) Reflux in the IJVs and/or vertebral
veins (VVs) in orthostatic and supine postures; reflux was considered pathologi-
cal when reversal flow lasted more than 0.88 s. (2) Reflux in the intracranial
veins. Reflux is defined as a reversal of flow direction during the inspiratory and
expiratory phase during normal breathing with mouth closed. The transcranial
color-coded duplex sonography study was carried out using the transcondylar
window which assesses the direction of flow in the petrosal sinuses.(3) B-mode
abnormalities/stenosis of the IJVs: (3a) Morphological stenosis: presence of se-
vere reduction of the Cross Sectional Area (CSA) of IJVs in the supine position
(\0 3 cm² which does not increase with Valsalva maneuver, performed at the
end of the examination; (3b) Hemodynamic stenosis: a significant stenosis with
simultaneous presence of intraluminal defects such as webs, septa or malformed
valves, and hemodynamic changes (block, reflux, increased velocity flow). (4)
Flow not Doppler-detectable in IJVs and/or VVs despite numerous forced in-
spirations, in both sitting and supine position. (5) Negative D CSA (DCSA) in
the IJV: the value is obtained by measuring the difference in IJV cross sectional
area between the supine and upright positions. The presence of two or more
criteria ensures a very high sensitivity for the diagnosis of CCSVI.[14].

3. Results

Thirty-two affected by definite MD (19 women and 13 men) were included in the study group. Twenty-one of these patients (65.6%) were positive for CCSVI at the ECD examination of the cerebrospinal venous flow, whilst 11 patients (35.4%) proved to be negative. The healthy control group consisted of 97 subjects (55 women and 42 men) and only 24 (25%) showed positivity for CCSVI. Demographic and clinical characteristics of the patients are summarized in Table 1. The ECD results (Table 2) showed a statistical significant difference (P\0.001) was observed for criteria 2 and 3b. The criteria 2 (intracranial veins reflux) was positive in 76.2 % of MD (16 patients) compared with 12.5% (three patients) in the control group. The criteria 3b (stenosis of IJV and hemodynamic changes)

Table 1. Demographics and clinical characteristics in the healthy controls and in patients with MD.

	Healthy controls Total : 97%	Ménière's disease Total: 32%	P Value
Age (years)	48.9 ± 8.1	(50 ± 9.1)	Ns
Men	42 (43%)	13 (40%)	Ns.
Women	55 (57%)	19 (60%)	Ns.
CCSVI POS %	24 (25%)	21 (65.6%)	< 0.001
S/M e MM.	36	67	< 0.001

Data are presented as mean values (range interval), or as number and percentage. MD = Ménière's disease; CCSVI = chronic cerebrospinal venous insufficiency; S/M = Septa/Membranes; MM = membrane occluding a vein; POS = positive.

Table 2. The distribution of the echo-color Doppler criteria between healthy controls and patients with MD.

	Healthy controls CCSVI POS.: 24 (25%)	Ménière's disease CCSVI POS.: 21 (65.6%)	P value
Parameter 1: IJVs and/or VVs reflux	12 (50%)	13 (61.9%)	NS
Parameter 2: Intracranial veins reflux	3 (12.5%)	16 (76.2%)	< 0.001
Parameter 3: IJVs stenosis a) morphologically	13 (54.2%)	13 (61.9%)	NS
b) hemodymamic	8 (33.3%)	14 (66.7%)	< 0.05
Parameter 4: Cervical veins blocked outflow	12 (50%)	12 (46,1%)	NS
Parameter 5: ΔCSA	0 (0)	0 (0)	NS

Data are presented as mean values (range interval), or as number and percentage. MD = Ménière's disease; CCSVI = chronic cerebrospinal venous insufficiency; DCSA = D cross sectional area; IJVs = internal jugular veins; VVs = vertebral vein; POS = positive.

was positive in 66.7% of MD (14 patients) and 33.3% of control subjects (eight patients) (p\0.05). In addition, the stenosis of the internal jugular vein ipsilateral to the ear affected was observed in 20 MD patients (62%) with CCSVI positive diagnosis. The ultrasound study of venous district of MD patients showed a 65 % positivity of CCSVI compared to 25% observed in the healthy. MD patients have a chronic venous insufficiency head-neck which is significantly higher than that reported from the control group. In particular the analysis of the second hemodynamic parameter, relative to the vascular intracranial reflux, showed a significant difference in the two groups. We observed that patients showed a prevalence of 76% compared to 12.5% in the control group. In MD patients the petrosal sinuses, more directly involved in the drainage of the inner ear, show the presence of a venous return which is not unidirectional as usually occurs physiologically.

4. Conclusions

The results obtained showed that CCSVI could be considered a new ultrasound vascular pattern of cerebrospinal venous system present in patients affected by definite MD. This vascular impairment significantly affects the vascular areas more directly involved in the venous drainage of the inner ear. The venous stasis of the head and neck veins may be considered a further etiopathogenetic mechanism which adds to many other already known and that define MD as a multifactorial disease.

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MÉNIÈRE'S DISEASE SYMPTOMATOLOGY IN RELATION TO THE AAO-HNS 1995 GUIDELINES

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Abstract

The study group consisted of 384 consecutive subjects affected by MD according to the AAO-HNS 1995 guidelines. In each case we have carried out an exhaustive anamnesis regarding all the aspects of the disease and audiometric threshold. According to AAO-HNS classification 228 subjects (59%) at the moment of the first control in our department were affected by definite DM, 30 (8%) by probable MD and 126 (33%) by possible MD. In this sample, among the 129 subjects of this group 90 (73%) were affected only by hearing loss and 36 (27%) only by vertigo. Age at the beginning of disease was not different among definite, probable and possible forms while subjects affected by the definite MD were older and presented a longer duration of the disease. Disability level was lesser in the possible forms, condition in which the lower degrees are more represented. Among the 228 definite forms, MD appeared with both vertigo and hearing loss together (temporal delay less than 24 hours) in 79 cases (35%), with hearing loss alone in 96 cases (42%) and with vertigo alone in 53 cases (23%). PTA mean threshold at 0.5-1-2-3 kHz at the first control in the 228 cases of definite MD is worse than in the 90 subjects affected by possible MD in its cochlear form. In conclusion definite form represents the most common form of MD at diagnosis and comprises the most disabling cases. However in the larger part of cases it begin as possible and transforms in definite later, normally within 5 years.

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1. Introduction

MD diagnosis is essentially clinical and specialized test equipment should not be required. In 1995 the Committee on Hearing and Equilibrium of the American Academy of Otolaryngology - Head and Neck Surgery published recommended guidelines for the diagnosis of the MD.[1] They defined Menière disease as:

– certain: definite MD plus histopathologic confirmation;
– definite: two or more definitive spontaneous episodes of vertigo 20 minutes or longer, audiometrically documented hearing loss on at least one occasion; tinnitus or aural fullness in the treated ear;
– probable: one definitive episode of vertigo; audiometrically documented hearing loss on at least one occasion; tinnitus or aural fullness in the treated ear, other causes excluded;
– possible: episodic vertigo of the Menière type without documented hearing loss or sensorineural hearing loss, fluctuating or fixed, with disequilibrium but without definitive episodes; other causes excluded.

The aim of this paper is to determine, in a large series of subjects affected by MD according to the AAO-HNS 1995 criteria, the distribution of the different forms, their relationship with clinical parameters and the aspects of the evolution of the disease over time.

2. Materials and methods

The study group consisted of 384 consecutive subjects affected by MD according to the AAO-HNS 1995 guidelines[1] and seen for the first time by the Authors (not necessarily at the first diagnosis). Age ranged from 14 to 86 years (mean age 53 years); 156 (41%) were males and 228 (59%) females. In all cases we have carried out an exhaustive anamnesis regarding all the aspects of the disease. In particular the following parameters were carefully evaluated: age, age at beginning of the disease, duration of the disease, symptoms referred at the first control, evolution of symptoms before the first control, associated diseases, controlateral ear status. Each patient has been submitted to a pure-tone audiometry (PTA) in a sound proof chamber. According to AAO-HNS 1995 guidelines[3],threshold reported in the paper refers to the average threshold at 0-5-1-2-3 kHz.

3. Results

Mean disease duration was 53 months, range 1-480 while median duration was 24 months. The side of the disease was the right in 171 cases (44%) and the left in 177 cases (46%). In 19 cases (6%) the disease was bilateral while in 17
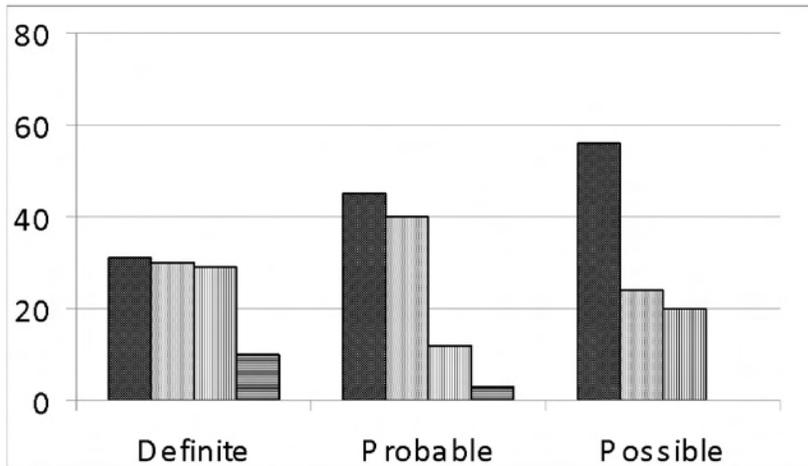
Fig. 1. Rate of subjects in relation to the degree of disability (I to IV) in the three different forms of MD. The degree of disability progressively decreases from the definite to the possible form. Degree I: oblique lines; degree II: points; degree III: vertical lines; degree IV: horizontal lines.

cases (4%) in was not possible to identify the side since they were possible MD without auditory symptoms.

Eighteen (5%) subjects were affected by ipsilateral delayed endolymphatic hydrops, 20 (5%) by contralateral delayed endolymphatic hydrops, 10 (3%) by Ohresser's premenstrual syndrome and 3 (1%) by Lermoyez's syndrome. In 14 cases (4%) patients were affected by Tumarkin's otolithic vertigo.

According to AAO-HNS classification 228 subjects (59%) at their first control in our department were affected by definite MD, 30 (8%) by probable MD and 126 (33%) by possible MD. The possible forms represent the patients affected only by hearing loss or vertigo. In this sample, among the 129 subjects of this group 90 (73%) were affected only by hearing loss and 36 (27%) only by vertigo. Age at the beginning of the disease was not different among definite, probable and possible forms while subjects affected by the definite MD were older and presented a longer duration of the disease. The disability level was lesser in the possible forms, condition in which the lower degrees are more represented. Since the degree of disability is evaluated by the AAO-HNS 1995 guidelines (3) on the basis of vertigo, the evaluation of this parameter among the possible forms has been carried out only in subjects affected by vertigo (90 cases, therefore the overall sample in this cases is limited to 348 cases). Among the 228 definite forms, MD appeared with both vertigo and hearing loss together (temporal delay less than 24 hours) in 79 cases (35%), with hearing loss alone in 96 cases (42%) and with vertigo alone in 53 cases (23%). MD therefore was immediately definite in 45% of cases and passed from possible to definite in 65% of cases. In the overall casuistry (384 cases) MD manifested immediately as definite in 79 cases (21%), appeared possible and became definite in 149
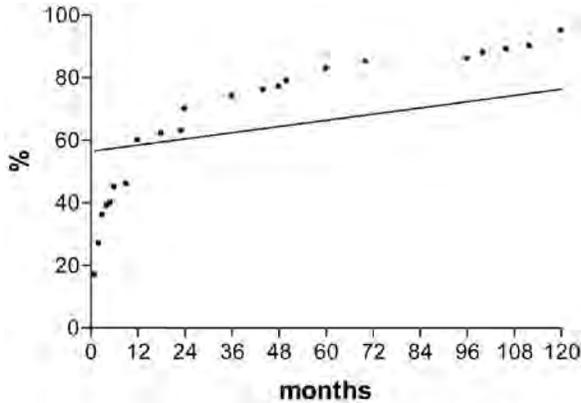
Fig. 1. Temporal delay (in moths) between the first appearance of the disease in its monosymp-
tomatic form (possible MD) and the transformation in definite form after the appearance of the
second symptom in the 149 subjects in which MD appeared as possible and become definite.
The rate of cases represents the cumulative percentage. The line represent the linear correlation
existing between the two parameters (slope 0.16, y-intercept 56, $p < 0.0001$).
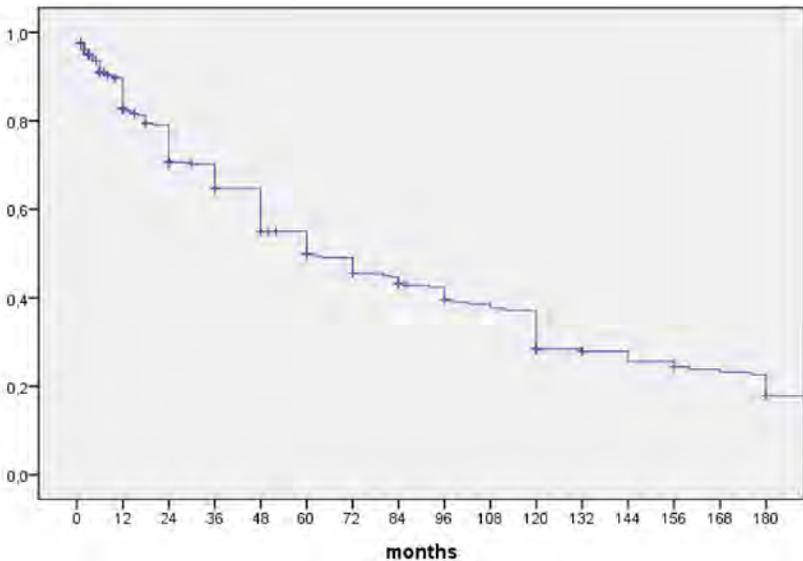
Fig. 2. Kaplan-Meier survival curve in the overall samples (384 cases). The event is represented
by the transformation from a possible form to a definite form.

cases (38%), remained probable in 30 cases (8%) and possible in 126 cases
(33%). In Figure 1 is reported the relationship existing between the temporal
delay, in months, and the rate of subjects, in term of cumulative percentage, who
developed a definite bi-symptomatic form from a possible monosymptomatic
form. This analysis is limited to those 149 cases that have changed from the
possible to the definite form. The figure shows how the transformation from

Fig. 2. Mean audiogram in subjects affected by definite (continued line) and possible (dotted line) MD.

the possible to the definite form has taken place in about 60% of cases within 12 months and in about 80% of cases within 60 months; this cannot considered as a conclusive data since in some cases, especially those who had a shorter duration of the disease, it is still possible an evolution of the disease. The line represents the linear correlation existing between the two parameters (p<0.001).
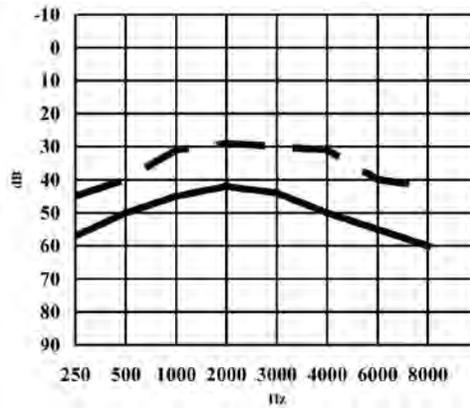
The risk of transformation from a possible-probable form into a definite one on the basis of disease duration in the overall sample (384 cases) has been evaluated, by means of Kaplan-Meier survival curve, in Figure 2. This analysis confirm that the risk to be affected by a definite form is higher within the first 2 years after the appearance of the first symptom and than progressively decreases over time, reaching a 50% risk at about 5 years.

Among the 149 cases in which MD transformed from a possible to a definite form this transformation took place quicker if the first symptom was vertigo, even if the difference is not significant at the Mann Whitney test.

In Tumarkin's and bilateral forms possible and probable MD is occasionally present while their frequency is higher in contralateral delayed endolymphatic hydrops. PTA mean threshold at 0.5-1-2-3 kHz at the first control in the 228 cases of definite MD is worse than in the 90 subjects subjects affected by possible MD in its cochlear form. Difference at the Student's T-test is significant (p<0.0001). In Figure 3 are reported mean audiograms in definite (continue line) and possible (dotted line) MD.

4. Discussion

In this study we have analyzed a large series of patients affected by MD on the basis of AOO-HNS 1995 guidelines[1] in order to determine the distribution of definite, probable and possible forms and to obtain some information about the

evolution of the disease. On the basis of anamnesis, it was possible to obtain data about the modality of MD appearance and its temporal evolution; obviously our results show the instantaneous situation while it was not our aim in this study the evaluation of the successive evolution, that can partly modify our conclusion, above all the distribution of the three clinical forms and their transformation.

The distribution among the three clinical degrees of the AOO-HNS 1995 guidelines[1] demonstrates that the most common form is the definite one, 59% of cases, followed by the possible form, 33% of cases. The rate of definite forms we have found is higher than that reported by Watanabe *et al.,*[2] 43% of cases, but in this study the classification was based on a different criterion.

MD manifested immediately in its definite form in about 20% of cases while in the remaining 80% of cases the pathology evolved in definite or remained in a lower degree. On this topic an interesting data emerging from our sample is the evolution of the disease over time and the risk of transformation from a lesser degree (possible MD) to a higher degree (definite MD). The possible form presents approximately 50% probability, within 1 to 530 months, of turning into definite form and about 50% to remain stable. In those cases, and according to previous reports[3,4], cochlear form is largely the most common form. A longer analysis could demonstrate that a higher rate of possible cases could turn into definite, but the existence of case who remained stable for more than 5-10 years demonstrates that MD can remain in its monosymptomatic form. On this topic, the analysis of the evolution of the possible forms shows that the higher risk of turning into definite is within 12 to 60 months. The transformation from a possible to a definite form is higher if the first symptom is vertigo. As regards the probable forms, they remained stable in 8% of cases at a 38 months mean follow-up. This finding suggests that in some cases MD attack can remain single, while it is also possible that with a longer follow-up some of these cases developed a definite form.

On the other hand, definite MD is more disabling, has a longer duration, determines a greater hearing loss and comprises a larger rate of subjects affected by delayed endolymphatic hydrops, Tumarkin's vertigo and bilateral MD than other forms [5] .

In conclusion definite form represents the most common form of MD at diagnosis and comprises the most disabling cases. However in the larger part of cases it begins as possible and later it turns into definite, normally within 5 years. If the first symptom is vertigo the transformation in the definite form occurs more quickly. However the stabilization of the possible monosymptomatic form is likely and relatively frequent.

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CERVICAL SPECIFIC PROTOCOL AND RESULTS FOR 300 MÉNIÈRE'S PATIENTS FOLLOWED FOR A MINIMUM OF FIVE YEARS

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Multiple factors and several common triggers contribute to Meniere's disease (MD), but there is only one cause. An upper cervical subluxation complex (UCSC) (Fig. 1) causes MD, the result of whiplash trauma, caused by vehicular accident or blow to head.[1] It takes an average of fifteen years (Fig. 2) from the time of injury until onset of symptoms.[2] Some presentation of one, two or all three of the following: translation of head, loss of normal cervical curve and/or head tilt. (Fig. 3) Translation means the head is fixated to one side, often the result of a 'T-bone' vehicular accident. Typical rear-ended whiplash causes reverse cervical curve. Head tilt will make you dizzy. Your body has two built-in gyroscopes, one made up of occiput, atlas and axis, the other L5, sacrum and ilium.[3] Your

Fig. 1. Right unilateral upper cervical subluxation complex, PIL (posterior and inferior on the left) Blair atlas listing. Lesion is highlighted in light blue circle.

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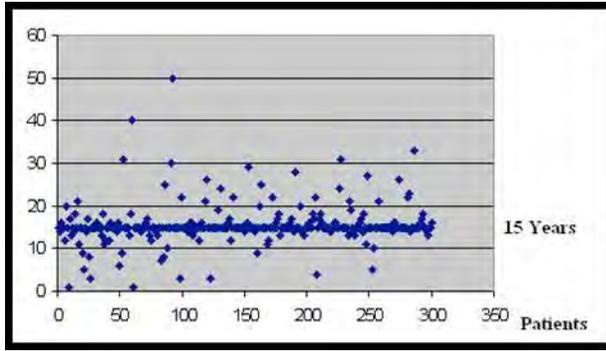
Fig. 2. It takes an average of fifteen years from the time of the trauma until the onset of symptoms.
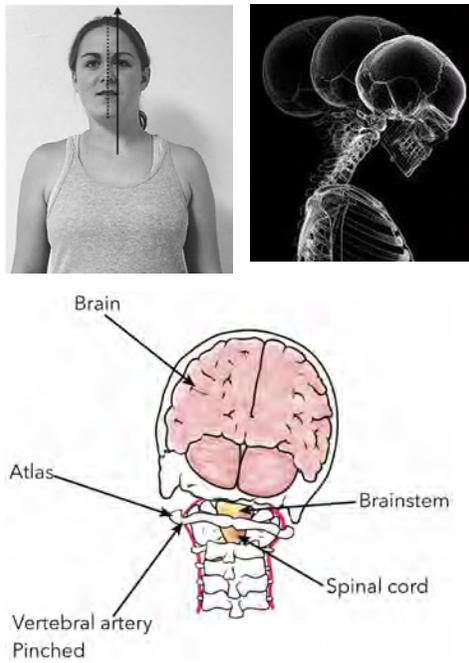
Fig. 3. Three presentations of whiplash: translation, forward head carriage, and head tilt.

innate intelligence will protect your brainstem, balancing the eyes and ears to the horizon by torquing the pelvis, creating a relative short leg.[4]

UCSC will create some combination of the following eight lesions: Auditory tube dysfunction near the opening in nasopharynx caused by atlas/axis subluxation[5] or near opening in middle ear via tensor vili palatina muscle caused by torquing of CN V,[6] traction of CN VIII,[7] insufficient blood supply to inner ear caused by 'cork in the bottle syndrome' causing venous backflow[8] or less blood flow in vertebral artery on side of the affected ear,[9] chronic CSF backjets into
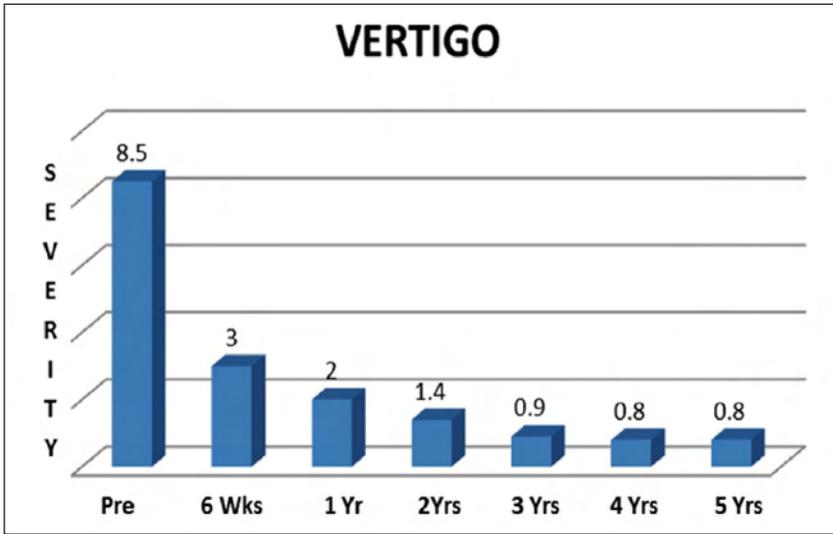
Fig. 4. Dramatic vertigo improvement six weeks post treatment, and over 90% improvement after four years.

the fourth ventricle,[10] confusion of Vestibular nuclei[11] and/or irritation of nerve supply to the endolymphatic sac.[12]

D.D. Palmer performed first chiropractic adjustment in 1895.[13] The C_2 correction restored the patient's hearing. D.D. and his son B.J. started the first Chiropractic College and in 1931 B.J. started researching upper cervical specific chiropractic.[14] He developed thermography to research pattern work,[15] which was used to determine when and where to adjust in this study, together with cervical specific ten-step relative leg length protocol.[16]

All 300 consecutive patients diagnosed with MD by otolaryngologists tested positive for UCSC. Three cervical x-rays were taken and analyzed: lateral, A-P open mouth and nasium. All showed evidence of UCSC and whiplash.[17]

There are four Blair atlas listings: ASR, PIL, ASL and PIR.[18] Ninety percent were Posterior and Inferior on opposite side of involved ear. Pierce Results adjustments were also performed in the lower cervicals on some patients.[19] Ninety percent reported dramatic improvement in vertigo after six weekly visits.[20] Four years post adjustment average improvement was over ninety percent.[21] (Fig. 4) Frequency and intensity of headaches worsened in three percent of the patients.[22]

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EPIDEMIOLOGICAL CHARACTERISTICS OF MÉNIÈRE'S DISEASE IN JAPAN: AN UPDATE

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1. Introduction

Nationwide surveys based on the same diagnostic criteria[1,2] have been conducted in Japan from 1976 to 2014 by the Meniere's Disease Research Committee,[3] the Peripheral Vestibular Research Committee,[1,3-5] and the Research Committee of Intractable Vestibular Disorders supported by the Ministry of Health, Labor and Welfare of Japan. These nationwide multicenter surveys have consistently used the same diagnostic criteria to evaluate the long-term trends of the epidemiologic characteristics of Ménière's disease (MD). In the present study, basic epidemiologic characteristics of MD found in the survey performed in 2014 were analyzed and compared with those of the four previous nationwide surveys to investigate the latest changes in the characteristics of MD in Japan.

2. Materials and methods

Sixteen committee members of The Research Group of Intractable Vestibular Disorders sponsored by the Ministry Health, Labor and Welfare of Japan conducted a nationwide survey from January to December 2014 at 15 universities and one private hospital located in various districts or areas of Japan. The committee members diagnosed de novo definite cases of MD according to the diagnostic criteria for MD decided on in 1976 by the Meniere's Disease Research Committee of Japan as follows: (1) repeated attacks of whirling

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vertigo; (2) fluctuating cochlear symptoms including hearing loss, tinnitus or aural fullness synchronized with attacks of vertigo; and (3) exclusion of central nervous system involvement, eighth nerve tumor, and other cochleovestibular diseases.[1] Cases fulfilling all three criteria were diagnosed as cases of definite MD. The diagnostic criteria for MD with bilateral fluctuant hearing loss were decided by the Peripheral Vestibular Disorders Research Committee of Japan in 1991 as follows: (1) repeated attacks of whirling vertigo; (2) fluctuating bilateral cochlear symptoms synchronized with attacks of vertigo; and (3) exclusion of central nervous system involvement, eighth nerve tumor, and other cochleovestibular diseases. Because the cochlear symptoms sometimes fluctuate, and the severity of symptoms is not the same in every ear, some patients complain of severe unilateral rather than bilateral symptoms. Cases fulfilling all three criteria were diagnosed as cases of definite bilateral MD. Survey data from 265 de novo definite cases of MD were stored and analyzed by database software at the Department of Otolaryngology at the University of Toyama. The sex distribution, unilateral vs bilateral involvement, and age at onset in both sexes in the latest (the present) survey were compared with those of the four previous nationwide surveys. The first survey was conducted by the Meniere's Disease Research Committee in 1975-1976, and the following three surveys were conducted by the Vestibular Disorder Research Committee in 1982-1984, 1990, and 2001-2006. To correct the age distribution of the population among the surveys, data from the national census for 1975, 1985, 1990, and 2005 and data from the estimate of population in 2014 reported by Statistics Japan were used. Chi-square tests were used to analyze the data statistically. A p value of < 0.05 was considered to indicate statistical significance. Some of the data in this study were presented in previous studies.

3. Results

In the latest survey, 79 males (29.8%) and 186 females (70.2%) were diagnosed with de novo definite MD (Table 1). The percentage of females in the population of Japan did not change significantly between 1975 to 2014; it was 50.8% in 1975 and 51.3% in 2014 according to national census data and data from the estimation of population by Statistics Japan. As in the fourth survey, the population-adjusted proportion of female patients in the latest survey was significant greater than that in the first surveys.

    The proportion of cases describing unilateral and bilateral involvement in the latest survey was 85.2% and 14.8%, respectively (Table 2). As with the third and fourth surveys, the proportion of bilateral MD cases counted in these three surveys was significantly greater than that in the first and second surveys.

    In the latest survey, age at onset of definite MD peaked between the third and fifth decades (Table 3). The proportion of patients with definite MD for whom the age of onset was 60 years or more was 29.0%, which was almost equal to that in the fourth survey (26.9%), and four times greater than that in the first

Table 1. Sex distribution.

Sex	1975-1976	1982-1984	1990	2001-2006	2014
Male	257 (49.4)	125 (43.1)	63 (42.6)	154 (37.6)	79 (29.8)
Female	263 (50.6)	165 (56.9)	85 (57.4)	256 (62.4)	186 (70.2)
Total	520 (100)	290 (100)	148 (100)	410 (100)	265 (100)

Values are number and (percentage) of patients.

Table 2. Unilateral and bilateral involvement.

Involvement	1975-1976	1982-1984	1990	2001-2006	2014
Unilateral	472 (90.8)	267 (92.1)	124 (83.8)	343 (86.2)	224 (85.2)
Bilateral	48 (9.2)	23 (7.9)	24 (16.2)	55 (13.8)	39 (14.8)
Total	520 (100)	290 (100)	148 (100)	398 (100)	263 (100)

Values are number and (percentage) of patients.

Table 3. Age at onset among all patients.

Age (years)	1975-1976	1982-1984	1990	2001-2006	2014
0-19	27 (5.3)	12 (4.3)	4 (2.8)	8 (2.0)	5 (1.9)
20-29	70 (13.7)	32 (11.5)	15 (10.4)	46 (11.3)	36 (13.6)
30-39	137 (26.9)	61 (21.9)	37 (25.7)	75 (18.4)	52 (19.6)
40-49	152 (29.8)	74 (26.6)	40 (27.8)	72 (17.6)	46 (17.4)
50-59	86 (16.9)	66 (23.7)	36 (25.0)	97 (23.8)	49 (18.5)
60-69	31 (6.1)	23 (8.3)	12 (8.3)	87 (21.3)	39 (14.7)
70	7 (1.4)	10 (3.6)	0	23 (5.6)	38 (14.3)
Total	510 (100)	278 (100)	144 (100)	408 (100)	265 (100)

Values are number and (percentage) of patients.

survey. The population-adjusted proportion of patients whose age at onset was 60 years or more in both the fourth survey and the latest survey was significantly greater than that in the first survey ($p < 0.05$ and $p < 0.1$, respectively).

4. Discussion

Recently, most studies around the world have shown a predominance of female patients with MD. In 2003, Morales Angulo et al.[6] reported the male:female ratio in Cantabria, Spain to be 0.38. In 2005, Havia et al.[7] reported that 56% of the patients with MD in southern Finland were female. In 2007, 64% of the patients with MD in West Africa were found to be female.[8] In 2010, there was a preponderance of female cases of MD (65%) as shown by a computer review of medical records in the United States.[9] In 2014, Tyrrell et al.[10] reported that the proportion of cases of MD was greater in females than in males in the United

Kingdom. This study showed that the number of de novo definite cases of MD in females increased over time relative to that in males. Some hormonal influence may explain these gender differences.[1,11]

Among the nationwide surveys in Japan, the proportion of MD patients with bilateral involvement in the last three surveys was higher than that in the first two surveys. The Peripheral Vestibular Disorders Research Committee of Japan drafted the diagnostic criteria for bilateral MD patients in 1988-1990.[2] The changes in the new diagnostic criteria may have been related to the increase in the reported proportion of patients with bilateral MD from the time of the third nationwide survey until last year in Japan.

Recent epidemiological studies have shown that the prevalence of MD increases with increasing age. In 2005, Havia *et al.*[7] reported a peak prevalence of 1709 per 100,000 in the age group of 61-70 years in Finland. In 2010, Harris and Alexander[9] described a prevalence of MD ranging from nine per 100,000 for patients under age 18 to 440 per 100,000 for patients 65 years and older. Because MD is a chronic non-lethal disease, cases of MD in the elderly comprise both long-standing cases that are reactivated and de novo cases.[5] In our previous studies, the proportion of MD patients aged 60 years and older increased significantly over time from the first to the fourth survey,[3-5] and the present study confirmed the recent tendency toward an increase in the proportion of elderly patients with MD.

5. Acknowledgement

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IDIOPATHIC ACUTE LABYRINTHINE DISEASES AND MÉNIÈRE'S DISEASE: THE NECESSITY OF A MULTIDISCIPLINARY APPROACH

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The persistent difficulty in finding a satisfactory and complete explanation for the etiopathogenesis of both Ménière's disease (MD) and other inner ear diseases is witnessed by the variety of the interpretations proposed and by the substantial lack of a specific therapy. As widely observed elsewhere, it is surprising how the related literature appears especially focused on the inner ear by itself, without sufficiently considering that its particular features imply a strict dependence on systemic conditions.

In 2009, our group outlined the necessity of a multidisciplinary approach to inner ear disorders,[1] starting from a series of observations about the influence of a hemodynamic imbalance on the labyrinthine homeostasis.[2-5] This influence is strongly supported by the characteristics of the inner ear blood supply, that is of terminal type and must support, with a small volume as compared to cardiac output,[6] a particularly high energy requirement; an additional not negligible factor is represented by the peripheral nature of this organ, and by the complexity of the regulatory mechanisms at the basis of its function.

Under these conditions, it is conceivable to postulate that a transient insufficient perfusion, even due to non-organic alterations (*e.g.*, an abrupt lowering of blood pressure levels followed by a brusque and exaggerated vasoconstriction), resulting in a more or less prolonged hypoxia can imply a real harm to the labyrinthine function.

Following this inference, a wide number of systemic affections may be considered as more or less directly involved in a possible inner ear acute dysfunction on the basis of an altered vasomotor function:[1] they range from chronic gastritis, that can be associated to microcirculatory disorders[7] to chronic renal

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disease, that can be linked to a sympathetic activity,[8] to a number of pathologic cardiovascular affections with different severity. An additional harmful factor must also be reported, and is represented by the cellular damage that can be due to various metabolic alterations as diabetes,[9] hyperuricemia,[10] hypercholesterolemia;[11] however, the most prominent factor of acute damage is represented, according to our studies, by the effect of sufficiently brusque and/or severe hemodynamic changes.[12,13]

In some previous recent papers,[14,15] we once more widely outlined the central role of an imbalance of autonomic nervous system in the genesis of these disorders. As a matter of fact, an autonomic imbalance may also be responsible for a series of organic diseases;[16] when deepening its possible consequences is not difficult to infer the crucial role of such an abnormal activity, that is notoriously responsible for ischemia/reperfusion phenomena in turn considered as a factor able to jeopardize also the inner ear.[17,18] From this starting point, it can be imagined a common damage mechanism shared with other organs, and related to the same alterations that are known to underlie a number of disorders of the latter;[18] as a consequence, it is also possible to imagine a common functional origin for inner ear 'idiopathic' and unexplained diseases including MD, the specific clinical picture depending on the extent and the duration of the hypoxic phenomenon: the particular sensitivity of a sensory organ as the inner ear could even permit a more detailed and rapid detection of the related symptoms.[19]

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TREATMENT

CURRENT STATUS OF THE TREATMENT PROCEDURE FOR THE PATIENTS WITH MÉNIÈRE'S DISEASE IN JAPAN – UNIQUE TREATMENT METHODS IN JAPAN

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Abstract

Ménière's disease (MD) is a disorder of the inner ear affecting hearing and balance to a varying degree. It is characterized by episodes of vertigo attacks lasting more than 20 minutes, low-pitched tinnitus, hearing loss and a feeling of fullness in the ear. There is currently no gold standard treatment for MD. A variety of medical and surgical treatments have been developed to treat or control the symptoms. Treatment can be divided into non-destructive and destructive procedures. Non-destructive methods aim to reduce the symptoms of MD through dietary restrictions as well as through the use of diuretics. Isosorbide, osmotic diuretic, is usually used to treat hydrocephalus and glaucoma. In Japan, isosorbide is also used for MD to improve the endolymphatic hydrops. As for destructive procedures, surgical decompression of the endolymphatic sac, surgical or chemical labyrinthectomy and vestibular nerve section are applied for intractable cases. Recently, improvement in vertigo and hearing in patients with MD were described after application of positive pressure to the middle ear using the Meniett device. However, in Japan, medical practitioners have been required to import the devices themselves to provide this middle ear pressure treatment because the Meniett device has not been cleared by the Ministry of Health, Labor and Welfare of Japan. Instead of a Meniett device, we are using the tympanic membrane massage (TMM) device as for middle ear pressure

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therapy. The usefulness of TMM to improve the vestibular symptoms and co-chlear symptoms in patients with MD and delayed endolymphatic hydrops as Meniett device is also reported in Japan.

1. Introduction

Ménière's disease (MD) is a disorder of the inner ear affecting hearing and balance to a varying degree. It is characterized by episodes of vertigo attacks lasting more than 20 minutes, low-pitched tinnitus, hearing loss and a feeling of fullness in the ear. There is currently no gold standard treatment for Ménière's disease. A variety of medical and surgical treatments have been developed to treat or control the symptoms. In this paper, unique treatment procedures for the patients with MD in Japan will be introduced.

2. Unique treatment methods for the patients with MD in Japan

2.1. Diuretic therapy in Japan

Treatment methods for MD can be divided into non-destructive and destructive procedures. Non-destructive methods aim to reduce the symptoms of MD through dietary restrictions as well as through the use of diuretics. Various types of di-uretic treatment have widely been used in MD to reduce excess accumulation of endolymphatic fluid. There are histological, biochemical and electrophysiological lines of evidence to support the efficacy of osmotic diuretics in the treatment of MD.[1-6] In particular, glycerol induces the most marked hearing improvement and is commonly used as a test substance to detect endolymphatic hydrops.[8] Histologically, glycerol-induced collapse is followed by a rebound increase in the endolymphatic compartment.[7] Due to these rebound effects, glycerol is not used therapeutically. Isosorbide, osmotic diuretic, is usually used as a diuretic mainly to treat hydrocephalus and glaucoma. In Japan, isosorbide is also used for MD to improve the endolymphatic hydrops. Clinically, isosorbide has been confirmed to be effective for the symptoms of dizziness, headache and tinnitus.[3] Further, hearing improvement, though in a limited number of cases, is expected with isosorbide.[8,9] Kakigi et al.[10] investigated the rebound phenomenon occurring with isosorbide using guinea pigs underwent surgical obliteration of the endo-lymphatic sac. Isosorbide reduced the cochlear endolymph volume, with a peak reduction six hours after intake. Thereafter, no prominent rebound phenomenon was noted. They concluded that in clinical use, the rebound phenomenon need not be considered since isosorbide is usually administered orally every eight hours.

Fig. 1. Tympanic membrane massage (TMM) device.

2.2. *Middle ear pressure treatment in Japan*

As for destructive procedures, surgical decompression of the endolymphatic sac, surgical or chemical labyrinthectomy and vestibular nerve section are applied for intractable cases. During the 1970s, Inglestadt *et al.*[11] observed that some patients reported improvement with pressure changes in a pressure chamber. Densert *et al.*[12] showed that manipulation of the middle ear pressure influences inner ear pressure. Later, improvement in vertigo and hearing in patients with MD were described after application of positive pressure to the middle ear. These reports eventually led to the development of the Meniett device. However, in Japan, medical practitioners have been required to import the devices themselves to provide this middle ear pressure treatment because the Meniett device has not yet been cleared by the Ministry of Health, Labor and Welfare of Japan. The tympanic membrane massage (TMM) device (Fig. 1) is an intermittent pressure treatment device initially used in western European countries in the late nineteenth century for patients with otitis media with effusion (OME) and without a tympanotomy. In Japan, the TMM device has been cleared for use in patients with OME by the Ministry of Health, Labor and Welfare of Japan, and currently medical practitioners can buy TMM device from a domestic supplier. Recently, TMM was used for the patients with MD and delayed endolymphatic hydrops as a middle ear pressure treatment instead of Meniett device in Japan. But it was not clear whether the TMM device is as effective on intractable MD and delayed endolymphatic hydrops as Meniett device. The TMM device is an electronically-controlled, low-pressure pulse generator that delivers pressure oscillating with a frequency of about 7 Hz. The intensity of the peak pressure can be controlled within 20 cmH$_2$O by the power control, *i.e.*, the output conditioning

dial, on the front panel. The mean peak amplitude of the pressure pulse is set to be almost equal (about 12 cmH_2O) to that in the Meniett device. The positive-negative pressure pulses are delivered to the ear canal through a rubber tube with a close-fitting plastic ear cuff covered by rubber. The pressure applications are always delivered continuously for three min. The TMM device delivers air pressure to the ear canal similarly to the Meniett device. Both devices produce an intermittent low pressure pulse within 20 cmH_2O. In the Meniett device, the peak positive middle ear pressure is 12 cmH_2O.[13] In the TMM device, the peak positive middle ear pressure is estimated to be 9 cmH_2O, while the peak negative middle ear pressure is estimated to be -5 cmH_2O. In both devices, it is thought that the middle ear pressure does not exceed the opening pressure of the Eustachian tube.[14] The frequency of the pressure oscillation is 6 Hz (Meniett device) or 7 Hz (TMM device). The pressure of the TMM device is biphasic (positive-negative pressure), while that of the Meniett device is monophasic (only positive pressure).

Watanabe *et al.*[15] investigated the efficacy of the TMM device on intractable vertigo in patients with MD and DEH. They compared the effects of pressure treatment between the TMM and Meniett device groups. Regarding vertigo outcomes in particular, all the patients in both groups experienced either complete or substantial control in the 7-12-month period after treatment. According to the Japan Society for Equilibrium Research (JSER) guidelines,[16] they calculated two types of numerical values (NVs) with the following formula: NV = average number of definitive spells per month in either the l-6-month period or the 7-12-month period after treatment/average number of definitive spells per month in the six-month period before treatment. In addition, remissions were defined as six consecutive months with no definitive spells.[17] The distribution of the NV according to the JSER guideline was not significantly different between the two groups. In addition, the time course of vestibular symptoms (*i.e.*, the frequency of vertigo per month and the period required for remission) was also not significantly different between the two groups. Hearing outcomes remained stable in the majority of patients (TMM device group, 92%; Meniett device group, 81%) in the 7-12 month period after treatment. The distribution of hearing outcomes according to the JSER guideline was not significantly different between the two groups. Because the efficacy of vestibular symptoms in the TMM device group is similar to the Meniett device group, they concluded that the TMM device might be a worthwhile option before use of tympanotomy tubes.

3. Conclusion

In this article, the current status of the treatment procedure for the patients with MD in Japan was introduced. As for diuretics, instead of acetazolamide, we usually used isosorbide, osmotic diuretics, for the purpose of improving endolymphatic hydrops for the patients with reduce MD and DEH. We also uses TMM device for the patients with MD and delayed endolymphatic hydrops as

a middle ear pressure treatment instead of Meniett device in Japan. The TMM device is also effective on intractable MD and delayed endolymphatic hydrops as Meniett device.

Acknowledgement

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ANTISECRETORY FACTOR AND MEDICAL FOOD – NOVEL THERAPY CONCEPTS

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The anti-secretory factor (AF) is a protein secreted in plasma and other tissue fluids in mammals. This 41-kDa protein provides protection against diarrheal diseases and intestinal inflammation. The AF protein is induced by cholera toxin and was first isolated and characterized by Swedish researchers Lange and Lönnroth in the 1980's. Immunocytochemistry has shown that AF is present in most tissues in the body.[1]

The endogenous plasma level of AF is increased by enterotoxins and surprisingly also by certain food constituents.[2] Based on these findings, AF-inducing medical and functional food and feed products have been developed by the Swedish R&D company Lantmännen AS-Faktor AB. SPC is an AF-inducing medical food, but the company has also developed an AF-rich egg yolk powder, Salovum (B221).[3]

SPC is short for Specially Processed Cereals. Tests with this product showed it to be effective in reducing diarrhea in various animal species. In human clinical trials SPC stimulates the production of the AF protein in patients suffering from IBD, reduces their symptoms and improves their quality of life.

In further clinical trials in Crohn's disease, secretory diarrhea and short bowel syndrome, SPC has been shown to exert both antisecretory effects (by inducing AF) and anti-inflammatory effects.

Because of the effects on hypersecretion in the GI tract, it was hypothesized that antisecretory treatment with SPC could be valuable in other instances where fluid imbalance is thought to play a role, such as Ménière's disease.

In an open pilot study, 24 MD patients received SPC for 14-30 days, and AF levels in plasma increased in 83% of the patients. Seventeen percent of the patients had no or very low increase of AF in plasma and none of these were improved. The attacks of rotatory vertigo were reduced in 12 patients and in three of these hearing was normalized. Studies in rats using immunohistochemical

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methods demonstrated that AF was localized to the cochlea and the vestibule of the inner ear, which led the authors to propose that AF could be a new regulator of endolymph.[4]

The second Swedish study was randomized, double-blind and placebo-controlled. Fifty-one adult patients with MD were included. Twenty-seven subjects were treated with SPC (one g per kg body weight per 24 hours in two servings) and 24 with control cereals for three months. The severity of MD was classified according to the American Academy of Otolaryngology – Head and Neck Surgery (AAO-HNS) grading system. Fourteen of the 27 patients in the SPC group reported decreased vertigo, compared to only two of 24 in the control group ($p < 0.001$). There were no side effects reported.[5]

The Swedish researchers have further demonstrated that an SPC diet fed to rats increases plasma levels of antisecretory factor compared to a standard rodent diet.[6] In a follow-up study, SPC was fed to rats in dietary concentrations of 5%, 10% or 15% for two weeks, and the AF activity in plasma increased in a dosage- and time-dependent manner.[7]

The Swedish results attracted the interest of a British research group, who performed a randomized, double-blind, cross-over and placebo-controlled study with SPC in 39 patients with Ménière's disease. The outcome measure was the AAO-HNS Functional Level Scale (FLS). The FLS score improved significantly after SPC, and the treatment was well tolerated by 91% of the patients. Fifty-nine percent of the participants reported an improvement in functional level.[8]

The presence of AF in the inner ear has been studied by Chinese researchers, who found that the range of localization of AF overlaps the distribution of aquaporin 1 (AQP 1) and aquaporin 2 (AQP 2). The authors suggest that an interaction between AF, AQP 1 and AQP 2 might be possible.[9] Aquaporins 4 and 5 have recently been implicated in the water permeability of the mammalian cochlea.[10]

Today, the antisecretory factor protein (AF) is recognized as a fundamental regulator of fluid balance in mammals. The relevance of water dynamics for Ménière's disease point the way for further clinical studies with AF-rich (Salovum, B221) and AF-inducing (SPC, SPC-Flakes) medical food products.

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ANTISECRETORY FACTOR-INDUCING THERAPY IMPROVES PATIENT-REPORTED FUNCTIONAL LEVELS IN MÉNIÈRE'S DISEASE

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1. Introduction

The myriad of treatments available to patients with Ménière's disease (MD) and the varying responses to treatment underscore the lack of knowledge about its pathophysiology. It has been hypothesised that the symptoms of MD are secondary to endolymphatic hydrops. Several studies have focused on the function of the endolymphatic duct and sac in the pathogenesis of hydrops, and there is histopathologic evidence that suggests decreased absorption or stimulation of secretion of the endolymph in these tissues.

The aim of this study was to evaluate the effectiveness of specially processed cereal (SPC) as a suitable adjunctive treatment for MD. Antisecretory factor (AF) is a 41 kd protein produced in the brain, gallbladder, lungs, kidneys, and intestine in response to infection. It has been postulated that AF acts as a modulator of water and ion transport by regulating chloride homeostasis and thereby counteracting excessive fluid secretion into the intestinal lumen. Interest in AF was spurred by the need to find a replacement for antimicrobial growth promoters in pig feed, as these were banned throughout the European Union in 2006 because of concerns about the risk of inducing antibiotic resistance.

Antimicrobial growth promoters had been used in animal diets to improve growth performance and prevent the side effects of early weaning such as infectious gastrointestinal and respiratory diseases. Endogenous AF synthesis can be stimulated by dietary modifications in animals and humans. Ingestion of specially processed cereal (SPC) that was optimised for increasing endogenous AF synthesis was shown to significantly increase plasma levels of AF in clinical trials, and the levels remained raised for four weeks after termination of the trial. Patients with long-standing symptoms of inflammatory bowel disease who

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received SPC reported improved clinical symptoms in comparison to a group of patients treated with placebo cereals.

2. Methods

We performed a randomised double-blinded, placebo-controlled, crossover study in a tertiary referral centre of patients who had a diagnosis of MD based on the guidelines of the Committee on Hearing and Equilibrium of the American Academy of Otolaryngology – Head and Neck Surgery (AAO-HNS). The main outcome measure was the AAO-HNS Functional Level Scale (FLS).

3. Results

Thirty-nine patients completed the study without any reported complications. The mean pre-treatment FLS score for the entire study cohort was 3.8 (median, 4; range, 1 to 6). The overall FLS score improved significantly ($p < 0.001$), to 2.8 (median, 3), after SPC treatment. No patients showed worsening on the FLS during SPC or placebo treatment. Of the 39 patients, 23 showed improvement on the FLS, and no change was observed in the remaining 16. The median improvement on the FLS in these 23 patients was 2 points (mean, 1.7; range, 1 to 4). The mean FLS score after placebo cereal treatment was not significantly different from baseline ($p = 0.452$), but was significantly higher than that after SPC treatment (mean, 3.7; $p < 0.001$). The marginal difference observed between the baseline FLS score and the placebo FLS score was due to the fact that five patients reported 1-point improvements on the FLS after placebo treatment. Nevertheless, significantly fewer patients improved on placebo than on SPC ($p < 0.001$).

Table 1. Efficacy Index of combined therapy in MD total sample and in the subgroup of MD patients without and with migraine.[1]

	Efficacy Index (EI)			
	< 25%	25%-50%	50%-75%	> 75%
MD total sample (n=25)	8 (32%)	8 (32%)	5 (20%)	4 (16%)
MD subjects without migraine (n=14)	4 (28%)	7 (50%)	1 (8%)	2 (14%)
MD subjects with migraine (n=11)	3 (27%)	2 (22%)	3 (27%)	3 (27%)

4. Conclusions

Treatment with SPC appears to be well tolerated by most patients (91%) without any complications. More than half (59%) of the study cohort reported subjective improvement in functional level.

The alternative to SPC may lie in Salovum egg yolk powder, which contains protein with antisecretory properties in a much higher (500 times) concentration than is found in normal hen eggs. It is made by feeding hens with SPCs capable of inducing production of protein with antisecretory properties in the yolk, from which an egg powder is produced. In view of its apparent antisecretory and anti-inflammatory effects, Salovum egg yolk powder was given to children with diarrhoea. After three days of treatment, their diarrhoea was significantly less frequent and severe than that of a group given placebo powder. It is possible that Salovum eggs or egg yolk powder could be a viable alternative to SPC.

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SPC-FLAKES® IN THE PROPHYLAXIS OF MÉNIÈRE'S DISEASE

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1. Introduction

A raised endolymphatic pressure (hydrops) is commonly accepted as the causal factor of episodic vertigo associated with hearing loss, fullness and tinnitus in Ménière's Disease (MD); nonetheless the etiology of the disorder is at present poorly known.[1] Probably as a consequence of this, a myriad of medical treatments have been proposed, including steroids, betahistine, calcium channel blockers and diuretics, none of them at present with a conclusive scientific evidence of their efficacy.[2] Recently, different papers focused on the possibility that fluid and ion homeostasis in the inner ear, possibly related to genetic factors, may play a role in the occurrence of MD.[3,4] Antisecretory Factor (AF) is a 41 kd protein produced mainly in pituitary gland in response to infection. It has been postulated that AF may act as a modulator of water and ions by regulating chloride homeostasis through membranes, and an interaction with aquaporins has been claimed.[5] In previous immunohistochemical studies, the AF and binding protein flotillin-1 has been demonstrated in the inner ear and cochlea of rat and humans.[6,7] SPC-Flakes® are especially processed cereal optimized to increase endogenous AF plasma levels. A 14-28 days-long period of intake of SPC-flakes® is necessary to obtain a significant AF plasma concentration, commonly followed by a positive clinical outcome. However, an increased AF response is achieved after only two to three days after a second period of intake of specifically processed cereals. Thus, there exists some sort of a 'biological memory' for the human capability of AF synthesis, and the first period of intake of specifically processed cereals, seems to be responsible for 'priming' of the secondary, enhanced AF response.[8]

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Recently, different authors reported a significant reduction of vertigo spells in MD patients treated with SPC-Flakes® ranging between 50% and 60%,[9,10] significantly other authors correlated the reduction of episodes of vertigo with an increase of AF plasma levels in MD patients performing the therapy.[11] The aim of our work was to assess the efficacy of the treatment with SPC-Flakes® in a sample of patients with definite MD and poorly responsive to betahistine.

2. Materials and methods

In the period between September 2014 and April 2015, 25 patients with definite MD according to AAO-HNS criteria[12] were enrolled in two different university centers in Italy.

They were enrolled among patients poorly responsive to betahistine (48 mg/day) and dietary measures (low salt and increased water intake) in the previous six months. Exclusion criteria were ongoing therapies with calcium channel blockers, beta blockers, diuretics and drugs active on Central Nervous System (SSRIs and benzodiazepines among them) or previous intratympanic injection with steroids or gentamicin.

Fifteen (60%) were females. The age at inclusion was 46.9 ± 7.7 y.o., while the age at the first vertigo attack was 41.8 ± 7.2 y.o. Eleven (44%) also referred headaches with migrainous features (pulsatile, associated with phono and photophobia, disabling and lasting hours).

Two of them (one male and one female) referred a positive familial history for MD, respectively for father and brother.

During the following three months they underwent a combined therapy with betahistine and SPC Flakes® (1 gr/Kg day). Dietary measures were observed.

Main outcome was considered the number of vertigo spells (per month) in the six months before therapy with SPC-Flakes® and in the three months of combined therapy. Moreover, an Efficacy index (EI) was calculated with the formula y/x per 100, where y is the number of vertigo spells (per month) during the three months of combined therapy and × the number of vertigo spells (per month) in the six previous months. Best therapeutic results are identified by lower numbers.

3. Results

The number of vertigo spells decreased from 1.8 ± 0.7 to 0.6 ± 0.3 per month during combined therapy ($p \leq 0.001$). On the opposite hearing level resulted unvaried in all subjects. Eight patients (32%) presented a lower than 25% EI, eight (32%) in the range between 25% and 50%, five (20%) in the range between 50% and 75% and four (16%) higher than 75%. On the total sample 16 out of 25 (64%) subjects presented a reduction of vertigo spells of at least 50%.

Table 1. Efficacy index of combined therapy in MD total sample and in the subgroup of MD patients without and with migraine.

	Efficacy index (EI)			
	< 25%	25%-50%	50%-75%	> 75%
MD total sample (n = 25)	8 (32%)	8 (32%)	5 (20%)	4 (16%)
MD subjects without migraine (n = 14)	4 (28%)	7 (50%)	1 (8%)	2 (14%)
MD subjects with migraine (n = 11)	3 (27%)	2 (22%)	3 (27%)	3 (27%)

Although non-migraineurs obtained better therapeutic results, no statistical difference was detected between MD patients with and without migraine, since five out of 11 (45.5%) migraineurs referred a reduction of vertigo spells of at least 50% while 11 out of 14 (78.5%) of non-migraineurs (p = 0.08). The results are summarized in Table 1.

Both patients with a familial history of MD presented a lower than 50% EI (30% and 50% respectively); both were migraineurs.

4. Discussion

Our work is mainly based on clinical observation and personal experience about therapeutic results of SPC-Flakes© on a sample of MD patients poorly responsive to betahistine, and no conclusive data can be drawn. Possible bias should be considered. Firstly, MD often present an unpredictable evolution and vertigo spells may be clustered in specific periods followed by months of absence of attacks; moreover, results may be influenced by the positive expectation of the patients with a consequent higher compliance toward the therapy.

Another potential limitation may be due to the poor number of our sample and the lack of a control group performing placebo. Finally, the limited time of observation prevented from collecting data on maintenance of therapeutic results.

Nonetheless, some interesting results may be underlined. The combined therapy demonstrated to be useful in 64% of subjects and our results are on line with previous works,[9-11] referring therapeutic benefits in 50%-60% of patients; in one of them data were collected in a randomized double-blind versus placebo study. Moreover, none of our patients referred side effects.

Although not statistically significant, a better trend for reduction of vertigo spells has been observed in non-migraineurs.

Mechanisms leading to hydrops remain at present uncertain, and previous papers focused on the possibility that hydrops is the consequence rather than the causal factor of symptoms.[13] Among possibilities, mechanisms related with water and ions homeostasis in the inner ear have been considered.[3,4,14] The AF demonstrated to be able to modulate water balance and ionic transport through membranes, possibly with a synergic action with aquaporins, widely studied in MD subjects; it should be also considered that other factors involved in the

regulation of the ionic transporters have been previously proposed as a predisposing factors to MD.[15]

5. Conclusion

Our results support the hypothesis that increased plasma levels of AF may decrease possibilities of vertigo spells in MD subjects and SPC-Flakes© may be considered among therapeutic possibilities in prophylactic therapy in MD.

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MÉNIÈRE'S DISEASE PATIENTS IN THE ACUTE STAGE

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Ménière's Disease (MD) is an idiopathic inner ear disorder characterized by spinning dizziness, fluctuating hearing loss (HL), ear fullness and tinnitus. The natural history of the disease is characterized by variable periods of exacerbation and remission of symptoms, although cochlear symptoms can also be observed among episodes. The hallmark of an acute attack is prolonged vertigo. Each episode of vertigo is characterized by a sudden unheralded intense sensation of movement, most commonly rotation or spinning, lasting 20 minutes to 12 hours.[1] Because of the brief duration of each attack the clinical examination of a patient with MD is often performed during the inter-critic period and no abnormalities are found. In the few cases where patients have been observed during a crisis, they appear quite unwell because of the unpleasant sensation of vertigo. They may be sweaty and pale, unable to stand up safely, nauseated and vomiting. They may have an horizontal nystagmus that changes direction as the attack progresses (beating toward the side of the disease at the beginning and toward the safe hear afterwards). An augmented amplitude in the contralesional of n10 of the ocular vestibular evoked myogenic potentials (oVEMP) is a behavior of the disease when this test was recorded.[2] Following an attack, patients are left with a sense of 'hangover' for a day or two before recovering to a normal function.

The treatment of MD remains one of the most controversial areas in the field of otolaryngology. The most significant barrier to define treatment is a lack of understanding of its underlying etiology and a variety of therapeutic options have been studied with conflicting results.

Once an attack is established, little can be done to alter its natural course. Vestibular suppressant and antiemetic medication have been used to control the vertigo, in association with electrolyte adjustment and rehydration. Because of the nausea and vomit complaints by the patient, the most adapted drug and form

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Table 1. Acute management of Ménière's disease.

Rest
Electrolyte adjustment
Rehydration

Main classes of drugs	Effect	Molecule	Side effects
▪ **Phenotiazine**	Anticholinergics with sedative and antiemetic activity on Chemo receptor zone	Promethazine Prochlorperazine Thiethylperazine	Extrapyramidal effects (dyskinesia) No association with neuroleptics and anticonvulsants
▪ **Antihistamines with anticholinergic activity**	Sedative and antiemetic activity	Scopolamine Dimenhydrinate Meclyzine	Blurred vision, drowsiness, glaucoma, Urinary retention
▪ **Benzodiazepines**	Vestibular suppressant and anxiolytic activity	Diazepam Clonazepam Lorazepam	Sedation
▪ **Antidopaminergics**	Antiemetic activity	Metoclopramid Levosulpiride	Extrapyramidal effects (dyskinesia), sedation, hypotension or endocrine dysfunction.

available should be the choice. Medications can be divided into different classes, including benzodiazepines, antihistamines, anticholinergics and antidopaminergics (Table 1); also calcium channel blockers may be used as vestibular suppressant, although their role in this context has not been cleared yet. Moreover, because of the possible autoimmune origin of MD, the use of oral or intratympanic corticosteroids has been also proposed both to reduce the acuity of the crisis and to promote the audio-vestibular recovery.[3] Last but not least is described the use of osmotic diuretics (mannitol, glycerol, etc.) administered intravenously. The rationale for their use is based on the supposition that these drugs can alter the fluid balance of inner ear, leading to a depletion of endolymph and a correction of hydrops. A study of 1982, to our knowledge the only one on this topic, described a greater efficacy of glycerol than mannitol in the acute phase.[4]

Benzodiazepines act on the cerebellar GABAergic system that inhibits vestibular nuclei response and on glycine receptors of the vestibulospinal reflex that regulate the postural tone.[5,6] Among this class, diazepam, clonazepam and lorazepam are the most used; molecules with extended release should be avoided in the treatment of vertigo. Because of their vestibular suppression as well as anxiolytic properties, benzodiazepines are favored for example in United States; however, their use should be limited because of their impairment on vestibular compensation increasing the risk of falling.

Antihistamines used in the control of vertigo, including meclizine and dimenhydrinate, have demonstrated efficacy in MD when compared to placebo, because of their potent antivertiginous and antiemetic activity also given by their anticholinergic properties.[7-9] Dimenhydrinate can be administered intra-

muscularly and intravenously; a typical side effect of this drug is its propensity to cause drowsiness. Meclizine is one of the most useful antiemetics to prevent and treat nausea and vomiting associated with vertigo of vestibular origin. Side effects of this drug include blurred vision and drowsiness. Scopolamine is a naturally occurring belladonna alkaloid with anticholinergic properties commonly used also to prevent nausea and vomiting associated with motion sickness; the availability of a transdermic administration allows to bypass the stomach and is a valid option for patients with nausea and vomiting. Because of their anticholinergic properties, care must be taken in using this class of molecules in patients with glaucoma or prostate disease; the dryness of the mouth constitutes another side effect.

Antidopaminergics like metoclopramid or levosulpiride can be administered orally, parenterally or rectally and therefore offers a convenient alternative in the acute setting. They reduce nausea and vomiting because of their action on the chemoreceptor trigger zone (CTZ). These drugs have fewer incidence of side effects like extrapyramidal symptoms (dyskinesia), sedation, hypotension or endocrine dysfunction.

Phenothiazines show central and peripheral anticholinergic activity and sedative and anti-emetic properties because of their action on the CTZ. Promethazine, a phenotiazine derivate with anticholinergic, antihistamine and antidopaminergic activity, has antiemetic and anxiolytic properties. This, coupled with versatility of administration and extremely low rate of extrapyramidal reaction, makes it an effective treatment in acute MD. Similarly, prochlorperazine and thiethylperazine belong to the phenotiazine group and may be used in the acute setting to treat severe nausea and vomiting. All these molecules can be administered rectally thanks to the availability of a suppository form that makes them extremely useful in an acute crisis in a patient suffering from nausea and vomiting and unable to take medications orally. Care must be taken in administrating thiethylperazine in patients using neuroleptics and anticonvulsants. In cases of psychomotor agitation, another phenothiazine derivate, promezine, can be used.

Calcium channel blockers show vestibular suppressant activity because of their anticholinergic and antihistamine properties.[10] Moreover, they may act on the calcium channel of the vestibular dark cells modifying the ionic concentration in the endolymph.[11] Side effects are tremors, drowsiness, weight gain and depression (usually only for long-term treatment). Available in this category are flunarizine and cinnarizine (not in the USA) and above all nimodipine and verapamil; these latter have been proposed in treating vertigo of vestibular peripheral origin[12] and MD particularly.[13]

All the molecules described above, separately or in combination, constitute a possible treatment during a crisis of MD, although to date there is no consensus on the recommendation of any of these drugs, or strong evidence of their effectiveness. The method of administration depends on the development of vegetative symptoms and the availability in the formulation of each molecule, and it could be orally, intramuscularly, intravenously or rectally. We underline how extremely

important it is to remember that, because of the action inhibiting the vestibular compensation of the most of these drugs, their use should be limited to the acute phase and stopped as soon as possible. In this phase a molecule as betahistine, that is described to favor vestibular compensation because of its action against H3 receptors, may play an important role and help patients to recover quicker after each episode.[14] Last but not least, we recommend rest, avoiding stress and exercise, hydration and electrolyte adjustment (especially in case of vomiting) as a therapeutic adjuvant in the acute setting.

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MÉNIÈRE'S DISEASE PATIENTS IN THE REHABILITATIVE STAGE

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1. Background

Ménière's disease (MD) is a clinical syndrome characterized by episodes of vertigo attacks associated with unilateral fluctuating sensorineural hearing loss, tinnitus and aural fullness. Vertigo and dizziness and the associated neuro-vegetative symptoms (nausea, vomiting) are extremely disabling for MD patients, who may show increased anxiety and depression, and they lead to dramatic socio-professional consequences. It is therefore necessary to stop vertigo quickly in the rehabilitative stage, and to reduce thereafter the frequency of the vertigo attacks and the anxiety level as well. Unfortunately, MD is an idiopathic aural and multifactorial disorder that still lacks an ideal management.

Several hypotheses have been proposed in the literature to explain the onset of the Ménière's attacks: (1) the endolymphatic hydrops (EH) in the affected ear, observed on autopsy in temporal bones,[1] is ubiquitous in MD and seems necessary but not sufficient to induce the vertigo attacks; (2) the impaired micro-circulation within the inner ear (ischemia) resulting from sudden large increases in endolymph pressure (the vascular theory);[2] (3) the elevated potassium level in the perilymph, due to EH-induced rupture of the Reissner's membrane, and inhibiting the hair cell transduction (the potassium intoxication theory).[3] However, EH *per se* does not explain all clinical features, and the vascular and potassium intoxication theories have been seriously criticized. The hypothesis of an ischemia/

reperfusion disorder of the inner ear sensory tissues has been proposed more recently.[4] According to this new model, vascular risk factors would combine with EH to cause the MD attacks. Three major interacting factors could be involved: a pre-existing EH on the affected side, a lowered threshold for intracerebral and intra-aural ischemia during the vertigo spells, and a differential sensitivity of the aural tissues to ischemia. Indeed, most animal models of EH do not induce vertigo spells,[5] but the attacks can be triggered in these models by middle ear injection of epinephrine[6] or blocking the vein of the vestibular aqueduct[7], both procedures that impair perfusion pressure.

2. Rehabilitation of MD patients

Today there is no ideal management of MD because specific treatments that should target the underlying causes of vertigo are still lacking, and because few well designed, placebo-controlled, double-blind, randomized clinical trials are available. However, three components must guide the rehabilitation of MD patients: the symptomatic treatment of acute vertigo and its associated nausea and vomiting symptoms in the acute stage, the prevention of future episodes, and the improvement of the recovery of the impaired vestibulo-ocular and vestibulo-spinal functions. The pharmacotherapy approach, the destructives procedures, and the vestibular rehabilitation therapy are the main ways to achieve these goals (Fig. 1).
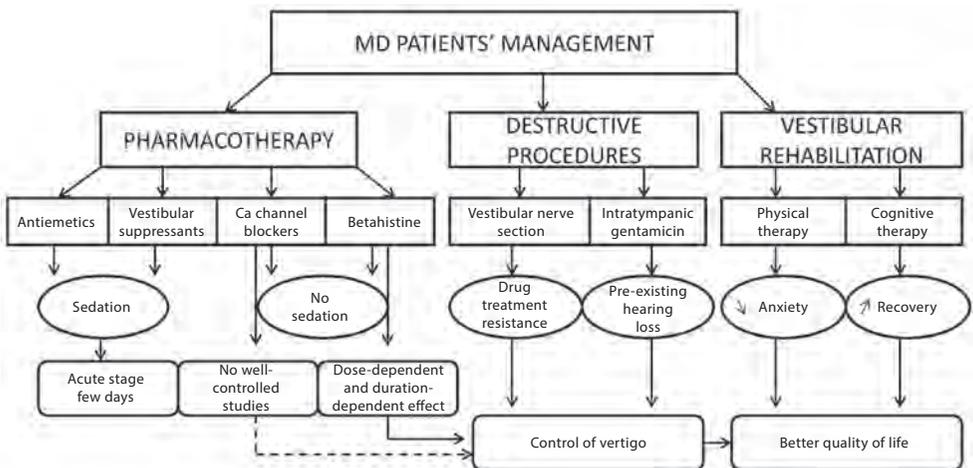

Fig. 1. Schematic illustration of the main approaches used to manage MD patients.

2.1 Pharmacotherapy

A wide variety of drugs are used to treat vertigo and the frequently concurrent nausea and vomiting in many peripheral vestibular pathologies including MD.[8-10] Vestibular suppressants and antiemetic medications are drugs modulating the cholinergic system (scopolamine, atropine), or the histaminergic system (dimenhydriate, promethazine), or the GABA system (benzodiazepines). They must be used for a few days only because of their side effects (sedation, drowsiness) that delay the spontaneous vestibular compensation and slow down the recovery process. On the other hand there are medications acting on voltage-gated ionic channels like the calcium channel blockers (nimodipine, flunarizine, cinnarizine). These drugs alter the neurotransmitter release at synaptic terminals, have no sedative effects, and because they act as blockers of the pressure sensitive potassium channels, they may be relevant when inner ear endolymphatic hydrops develop.[11] Unfortunately, no controlled investigations have evaluated carefully the therapeutic effect of these drugs.

Betahistine, an analogue of histamine with strong inverse agonistic action on the H3 histamine receptors (and weak H1/H2 agonistic actions), is currently used in the European countries for the management of MD. It significantly reduces the incidence and severity of vertigo and controlled studies reported that betahistine was the most effective when compared to cinnarizine, flunarizine or Ginkgo biloba (see Refs 8, 9). In MD patients, a combination of betahistine (32 mg/day for six months) plus nimodipine (40 mg/day for six months) showed a significant improvement compared to monotherapy with either drug.[12] Betahistine efficacy can be explained by mechanisms targeting the histamine receptors at three different levels: the vascular tree, the central nervous system, and the peripheral labyrinth.[13] Indeed, investigations in animal models showed that betahistine increased cochlear blood flow,[14] increased histamine turnover in the central nervous and vestibular systems,[15] and decreased vestibular input in the peripheral vestibular system.[16,17] In MD patients, betahistine reduced the intensity and frequency of vertigo spells significantly,[18] and in the only investigation reflecting the state of the art (*i.e.*, randomized, double-blind, placebo-controlled), betahistine treatment strongly accelerated the recovery of posture and balance functions.[19] It must be mentioned, however, that the therapeutic effect of betahistine is dose- and duration-dependent, as demonstrated both in animal models[14,20] and MD patients.[18,21]

High doses of betahistine (at least 48 mg/day) and long-term betahistine treatment (six to nine months) seem two necessary requirements for the prophylactic treatment of MD and the improvement of vestibular compensation.[11] This could be explained by an accumulation process in the brain, and/or an enhanced and prolonged effect in time by betahistine metabolites (manuscript in preparation), which have the same affinity at rodent H3 receptor binding sites than betahistine.[22] Interestingly, improvement of labyrinthine microcirculation was observed in guinea pigs *in vivo* receiving aminoethylpyridine and hydroxyethylpyridine,[23]

two major metabolites of betahistine that alter also the output of the vestibular sensory organs[17] and the histamine turnover.[22] According to the new theory of the Ménière attack as an ischemia/reperfusion disorder of inner ear sensory tissues,[4] betahistine could therefore regulate and normalize the reduced perfusion pressure in the inner ear of patients with EH.

2.2 Destructive procedures

If relief of vertigo associated with MD cannot be obtained with medical treatments, vestibular nerve section (VNS) can be proposed using microscopic technique described by William House (and popularized in the 1980s with the retrolabyrinthine and the retrosigmoid-internal auditory canal approaches). High rates of vertigo control and hearing preservation were obtained with the VNS techniques, but uncommon risks associated with the surgery (bleeding, meningitis, cerebrospinal fluid leak) lead many otologists to prefer gentamicin injections as the first line treatment for MD patients. The first results with intratympanic aminoglycoside administration were published in 1956,[24] and since this publication hundreds of reports have been published. They showed that intratympanic gentamicin significantly decreases the vertigo attacks, a result supported recently by a Cochrane analysis.[25] Limitation of this ablative treatment is impairment of hearing in about 20% of the MD patients,[26] the reason why it is recommended to treat only patients with pre-existing hearing damage.[10]

    When AAO-HNS criteria were used to compare the two ablative treatments, the vestibular nerve section has a higher rate of excellent vertigo control (92%) than intratympanic gentamicin (66%), but the post-treatment balance dysfunction rates were similar with the two treatments (56% and 53%, respectively).[27] However, it must be kept in mind that the scores regarding these two destructive procedures may vary considerably depending on the outcome measures (performed at different follow-up times), the natural history of the MD patients (severity and frequency of the attacks), and the protocols used (surgery approach, concentration and number of gentamicin injections). The MD patients have to know the different complication profiles and efficacy rates of these destructive procedures regarding vertigo control and remaining hearing, to evaluate the risks, and to choose.

2.3 Vestibular rehabilitation

Pharmacotherapy prescribed in MD patients must be considered in its relationship to central compensation. Indeed, the spontaneous or naturalistic compensation of the vestibular deficits is not optimal and, in addition, it can be impacted negatively by drug treatments. Symptomatic therapies for nausea and vomiting (antiemetics) and for acute vertigo (vestibular suppressants with sedative side effects) are known to impair the central compensation in the vestibular nuclei and to slow down the recovery process. Vestibular rehabilitation (VR) therapy can help both to counteract pharmacotherapy side effects and to optimize the

central compensation. Based on a number of highly quality randomized controlled trials, the Cochrane DataBase[28] indicates that VR is a safe and effective management for unilateral peripheral vestibular dysfunction. The ten recommendations we have recently published for optimal functional recovery in vestibular loss patients[29] remain available for MD patients.

The emotional state is particularly altered in MD patients who experience frequently vertigo attacks. The clinician's impression and psychometric and psychiatric studies as well have confirmed that patients with vertigo and dizziness often suffer from anxiety and depressive components.[30] Using the Short Anxiety Screening Test, the Dizziness Handicap Inventory Test, and subjective scales aimed at examining the fear of height and avoidance of risky situations, we showed that MD patients had significantly higher scores compared with healthy subjects.[31] Patients with vertigo in general showed also higher scores as measured by the hospital anxiety and depression scale.[32] VR therapy significantly ameliorates all emotional aspects in chronic vestibular patients,[33] and vestibular exercises improve also gaze stabilization and balance control. By improving the psychological symptoms, VR therapy increases patient confidence and provides reassurance, both factors that participate to regain a good quality of life for MD patients.

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INTRATYMPANIC TREATMENT

FIFTEEN YEARS OF INTRATYMPANIC PRESSURE TREATMENT FOR DISABLING MÉNIÈRE'S DISEASE

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1. Introduction

Pressure treatment is regarded to be one of the therapeutical options to offer for disabling Ménière's disease (MD) when the patients are not responding to any medical treatment. As a matter of fact, after the first experimental investigations of the 1970s,[1] preliminary clinical studies were emphasizing the positive role played for MD by a pressure treatment performed inside a chamber pressure[2-4] as well as by means of a portable device.[5,6] Further experimental support was given, during the same period, from the studies by Sakikawa and Kimura[7] and Kimura and Hutta[8] that could demonstrate a positive effect of the simple middle ear ventilation on limiting the degree of endolymphatic hydrops experimentally-induced on Guinea pigs.

The Meniett® is a portable device that allows a patient to self-administer the treatment at home, whenever it is required. Although the mechanism at the basis of its functioning still remains to be elucidated, an increased oxygenation of the inner ear, a direct stimulation of baroreceptors at the round window level or an impulse to the longitudinal flow of endolymph towards the endolymphatic sac have been taken into consideration.

Since 1999, Meniett® has become at our Center the pivotal treatment for MD recalcitrant to any medical treatment. Due to our policy to select conservative procedures without risking additional damage to the inner ear, such as when applying intra-tympanic gentamicin, this pressure therapy started to be always proposed to those Ménière's subjects already selected for our gold standard surgical

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procedure, *i.e.*, vestibular neurectomy. After more than 15 years, Meniett® still plays the same role in our practice so that the purpose of the present report is:

- to present long-term data on a consistent series of subjects;
- to comment on the role of this local pressure treatment in the staircase of the therapeutical options offered for this disease;
- to comment on the possible mechanism of action, as derived by the recently applied electrophysiological investigations.

2. Material and methods

Ninety-three subjects diagnosed as having a definite MD according to the 1995 AAO-HNS guidelines[9] received a local pressure treatment with Meniett® device (Medtronic, St. Paul, USA) from 1999. This treatment has been reserved to the most severe cases of MD, classified as Class D/E and with a Functional Level Scale (FLS) equal to 5/6,[9] due to relapsing, invalidating vertiginous spells occurring during the previous three months. Our protocol was arbitrarily chosen for allowing the patients to receive the device free of charge, and consisted in one-month treatment that started the same day of insertion of a short-term, trans-tympanic ventilation tube. Each patient was instructed to use the device five times per day, considering that each session – started by pressing a button displayed on the front of the device – was running automatically for three minutes. During the treatment period, *i.e.*, one month, each patient was asked to fill a diary for annotating any symptom related to MD, mostly vertigo episodes, as well as eventual changes of hearing, fullness or tinnitus. At the end of the treatment, the evaluation regarded the rate of vertigo control that allowed framing each patient in the same or a new Class or FLS,[9] the hearing threshold assessment and the Dizziness Handicap Inventory.

A last series of subjects underwent an electrophysiological assessment with electrocochleography (ECochG) before starting the pressure treatment, in order to get evidence of the presence of a hydropic pattern,[10] and one, three and six months after the treatment, in order to shed some light on the possible correlation between the outcome of the treatment and the ECochG-related hydropic pattern. The present report takes into consideration 41 patients who, at the present time, have at least two years from the end of the treatment, while the remaining 50 treated patients were excluded for different reasons (less than two-years follow-up, decease, change of address, etc.).

3. Results

The majority of the subjects performed only one pressure session, and only few cases required additional cycles for a delayed recrudescence of the disease, with the patient still wishing not to undergo a surgical procedure.

The 6 Class E (FLS 5) subjects reported a symptomatological improvement at the end of treatment, with a DHI score that diminished from 59 to 49.7, whilst only 16% of them reached a Class A or B. At the time of the present examination, the DHI score was 39.8 and all these subjects became Class A or B.

Out of the 35 Class D (FLS 4) subjects, 42% reached Class A or B soon after the treatment, while 63% were reaching Class A or B at the present evaluation. Overall, the success rate – that in this study signified avoiding vestibular neurectomy surgery – was equal to 68.3%.

The ECochG findings of the last series of Menière's subjects showed that all of them presented with a hydropic electrocochleographic pattern, *i.e.*, an SP/AP ratio equal or greater than 0.48.[10] At the end of one cycle of Meniett® treatment, the hydropic ECochG pattern was still present in the majority of the subjects, while all of them but two referred a remarkable improvement of their symptoms. Three months later, however, the ECochG pattern was found to be normalized in nearly all the subjects but in two of them, who were also referring an unsatisfying relief from vertigo and were addressed to vestibular neurectomy. A similar ECochG finding was also confirmed six months after the end of Meniett® treatment.

4. Discussion

In Menière's patients recalcitrant to any type of medical treatment, several but not univocal solutions are advised by the different Otologic Centers. Among them, the intratympanic gentamicin treatment represents the most applied, enabling to achieve a favorable outcome in a great percentage of patients. It is also known that gentamicin's effect is related to its toxic action on the vestibular end organs. In our clinical practice, contrarily, this form of therapy has always been considered paradoxical, as it would appear so any treatment that, in order to cure a disease, will provoke an additional toxic effect to the diseased organ. If this is the auspice for the action on the vestibular structures, it is certainly not for the auditory ones, already threatened by the disease itself and its natural course. This is the actual motivation for selecting, instead, a conservative approach, such as with the pressure treatment, in the wait of validation of similar conservative therapies, such as with intratympanic steroids, for example.

The Meniett® device has been clinically experienced by several centers worldwide, with results in agreement with our positive outcomes.[11-13] Nevertheless, this form of treatment has not achieved a validation also on the light of the last 'negative' Cochrane review.[14] Among the possible reasons, costs and, mostly, the lack of knowledge of its mechanism of action are surely playing a preeminent role in this regard.

When looking at the electrophysiological findings recorded in the last series of treated subjects, however, it would seem that the effect of Meniett® on endolymphatic hydrops is mostly relevant, not immediately after the treatment, but

only at a later stage, as evidenced three and six months after the treatment. This finding would appear of outmost importance if one consider that endolymphatic hydrops was present in all the treated subjects before the pressure treatment, as a confirmation that the selection of the patients was very accurate not only on the ground of the symptomatological aspects.

The pressure treatment with Meniett® has been shown to be efficient for avoiding to a selected, and severely impaired cohort of Ménière's subjects, recalcitrant to medical treatment, to undergo a surgical procedure. Furthermore, Meniett® has shown not to induce any side effect, although not acting on the auditory symptoms (hearing, fullness and tinnitus). It can therefore be proposed as conservative, first option treatment when the medical treatment fails.

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A STUDY FOR LOCAL TREATMENT USING THREE DIFFERENT POLYMERS AIMED FOR MIDDLE EAR ADMINISTRATION

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Abstract

Objectives: Recent advances in inner ear research support the idea of using the middle ear cavity for drug administration to target the inner ear. Rheological and safety assessments of three candidate polymer formulations for intra-tympanic drug administration are tested.

Method: The formulations were based on sodium carboxymethyl cellulose (NaC-MC), sodium hyaluronate (NaHYA), and poloxamer 407 (POL). Rheological studies were performed with a controlled rate instrument of the couette type. Safety studies were performed in guinea pigs subjected to an intra-tympanic injection of the formulations. Hearing function was explored with ABR before and 1, 2, and 3 weeks after the injection. Elimination of the formulations marked with coal was explored with an endoscopic digital camera 1, 2, and 3 weeks after injection. Middle and inner ear morphology was examined with light microscopy 6 days after injection.

Results: NaHYA showed a high viscosity solution rather than a real gel. It did not cause prolonged hearing threshold elevations. The mucosa of the middle ear showed no sign of inflammatory reaction and the inner ear structures remained intact. The results of the elimination and morphological investigations support the conclusion of NaHYA being most promising.

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Conclusion: A formulation based on sodium hyaluronate (NaHYA) was the best candidate vehicle for intra-tympanic drug administration regarding conductive hearing loss, inflammatory reactions, and elimination.

Keywords: ABR, carboxymethyl cellulose, guinea pig, hyaluronan, intra-tympanic, morphology, poloxamer 407, polymer, targeted drug delivery, toxicity

1. Introduction

To be able to transport drugs into the inner ear, through an easy route and under stable conditions, is important if a patient is required to take ototoxic medication like drugs for treatment of cancer. Different techniques for drug application to the round window in the middle ear have been developed since it is believed to be the most important interface. The MicroWick is composed of polyvinylacetat which can be loaded and inserted into the round window niche through a ventilation tube in order to transport Gentamycin to the inner ear for treatment of Ménière's disease (MD).[1] Implantable micro-catheters and osmotic pumps can be used to deliver medication continuously like steroids[2,3] and biodegradable vehicles like chitosan, gelatin and poly lactic co-glycerid can experimentally transport different growth factors and dexamethasone.[4] The nanotechnology can deliver high concentrations of loaded particles to the inner ear, but this experimental area is still to be expanded.[5] There are some disadvantages in all systems today, like difficulties to stop and restart an osmotic pump without removing the system and the fact that dosages cannot be varied. Constant infusion has been difficult to use due to the low volume in the inner ear and thereby the low clearance. All intra-cochlear deliveries have a greater risk of infection.

There is also a systemic way to deliver drugs to the inner ear but then the blood-brain barrier, which is the same as the blood-cochlear barrier has to be passed.[6]

The round window membrane (RWM) and lately also the oval window have been in focus as a route for drug delivery. The RWM is 70 μm thick and consists of three layers: inner and outer epithelium and a core of connective tissue; its function is to be a pressure regulator when the sound waves are transported from the stapes footplate through the cochlea, as well as being a local defense barrier. The passage of substances over the membrane depends on the size and structure of the particles as well as the charge. Small molecules (less than one kDalton) pass easily, but larger particles have to use pinocytosis. To deliver drugs to the inner ear can be performed either by a cochleostomy through the otic capsule or by injections through the RWM when the window is acting as a semi-permeable membrane. The transport through the oval window is still an uncertain pathway and is believed to only be able to transport smaller amounts of drugs with specific drug size and charge.

In the study design three gels, sodium sodium hyaluronic acid, carboxymethyl cellulose and poloxamer 407 (NaHYA, NaCMC and POL), were tested as vehicles for transportation into the inner ear.

2. Material and methods

Albino guinea pigs were injected under Ketamin-xylazine anesthesia (Ethical permission N334/05) with NaHYA, NaCMC or POL through the tympanic membrane into the auditory bulla in order to test the safety of the different gels. Acoustically evoked ABR was used with a TDT system II using 2 ms sine waves with tone bleeps at 3, 6, 12, 20 and 30 kHz delivered into the ear canal in a sound proof box. Wilcoxon signed rank test was used for statistical evaluation. ABR assessments were repeated weekly for three weeks after the administration.
Visual semi-quantification of elimination of the polymer formulations was established by loading each gel with coal then quantifying the remnants in a four-grade scale and visualization by an endoscopic digital camera.
Light microscopic evaluation of prepared and serially sectioned specimens with emphasis on the inflammatory reaction in the TM, middle ear mucosa, the RWM, the hair cells and the stria vascularis were performed after injection with the three different gels.

3. Results

Ears injected with NaHYA had significantly lower threshold shifts after one week in all frequencies and in the higher frequencies also after two and three weeks compared to animals injected with the other two gels (Fig. 1).
Concerning elimination of the coal-loaded gels semi-quantification did not show full elimination in any animal injected with any of the gels after three weeks.
In the light microscope after using injections with NaHYA, neither signs of inflammation in the mucosa nor any ABR threshold elevations were noted. No remnants of the polymer formulation could be found six days after injection. A swollen mucosa could, however, be seen in the middle ear of animals injected with both NaCMC and POL, but no remnants of the polymer formulation could be seen in the NaCMC group.

4. Discussion

The results of the elimination and morphological investigations support the conclusion that NaHYA is the most promising candidate for intra-tympanic administration, especially in contrast to NaCMC and POL.
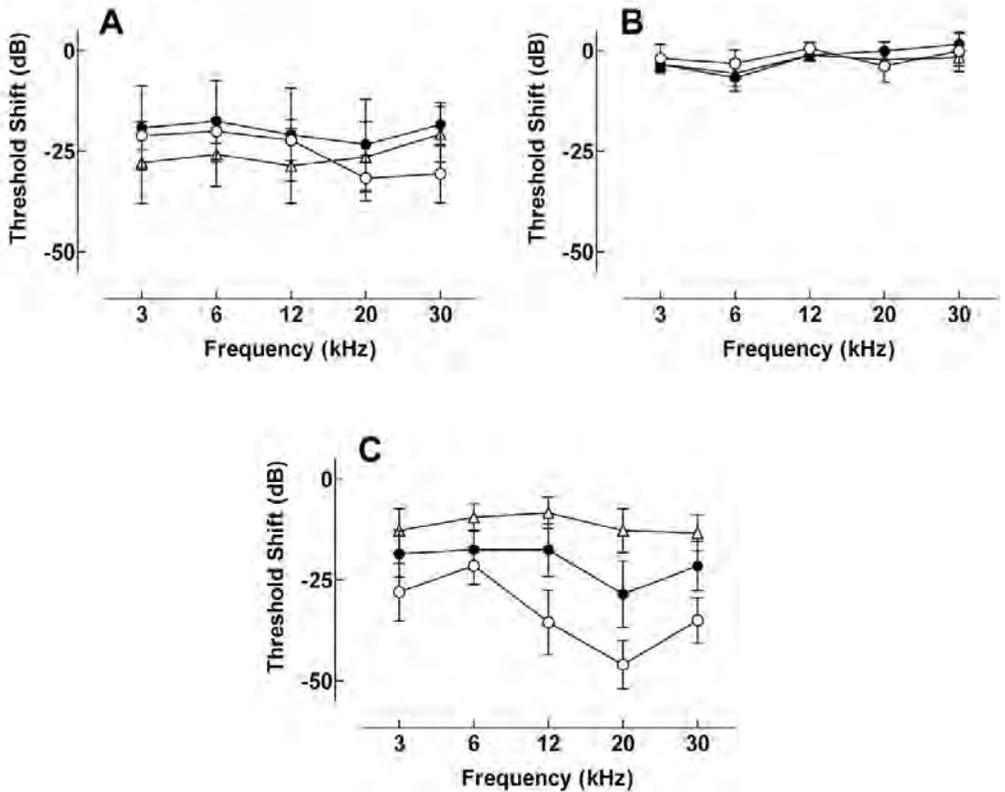
Fig. 1. ABR was performed in guinea pigs subjected to an intratympanic injection (0.15 mL) of NaCMC (A), NaHYA (B), or POL (C) one (open circles) two (closed circles), and three weeks (open triangles) after injections. Data are expressed as mean± SEM.

Hyaluronan is naturally occurring in humans, even in the inner ear, and consists of a repeating disaccharide. It has been used earlier in otologic research, both in animals and humans, and is well-tolerated. Most of the elimination should take place through the Eustachian tube but further elimination studies on the kinetics have to be performed since the elimination route is not clear and there are differences in time between species as well as gel concentration. Low viscosity solutions will disappear more rapidly. There were no statistically significant hearing threshold shifts using NaHYA one, two, and three weeks after administration and no remnants of NaHYA found in the light microscopic investigation six days after its administration. NaHYA has been used for testing loading and elimination of substances like gadolinium, a paramagnetic agent enhancing radiologic contrast in order to visualize distribution after intratympanic administration of NaHYA and has shown that there is a total elimination within 24 hours.[7]

Concerning the results with the coal-marked polymer, it is known that coal is not soluble in NaCMC, NaHYA, or POL. The fact that there was not a total

elimination of coal remnants in any ear does not prove that remnants of NaCMC, NaHYA, or POL were still present in the middle ear.

The coal remnants found around the middle ear ossicles, as in all NaCMC-administered ears, up to three weeks after the administration could also have an effects on the hearing function documented presently. The substance might even be ototoxic, according to an earlier guinea pig study.[8]

POL seems to be less suitable for intra-tympanic administration because of the induction of a striking thickening of the tympanic membrane and severe effects on the hearing thresholds.

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INTRATYMPANIC DEXAMETHASOME: POTENTIAL IN THE PREVENTION OF CISPLATIN OTOTOXICITY

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1. Introduction

Cisplatin is a widely used chemotherapy for the treatment of various malignant neoplasms.[1-4] Nephrotoxicity, neurotoxicity and ototoxicity are the main dose-limiting side effects of Cisplatin. While nephrotoxicity can be controlled with hydration therapy, there are no known preventive treatments available for neurotoxicity or ototoxicity.[2,4]

Cisplatin-induced hearing loss (CIHL) is characterized by bilateral, symmetric, progressive and irreversible sensorineural hearing loss. Hearing impairment is dose related, cumulative and takes place within hours to days from the administration of Cisplatin.[1-4] The reported rate of CIHL ranges between 11-97%, with an average incidence of 62%.[5-7] When the cumulative dose of Cisplatin is greater than 300 mg, the risk of hearing loss is directly correlated with the increase in its dosage.[8]

The pathogenesis of CIHL is attributed to the creation of reactive oxygen species (ROS) and depletion of antioxidant enzymes. This results in lipid, protein and nucleic acids peroxidation with Caspase system activation and apoptosis of inner ear cells.[4,9-11] The outer hair cells at the basal turn of the cochlea are initially lost to be followed by the remaining outer hair cells, inner hair cells, supporting cells and spiral ganglion neurons.[12]

Various chemoprotectants have been suggested to ameliorate CIHL. These include sodium thiosulfate, amifostine, diethyldithiocarbamate, 4-methylthiobenzoic acid, D- and L-methionine, N-acetylcysteine, and glutathione ester.

The mechanisms of action include direct antioxidant activity and maintenance of glutathione levels which is the main natural ingredient protecting against ROS.[1,2,4] Unfortunately, many of these compounds fail to cross the blood-inner ear barrier and the systemic administration resulted in significant side effects including the reduction of Cisplatin anti-neoplastic effect.[1] Therefore, these drugs are currently considered not suitable for human clinical use.[1,2,13] Recently, gene-therapy for Cisplatin ototoxicity has been studied in cell cultures and experimental animal models using different viral vectors, plasmids, or Si-RNA. This potentially promising approach requires further investigation regarding safety, immunogenicity, and consequences of genetic manipulation in the inner ear tissues.[14,15]

Abundant corticosteroids receptors were demonstrated within the critical inner ear structures suggesting active role of these compounds in inner ear homeostasis.[16-18] Corticosteroids has been reported to up-regulate cochlear anti ROS enzymes activity, reduce the formation of inflammatory molecules, and to decrease ototoxicity-induced inner ear cellular apoptosis.[2,3,19-21] Currently, systemic steroids treatment is practiced for various inner ear pathologies in which ROS are involved. These include sudden sensorineural hearing loss, noise-induced hearing loss, autoimmune inner ear disease, Ménière's disease, salicylate and aminoglycoside ototoxicity.[2,3,17] Since Cisplatin ototoxicity involves ROS production and depletion of antioxidant enzymes, a rationale for its prevention by corticosteroid is suggested. However, previous studies reported that systemic steroid treatment might result in reduced tumoricidal activity of chemotherapy – which might worsen patient prognosis.[22,23]

Intratympanic (IT) delivery of drugs is a contemporary method based on the diffusion of the active remedy across the round window into the inner ear where it exerts its therapeutic effects. Higher inner ear concentrations are gained when compared to oral or parenteral administration while avoiding the undesirable systemic side-effects including the potential reduced efficacy of chemotherapeutic agents.[22-26]

IT administration of steroids, especially Dexamethasone, has been vastly used over the last decades for the treatment of sudden sensorineural hearing loss and Ménière's disease.[24,26,28]

The purpose of the present study was to examine the possible role of Intratympanic Dexamethasone (ITD) in the prevention of CIHL in a clinical setup.

2. Methods

Twenty-six patients suffering from a neoplastic disease for which the primary curative-intent treatment protocol included Cisplatin were recruited to a prospective randomized controlled study. Most subjects received additional cytotoxic agents in combination with Cisplatin. These included Bevacizumab, Capecitabine, Docetaxel, Doxorubicin, 5-FU, Gemcitabine, Pemetrexed, Topotecan, Vinorelbine, and VP16. Ototoxicity is not a reported side effects in any of these chemotherapeutic agents.

After signing an informed consent the patients had a baseline evaluation that included detailed history with emphasis on previous or existing ear disease, microscopic otoscopy, pure tone, speech and impedance audiometry, and Distortion Product Otoacoustic Emissions (DPOAEs) testing. The latter was reported to be particularly useful in monitoring early injury to outer hair cells resulting from various etiologies including Cisplatin ototoxicity.[7,11,28-30]

Exclusion criteria were: (1) age < 18 years; (2) pathological findings on otoscopy which did not allow safe IT drug delivery and reliable DPOAEs testing; (3) lack of stapedial reflex and type A tympanometry on impedance audiometry; (4) previous inner ear disease which might result in sensorineural hearing loss; (5) conductive hearing loss > 5 dBHL; (6) pure-tone thresholds average (PTA) > 40 dBHL for the frequencies 500-3000 Hz or 4000-8000 Hz; (7) asymmetry of PTA between the ears > 10 dBHL for the frequencies 500-3000 Hz or 4000-8000 Hz; (8) previous treatment with Cisplatin; and (9) history of radiation therapy to the head and neck regions.

The ears of patients that matched the inclusion criteria for the study were randomly assigned employing a computerized 'randomizer'[31] to the study or control groups.

Prior to each Cisplatin treatment session ITD was injected to the baseline randomly assigned ear while the other ear of the same patient served as the control.

Follow-up audiometry and DPOAEs testing were carried by one of two clinical audiologists who were blinded to the side treated. These were performed one week after the cumulative dosage of Cisplatin reached at least 400 mg, a level at which a maximal ototoxic effect is anticipated.[1-4,8]

2.1. Statistical analysis

Clinically significant CIHL was defined according to the guidelines of the American Speech-Language-Hearing Association (ASHA) as hearing loss of 20 dBHL or more in a single-frequency or hearing loss of at least 10 dBHL in two or more adjacent frequencies.[32]

The incidence of clinically significant hearing loss between the study and control ears was compared by the Fisher exact test.

The following variables were compared between and within groups: pure tone thresholds (PTT) at 500, 1000, 2000, 3000, 4000, 6000 and 8000 Hz; Pure tone averages (PTA) at the frequency range 500-3000 and 4000-8000 Hz; DPOAEs signal to noise ratios (SNR) for the f2 frequencies 1031, 2063, 2953, 4172, 4969, 5953, 7031 and 8391 Hz; and average DPOAEs SNRs at the f2 frequencies of 1000-3000 Hz and 4000-8000 Hz.

Comparison between groups was carried out by the Student's unpaired two tailed t-test and within groups by the Student's paired two-tailed t-test. Bonferroni correction was employed to compensate for multiple comparisons.

Sample size calculation determined that 16 ears in each group were required to complete the study. The calculation was based on the following parameters:

$\alpha = 0.05$, $\beta = 0.20$, power $= 80\%$, estimated difference $= 10$, and standard deviation $= 10$.

Statistical analysis was done using SPSS.

3. Results

Eleven of the 26 patients recruited dropped out from the study. Three patients died of their disease, two had cerebrovascular accident that might have added confound parameter of central hearing loss, in three patients the treatment protocol was changed by the attending oncologist before a cumulative dose of 400 mg Cisplatin was reached, and three opted to withdraw their consent for participation in the study.

The average cumulative dose of Cisplatin was 517 ± 184 mg (mean $\pm$ standard deviation; range 410-1165 mg) and the average number of Cisplatin treatment sessions 5.6 ± 2.5 (mean $\pm$ standard deviation; range 3-11) spanned over two to six months. The time elapsed from ITD injection to Cisplatin treatment was 150 ± 59 minutes (mean $\pm$ standard deviation; range 105-315 minutes).

No differences were found between the study and control ears in the baseline audiometric and DPOAEs evaluations.

Significant CIHL as defined by ASHA criteria[32] was diagnosed in six (40%) ears of the control and five ears (33.3%) of the study group. The differences between the groups in did not reach statistical significance.

Significant increases in the average PTTs at 8000 Hz were found in both the study and control groups: 7.3 ± 9.2 dBHL and 11.3 ± 11.7 dBHL, respectively ($p < 0.005$, 95% confidence interval 2.2 to 12.5; $p < 0.01$, 95% confidence interval 4.8 to 17.8, respectively; paired t-test).

Significant increase in the PTT for 6000 Hz of 8.7 ± 11.4 dBHL was observed in the control group ($p < 0.02$, 95% confidence interval 2.3 to 15; paired t-test). For the ITD treated group non-significant threshold increase of 2.3 ± 8.8 dBHL was found (Fig. 1).

On the completion of the study 5 (33.3%) ears of the study group had PTTs for both 6000 and 8000 Hz that were at least 10 dBHL lower when compared to those of the contralateral matched ears.

Within the groups comparison of the PTA for 4000-8000 Hz showed significant average increases of 3.9 ± 5.8 and 7.2 ± 7.9 dBHL in the study and control groups respectively ($p < 0.03$, 95% confidence interval 0.68 to 7.1; $p < 0.005$, 95% confidence interval 2.8 to 11.6, respectively; paired t-test).

Significant decrease in the DPOAE SNR for 8391 Hz and 7031 Hz of 4.2 ± 1.7 and 2.4 ± 3.9 dBSPL respectively were found only in the control group ($p < 0.04$, 95% confidence interval 0.13 to 4; $p < 0.04$, 95% confidence interval 0.16 to 4.7, respectively; paired t-test).

Significant decrease of 2.6 ± 4.4 dBSPL in the DPOAEs average SNR for the f2 frequencies of 4000-8000 Hz was found in the control group ($p < 0.04$,
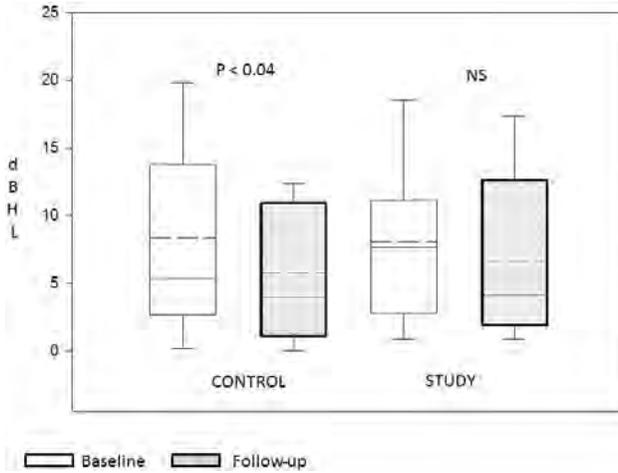
Fig. 1. Box plots of the pure tone thresholds at 6 kHz (dBHL), on the baseline and follow-up evaluations in the control and research groups. The boundary of the box closest to zero indicates the 25th percentile, the solid line within the box marks the median, the dashed line marks the mean, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers above and below the box indicate the 90th and 10th percentiles, respectively. The threshold increase in the control group was statistically significant ($p < 0.02$). NS = Not significant.
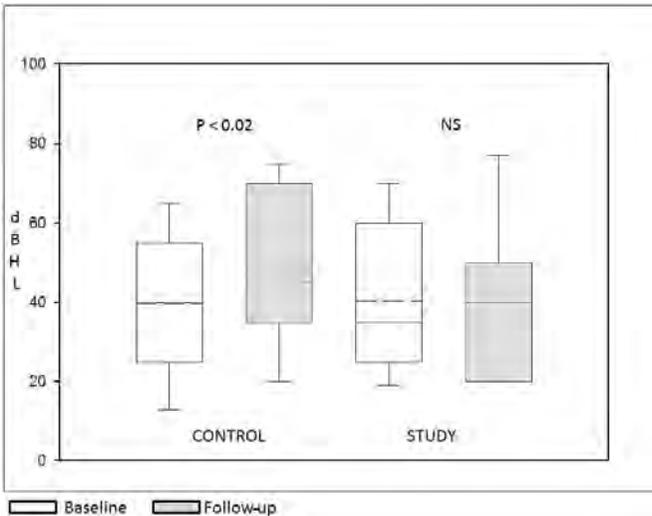
Fig. 2. Box plots of the DPOAEs SNR averages (dBSPL) over 4-8 kHz, on the baseline and follow-up evaluations in the control and research groups. The SNR decrement in the control group was statistically significant ($p < 0.04$). NS = Not significant.

95% confidence interval 0.16 to 5; paired t-test). The SNR decrease of 1.35 ± 4.1 dBSPL in the study group was not of statistical significance (Fig. 2).

4. Discussion

The main finding of the study is that ITD delivered shortly before each Cisplatin session provided minimal protection against CIHL. Although significant threshold increase was observed in both the study and control groups for 8000 Hz, and the PTA of 4000-8000 Hz, the increase at 6000 Hz and reduced DPOAE's SNR for the f2 frequencies 7031 and 8391 Hz and SNR average of 4000-8000 Hz was significant only in the control group. Also, on the completion of the study third of the study group ears had at least 10 dBHL better hearing thresholds in both 6000 and 8000 Hz when compared to the contralateral control ears.

The specific mechanisms involved in the attenuation of CIHL by Dexamethasone are not known yet. However, recent studies suggest the relevance of various cellular processes that might explain this otoprotection.

High presence of mineralocorticoid and glucocorticoid receptors was observed in the outer and inner hair cells, spiral ganglion neurons and spiral ligament.[16,18,33]

The mineralocorticoid receptors demonstrate higher affinity to steroidal hormones which points to the significant role of steroids in maintaining fluid regime and homeostasis of the inner ear.[16,17] The blood-labyrinthine barrier is composed of capillary endothelial cells with tight-junctions which inhibit absorption of chemicals from the systemic circulation into the inner ear. Rising level of ROS operates the vascular endothelial cells to secreting of different cytokines which damage the tight junctions, cause violation of the inner ear fluid homeostasis, damaging the endocochlear potential and hence lead to apoptosis of cell lines in the organ of Corti.[34] Glucocorticosteroids were reported to bring a new creation of the tight junctions between endothelial cells, thus restoring endothelial function in the stria-vascularis.[35]

The inner ear pharmacokinetics of Dexamethasone following intratympanic injection on one hand and that of Cisplatin after intravenous administration on the other hand indicate that ITD in a controlled timing may provide effective protection against Cisplatin ototoxicity. Maximal concentration of Cisplatin in the perilymph was measured 20 minutes after intravenous delivery, declined to about half from its peak after 40 minutes and was below detection after 90 minutes.[36] For Dexamethasone, maximal perilymph level was recorded one hour following intratympanic injection.[37] Employing high pressure liquid chromatography and mass spectrometry significant perilymph levels of Dexamethasone were measured within one hour from intratympanic injection which decreased by by 50- to 100-fold within 12 hours.[38]

Maximal therapeutic effects of Dexamethasone are anticipated when its peak concentration in the perilymph correspondences that of the intravenously administered Cisplatin. Animal studies indicate that the optimal timing for ITD would be about 40 minutes before Cisplatin delivery.[9,16,36,37] In the clinical realm we found it difficult to follow the ideal timing for ITD injection prior to Cisplatin treatment. This might decrease the potential maximal protective effect of Dexamethasone. Also, larger therapeutic effect might be reached by higher concentration of the drug.

In conclusion, the study shows minimal effect of ITD towards the reduction of Cisplatin ototoxicity. A larger scale research, employing various concentrations to be delivered in precise timing, is required to further investigate the potential role of ITD in the prevention of Cisplatin induced hearing loss.

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INTRATYMPANIC DEXAMETHASONE AND GENTAMICIN IN THE TREATMENT OF DISABLING VERTIGO IN MÉNIÈRE'S DISEASE

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1. Introduction

A variety of conservative approaches have been the mainstay of initial therapy of Ménière's disease (MD) over time, including dietary sodium restriction, diuretics, oral steroids, calcium channel blockers, and vasodilators.[1] Medical therapy is successful for regulating the symptoms in at least 70% of MD patients.[2] For medically intractable MD, various surgical techniques have been developed including endolymphatic sac decompression, labyrinthectomy and vestibular nerve section.[3] However, some of these techniques have been criticized because of their poor long-term control of vertigo, their definitive hearing damage, or the morbidity associated with the procedures.[1,3] Intratympanic (IT) gentamicin perfusion as a treatment for MD was first described by Lange more than three decades ago.[4] Since that time, several different protocols and treatment schedules have been developed to obtain partial or total destruction of the labyrinth. Numerous studies have evaluated the efficacy of these various methods of IT gentamicin and the outcomes have varied.[2,5-6] In the last decade, the use of IT corticosteroid injections has gained popularity as there are minimal associated adverse effects. Parnes *et al*. demonstrated in animals how hydrocortisone, methylprednisolone, and dexamethasone injected into the middle ear crossed the oval window, obtaining higher endolymphatic levels than those obtained with systemic administration.[7] Similar to gentamicin, there are many different treatment protocols for IT corticosteroids.[8] Published results vary, and no consensus among the investigators has been reached for the recommendation of one single protocol.

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Silverstein introduced the MicroWick™ procedure in 1998 as a new method for the administration of IT dexamethasone or gentamicin directly onto the round window for an extended period of time. Previous studies examining the efficacy of this delivery method for gentamicin reported vertigo symptoms were relieved in 76.8-85% of patients.[9-10]

The limitations of the previous studies that have investigated the various treatment protocols for IT gentamicin and corticosteroids includes lack of direct comparison of the different treatment protocols. Furthermore, there is a gap in the current literature that provides evidence for the percentage of patients who require repeat injection or more advanced surgical intervention after relapsing from an initially successful intra-tympanic therapy. The aims of this study were: (1) to determine the effectiveness and long-term vertigo control of IT dexamethasone and IT gentamicin in patients with MD; (2) to compare the effectiveness of various doses of IT gentamicin and dexamethasone versus the MW procedure; (3) to determine the percentage of patients who required IT gentamicin after undergoing IT dexamethasone treatment; and (4) to determine the subset of patients who failed initial gentamicin treatment and required repeat gentamicin treatment or more advanced surgery such as labyrinthectomy or vestibular nerve section.

2. Method

Following local Institutional Review Board (IRB) approval, the investigators of this retrospective study examined the medical records of patients that received treatment for MD at an outpatient tertiary referral center between May 2011 and May 2013. Utilizing the American Academy of Otolaryngology – Head and Neck Surgery's (AAO-HNS) Committee guidelines for the diagnosis and evaluation of therapy in MD,[11] the data extracted from the patient's chart included clinical and audiometric data that were initially collected prior to treatment and at one, 12 and 24 months post treatment. Adults 18 years or older with a definite diagnosis of MD who underwent treatment with IT dexamethasone or gentamicin delivered by single weekly injection or extended daily treatment via the MicroWick were included in the data analysis. Individuals without a definite diagnosis of MD according to AAO-HNS guidelines or those with bilateral MD were excluded. Audiograms were used to calculate a four-frequency pure tone average (PTA) of 0.5, 1, 2 and 3 KHz and the speech discrimination score. Videonystagmography (VNG), including bithermal and ice caloric results of the affected ear was collected. In addition, clinical data was obtained including the average number of definitive vertigo attacks at each interval; the date of procedure, type of IT treatment protocol, drug dosage and duration of therapy. The effect of the IT procedure on vertigo attacks on vertigo control was calculated and then classified according to number of residual attacks according to AAO-HNS criteria (class A-F) (Table 1). The two subjective symptoms, aural fullness and tinnitus, were also investigated. For the purposes of this retrospective analysis, the patient's

Table 1. AAO-HNS vertigo control criteria.

Class	Number of attacks
A	0 (complete control)
B	1-40 (substantial control)
C	41-80 (limited control)
D	81-120 (insignificant control)
E	> 120 (poor or worse control)
F	Secondary treatment required due to disabling vertigo

subjective report of these symptoms following treatment were collected and then categorized as 'same', 'better' or 'worse'. Demographic data (duration of MD symptoms, affected ear age at IT treatment and gender were also extracted from the medical record.

Two main groups were formed: a dexamethasone and a gentamicin group. Participants were further classified into sub-groups according to the treatment protocol they received (single weekly injection or MicroWick). The single injection technique and the MicroWick procedure, both performed in the office under local anesthesia are described elsewhere.

2.1. Post-treatment assessment

For the dexamethasone group, regardless of the treatment protocol, patients were evaluated at weekly or bi-weekly intervals. Audiometric testing and electrocochleography (ECOG) were performed and vertigo control was assessed by subjective report of vertigo attack frequency and severity. In addition, patient symptoms of aural fullness, tinnitus and hearing loss were recorded. If there were no vertigo attacks reported, then no further therapy was recommended at that time. If there were any vertigo attacks but the patient was satisfied with a reduction in the frequency and severity compared with what they had experienced before the IT dexamethasone, then the patient was offered another IT dexamethasone. However, if the patient felt that the effect of the last IT dexamethasone injectable was unsatisfactory, IT gentamicin or ablative surgery was recommended.

Irrespective of the treatment protocol, weekly follow-up examinations for the gentamicin group included audiometric and VNG caloric testing (bithermal and ice-air). In addition, patient symptoms including vertigo frequency and severity; aural fullness, tinnitus, hearing loss and gait abnormality was also assessed. For both the single weekly IT injection and the MicroWick protocol, treatment was titrated at each visit based on the caloric and hearing test result as well as the subjective symptom report. However, the aim of treatment was to achieve 100% reduced vestibular response (RVR) on at least bithermal caloric testing while preserving hearing in the affected ear. Consequently, treatment was discontinued when the vestibular function reached 100% RVR or there was a significant drop in the speech discrimination.

2.2. Statistical analyses

Descriptive statistics were described using the mean, standard deviation and range. Ninety-five percent confidence intervals were also estimated when appropriate. Categorical variables were expressed as frequencies and percentages. Due to the small sample sizes in both groups, no parametric statistical testing was performed. However, Chi square testing was performed for the categorical variables 'tinnitus' and 'aural fullness', to examine the significance of the distribution of the symptom report scores post-treatment. The level of statistical significance was defined by a *p*-value < .05. All statistical analyses were performed using Microsoft Excel (2010).

3. Results

3.1. Dexamethasone group

This group included 30 patients: 14 were male and 11 were female. Demographic analyses are provided in Table 2. The mean vertigo recurrence duration was 10.2 months following treatment, with an observed range between 0.5 and 45.1 months post-treatment. The mean PTA (95% confidence interval = CI) before was 43.8 dB (CI, 36.7 – 50.1 dB). After one month, the mean PTA value was 49.0 dB (CI, 40.8-57.2 dB) and at 24 months 51.8 dB (CI, 41.7-61.9 dB). Mean speech discrimination scores pre-treatment were 75% (CI, 65.8-84.1%) and were 67% (CI, 54.4-79.6%) at 1one month and 59% (CI, 43.2-74.8%) at 24 months post-treatment.

According to the AAO-HNS classification index for vertigo control, complete vertigo control (class A) was achieved in 13 patients (43%), and substantial control (class B) was achieved in three patients (10%) at the 24-month follow-up evaluation. Fourteen patients (47%) received the following secondary treatments because of lack of vertigo control (Class F): (**a**) gentamicin 40 mg/cc single injections (36%, n = 5); (**b**) dexamethasone 24 mg/cc single injections (21%, n = 3); (**c**) gentamicin 10mg/cc single injection (7%, n = 1); (**d**) vestibular neurectomy (7%, n = 1); and (**e**) medical management (29%, n = 4). Prior to treatment, aural fullness and tinnitus were reported in 90% (n = 27) and 97% (n = 29) of patients respectively. Following treatment, the majority of patients (67%, n = 20) reported their aural fullness as 'better'. In contrast, tinnitus was categorized as 'better' in only 47% (n = 14) of patients. In order to assess whether there was a significant change in the proportion of aural fullness and tinnitus reported in each category ('same', 'better', or 'worse') post-treatment, Chi square tests were calculated for these categorical variables. There was a statistically significant change in the distribution of aural fullness responses (χ^2 = 10.9, df = 2, *p* = < .05). However, there was no significant change in the distribution of tinnitus responses post-treatment (χ^2 = 4.1, df = 2, *p* = > .05). VNG was not

Table 2. Demographic characteristics of dexamethasone group.

	N = 30
Gender	Male: 14 (46.7%)
	Female: 16 (53.3%)
Age	Mean: 60
	Range (35-81)
Affected ear	Right: 17 (56.7%)
	Left: 13 (43.3%)
Duration of MD	Mean: 38 months
	Range: (1-120 months)
Tinnitus	27 (90%)
Aural fullness	29 (97%)

routinely performed in patients that received dexamethasone IT, so no results will be reported for this group of subjects. Only one subject (3%) in this group had a persistent tympanic membrane perforation requiring tympanoplasty at an outpatient surgery center.

3.2. Gentamicin group

In this group, there were five male and 11 female subjects, totaling 16 subjects. Please refer to Table 3 for the demographic analyses. In this sample, vertigo recurrence was observed from one month to 35 months following treatment, with a mean of 12.1 months. Analysis of audiometric measures revealed a mean PTA of 51.6 dB (CI, 47.8-55.4 dB) pre-treatment. After one month, the mean PTA values were 59.9dB (CI, 48.5-71.3 dB) and 67.2 dB (CI, 51.3-83.1 dB) at 24 months post-treatment. Mean speech discrimination scores were 56% (CI, 40.7-71.3%) pre-treatment and at one month and 24 months following treatment were 53.5% (CI, 35.2-71.8%) and 48.0% (CI, 26.9-69.1%) respectively. Examination of the VNG results before treatment showed a mean (standard deviation = SD) bithermal and ice caloric reduced vestibular response (RVR) of 43.1% (SD = 27.3%) and 56.3% (SD = 32.3%) respectively. Mean bithermal and ice caloric RVR scores were 97.2% (SD = 8.9%) and 62.2% (SD = 32.1%) at one month and were 95.3% (SD = 9.5%) and 82.0% (SD = 33.7%) at 24 months post-treatment.

Following the index suggested by the AAO-HNS, complete vertigo control (class A) was achieved in nine patients (56%), and substantial control (class B) was achieved in four patients (25%) by the 24-month follow-up. Three patients (19%) received a secondary treatment because of lack of vertigo control (Class F): Two patients (13%) received additional doses of IT gentamicin 40 mg/cc by single injection and one patient (6%) underwent labyrinthectomy.

Among the 16 patients, the majority (94%) reported tinnitus prior to treatment and only five (31%) described their tinnitus as 'better' post-treatment. Tinnitus

Table 3. Demographic characteristics of gentamicin group.

	N = 16
Gender	Male: 5 (31%)
	Female: 11 (69%)
Age	Mean: 68
	Range (44-83)
Affected ear	Right: 7 (44%)
	Left: 9 (56%)
Duration of MD	Mean: 75 months
	Range: (3-420 months)
Tinnitus	15 (94%)
Aural fullness	14 (88%)

remained the 'same' in nine (56%) patients and 'worse' in only two (13%).The symptom of aural fullness was classified as the 'same' or 'worse' in six (37%) and three (19%) patients respectively post-treatment. Seven (44%) patients reported their aural fullness as 'better'. As with the dexamethasone group, Chi square tests were performed to evaluate whether there was a significant change in the distribution of tinnitus and aural fullness report scores post-treatment. The results did not reveal a significant change in either tinnitus ($\chi^2 = 4.64$, df = 2, $p => .05$) or aural fullness ($\chi^2 = 3.62$, df = 2, $p => .05$) following treatment. Only one patient in the gentamicin group required tympanoplasty for a persistent tympanic membrane perforation.

4. Discussion

The natural course of MD is an episodic pattern of vertigo, hearing loss, aural fullness and tinnitus with variable symptom severity and frequency as well as periods of quiescence. This unpredictability, along with the lack of definitive insight into the etiology underlying this condition often poses a challenge for the physician in the diagnosis and treatment of MD.[5] Silverstein *et al*. studied 50 patients who refused surgery for MD. Fifty-seven percent had vertigo resolution in two years and 71% had vertigo resolution in 8.3 years.[12] Thus, physicians are often conflicted as to whether perform an ablative procedure or consider a more conservative treatment to diminish patient's symptoms when the MD symptoms may spontaneously abate. IT perfusion of the inner ear with gentamicin and dexamethasone has enhanced the physician's treatment armamentarium. As a minimally invasive procedure, it is effective in reducing the frequency of vertigo attacks in patients that have failed medical therapy with less morbidity than more invasive surgical interventions.[2,6,8,13,14]

The findings of our study support the results of previously published studies that have demonstrated IT dexamethasone and gentamicin is effective in the management of vertigo in patients with MD. We have described our Institute's experience using IT dexamethasone as an adjunctive therapy when MD patients' vertiginous symptoms worsen and are no longer controlled with medical therapy. Significant vertigo control (Class A and B) with IT dexamethasone was achieved with 53% of our cohort which is consistent with previous reports. The reported vertigo control rates for IT dexamethasone vary widely in the literature (47-91%).[15-16] While IT dexamethasone is less effective than gentamicin in vertigo control of patients with MD, IT dexamethasone could be offered in place of IT gentamicin with lower expectations of vertigo control in individuals with bilateral disease; when treating the only hearing ear affected with MD; in patients that refuse chemical labyrinthectomy, and in the elderly, when there is concern regarding incomplete vestibular compensation post-treatment.

It should be noted that the majority (63%) of patients who received IT dexamethasone reported improved aural fullness following treatment. This finding was statistically significant in our analysis, suggesting that IT dexamethasone may be effective treatment for this symptom associated with MD.

In the gentamicin group, total vertigo control (Class A) was observed in 56% of patients. In addition, substantial vertigo control (Class B) was seen in 25% of patients, for a total (Class A and B) rate of 81%. This is consistent with the results of a meta-analysis that estimated total vertigo control (Class A) rates of 65.2% and 94.1% for substantial vertigo control (Class A and B) from the results of previously published retrospective studies.[6] These results indicate that IT gentamicin generally provides better results than IT steroids. However, gentamicin achieves better vertigo control because it reduces vestibular function. while IT steroids have an anti-inflammatory and vasomotor effect with no deleterious effect on hearing or balance.[13] Interestingly, when we compared the gentamicin 40 mg/cc single weekly injection technique to the slow perfusion of gentamicin 10 mg/cc delivered by the MicroWick procedure, there appeared to be no significant difference in the vertigo control rate between the two protocols. Furthermore, the degree of hearing loss was similar with both treatment protocols. For the associated symptoms of tinnitus and aural fullness, our analysis found no significant difference in the proportion of responses of 'same', 'better' or 'worse' following treatment, indicating that gentamicin had no therapeutic benefit for these symptoms.

This study has several limitations. It is a retrospective analysis and there was no control group utilized for comparison. The use of a control group is essential for determining if the outcome is due to the natural course of MD or an actual treatment effect. Further, the small sample size of the group increased sample variability and decreased statistical power.

5. Conclusion

Our results offer supportive evidence to the existing body of literature that has examined the efficacy of IT dexamethasone and gentamicin in the treatment of MD. Although, IT dexamethasone is an effective treatment, gentamicin appears to offer better vertigo control. IT Dexamethasone may offer additional benefit by reducing the symptom of aural fullness associated with MD. Further prospective investigation of the various IT treatment protocols, utilizing control groups is needed.

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INTRATYMPANIC OR TRANSTYMPANIC DRUG THERAPY?

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We started intratympanic (IT) drug therapy for Ménière's disease in Alexandria, Egypt, in 1993. In light of the results of IT therapy, the anatomical and operative observations of the presence of false round window (RW) membrane in almost 50% of the cases, we started transtympanic (TT) drug therapy: microscopic (2003) and endoscopic (2013). In this paper we describe the indications, equipment, rationale and technique of this therapy.

The (RW) is the main gateway to the inner ear. Other gates include the oval window and the semicircular canals. Drug delivery to the inner ear through IT via the RW membrane can be done, or by intralabyrinthine injection via the RW or semicircular canals.

In 1981, Stewart and Belal studied the surgical anatomy and pathology of the RW in 272 normal temporal bones in persons varying in age between infancy to 87 years.[1] The niche was like a triangular prism with the base at RW membrane and three walls. The mean horizontal diameter was 1,5 mm, while the mean vertical diameter was 1,2 mm.

The entrance to the RW may be altered within the entrance or by structures outside the niche. False RW membrane was found in 55% of the temporal bones examined (Figure 1). Silverstein found a partial membrane in 17% and a complete membrane in 12%.[2] While Alzamil and Linthicum (2000) found RW obstruction in 33% of 202 temporal bones: fat or fibrous tissue plugs 13% and membrane in 20%.[3]

In IT drug therapy, if the target site is the basal coil of the cochlea or vestibular system, one shot of IT treatment suffices. If the apical coils of the cochlea are the target, multiple shots, pumps, catheters, biodegradable polymers and cochlostomy may be needed. IT drug therapy may be done in acute sensorineural hearing loss due to idiopathic sudden HL, nose trauma, ototoxicity, ischemia,

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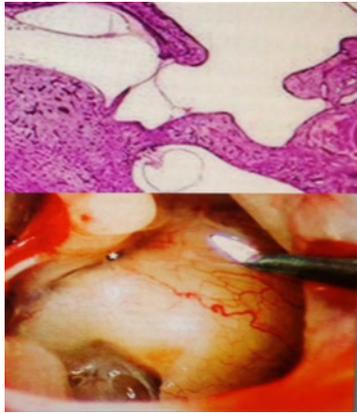
Fig. 1. False round window membrane in temporal bone section (above) and as seen by endoscope through the myingotomy incision (transtympanic endoscopy).

infection, head trauma, etc., or in autoimmune hearing loss. Some tinnitus cases may benefit from IT therapy.

Also, IT therapy can be done in case of vertigo due to Ménière's disease, hydrops or pre-operative before acoustic tumor surgery or cochlear implantation.

The following drugs have been used in IT drug therapy: Glucocorticoids (most commonly dexamethasone), amynoglycosidis (gentamycin), anesthetic, neurotransmitters for tinnitus, monoclonal antibodies for autoimmune sensori-neural hearing loss, apoptosis inhibitors for noise induced hearing loss, stem cell, gene vectors, growth factors for hair cell loss.

Many factors play a role in to what extent the drug reaches the inner ear, for example: Concentration gradient, round window membrane permeability, contact period, drug particle size, electric charges, etc.

Of concern with regard to the IT is the presence of round window false membrane and the fact that almost 90% of the injected drug goes down the Eustachian tube at the time of the injection (Figure 2).

Therefore, the most important variant in IT drug therapy is the technique of injection whether it is done by IT exploratory tympanotomy or TT injection.

IT drug therapy may be done by office injection, sub-annulus catheter or via exploratory tympanotomy.[3,4]

TT drug therapy is done by endoscopic-assisted techniques using round window gel foam or RW pumps.

The office setup in IT drug therapy is simple. It is usually done in the office under local anesthesia. The drug is injected in the antero-superior quadrant of the eardrum.

Success rate is usually 50%.

The aim of TT drug therapy is to be sure of round window patency i.e. there is no false round window membrane and if there is one it is removed by fire go degree hooks.
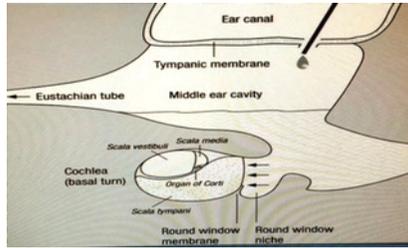
Fig. 2. Paths of intratympanic drug after its injection in the middle ear

TT therapy also allows putting gelfoam impregnated in the drug thus making sure of sustained drug delivery to the inner ear at least for 7-10 days after the injection.

TT drug therapy can be performed either microscopically or endoscopically.

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SURGERY

SAC SURGERY FOR TREATMENT OF MÉNIÈRE'S DISEASE

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Abstract

We describe the results of endolymphatic sac decompression and drainage. The method described by Paparella[1] to treat cases of severe vertigo in patients with Ménière's disease. The results of this procedure in 95 patients are discussed. In patients with unilateral disease, good results were obtained in control of vertigo in 94,5% of patients and significant improvement in cochlear function in over 15%.

1. Introduction

Most vertigo due to endolymphatic hydrops, whether secondary to known etiology or idiopathic (Ménière's disease), is amenable to medical therapy.

The clear-cut history of episodic vertigo, a sense of aural fullness, fluctuating progressive hearing loss and tinnitus is characteristic of hydrops. In addition, loudness intolerance and diplacusis are often present. In most of these cases it is impossible to stablish the etiology.

ldiopathic endolymphatic hydrops is then usually treated symptomatically with anti-emetics, sedatives, tranquilizers, diuretics and vasodilatory agents on an empirical basis. Psychological support of these patients is a most important part of treatment.

When intractable incapacitating vertigo cannot be controlled by other means, surgical intervention must be considered. In this situation it is desirable to recommend a procedure which offers a high likelihood of vertigo control with maximum hearing preservation.

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Many surgeries for intractable vertigo were described. We can divide them into conservative (endolymphatic sac surgeries, saculotomy, crioterapy, fistulization of the labyrinth) and destructive (vestibular neurectomies and labyrintectomies).

The first conservative surgery for Ménière's disease was described in 1927 by Portmann,[2] a technique for endoIymphatic sac decompression following the the hypothesis of some pressure increase in the labyrinth. In 1938, Hallpike and Cairns[3] demonstrated the pathological findings of endolymphatic hydrops in patients with Ménière's disease.

The operation had, however, been used relatively infrequently, and reports by other otologists were often not enthusiastic about the procedure's value.

Paparella[1] published very good results of a technique with decompression and drainage. In his paper he emphasized that the experimental demonstration of the endolymphatic sac's role has not been conclusive, however, it appears that the sac does have a significant function in endolymphatic homeostasis. Kimura[4] in 1967, reliably produced endolymphatic hydrops in guinea pigs by obliterating the endolymphatic duct and sac.

When endolymphatic homeostasis is disturbed and hydrops occurs, the sac appears a conceptually excellent site for decompression of the endoIymphatic space. The sac is relatively distant from the cochlear and vestibular organs. There is also some evidence that flow within the endolymphatic duct is away from these organs toward the sac.

2. Method and results

This study was performed in the ENT Department of the Faculty of Medicine of the University of São Paulo, Brazil.
I operated 95 patients using this method.

Data on patients who received this surgery from 1986 to 2012 are presented here. Patients in this series became candidates for surgery when their vertigo became incapacitating and could not be controlled after extensive medical and psychological management. Some of these patients were candidates for laby-rinthectomy and had 50 dB or greater hearing loss and/or less than 50% discrimination in the affected ear. Symptoms had been present in these patients for from one to ten years. Average duration of significant vertigo was one year prior to surgery.

Demographic data: Male patients: 36 (38%); female patients 59 (62%). Age: 28-67 years old, average 45. Unilateral disease: 88 cases; bilateral disease: seven cases.

Table 1. Unilateral disease HL*

Cases	Hearing loss
3	40 dB
32	50 dB
39	60 dB
14	70 dB

* Hearing average bone conduction HL from 500 1000 2000 Hz

The method of endolymphatic sac decompression and drainage that we employed was described by Paparella.[1]

Wide exposition of the mastoid cortex, mastoid ectomy by complete exenteration of mastoid air cells including tip cells, and widening of the aditus.

The horizontal semicircular canal and the location of the posterior semicircular canal are identified. Bone covering the lateral sinus and posterior cranial fossa and a portion of tegmen mastoideum is thinned. The distal portion of the lateral sinus, all accessible dura in Troutman's triangle, and often an adjacent small portion of dura of the middle cranial fossa are exposed by removal of the thin bone with curettes and elevators. The sac is then identified as a white, dense thickening in the dura, pointing toward the inferior portion of the posterior semicircular canal.

The white sac in most cases is clearly differentiated from adjacent dura by a lack of blood vessels coursing across the dura and also by the thickness of the white sac as compared to the thinness of the dura elsewhere.

We make a small opening in the sac with a joint knife, often beneath the bony ledge. Retracting the dura with a suction tip or elevator allows further identification of the sac under the bony ledge. The lumen of the sac is then identified by blunt probing with right and left curved probes. A Silastic® T is cut from silicone rubber .005-inch sheeting. The T portion of the tube is then pushed into the small opening and is allowed to coil upon itself, thus forming a bubular drain. Absorbable gelatin sponge (Gelfoam®) saturated in a steroid antibiotic solution is then placed in the mastoid cavity.

The same classification as described by Paparella[1] was used.

No major complications occurred in these operations.

Follow-up from three to 15 years after surgery.

Table 2. Classification of treatment results in endolymphatic hydrops.

Group	Vertigo	Hearing
A	Controlled	Improved (> 20 dB and/or 20% discrimination score)
B	Controlled	Unchanged
C	Controlled	Worse (> 20 dB and/or 20% discrimination score)
D	Uncontrolled	– –

Table 3.

Unilateral disease patients			BBilateral disease patients		Total patients %
Group	Number	%	Number	%	
A	12	14	3 ears (2 patients)	16	15
B	65	73	7 ears (6 patients)	60	66,5
C	6	8	2 ears	19	13,5
D	5	5	2 ears	6	5,5

Group	Number	Unilateral patients Followed > one year %	Unilateral patients Followed < one year Number	%
A	6	31	3	27
B	8	43	8	73
C	3	16	0	
D	2	10	0	

3. Discussion

While it is difficult to evaluate subjective symptoms, we have attempted to assess these results as objectively as possible. Criteria for hearing must take into account the fluctuating nature of the disease. We consider as improved hearing a > 20 dB change in hearing or 20% change in discrimination score.

Our primary objective in these operations was the elimination of incapacitating vertigo. Total absence of vertiginous symptoms was accomplished in many patients, most of whom had previously been incapacitated because of vertigo.

The patients with advanced unilateral idiopathic endolymphatic hydrops in this series obtained control of vertigo in 94,5% of the cases (Groups A, B and C). Of these patients, 15% also had significant improvement in hearing following the procedure. In 13,5% the hearing subsequently deteriorated significantly.

Any procedure or treatment for vertigo is difficult to evaluate. In our hands the procedure has been safe with no major complications.

In some of our patients with bilateral disease, improvement was noted even though only the more severe ear was operated on. The rationale for good results in these cases could be based on either psychological or physiological grounds.

These observations indicate to us that endolymphatic sac drainage is a safe procedure to be considered in patients with uncontrolled vertigo with medical and psychological treatment.

Our results with this procedure have been gratifying in that 94,5% of our incapacitated endolymphatic hydrops patients returned to a functional life free of uncontrolled vertigo; there was significant improvement of cochlear function in over 15% of these patients.

Endolymphatic sac drainage and decompression is a significant adjunct to our care of the patient suffering with severe endolymphatic hydrops and has important advantages over other forms of clinical and surgical treatment for this disease.

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OSTEOPLASTIC REFINEMENT IN ENDOLYMPHATIC SAC SURGERY

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1. Introduction

The surgical approach to the endolymphatic sac requires a cortical mastoidectomy. In a normally developed mastoid air cell system, removal of the mastoid cortex *en bloc* may be standard practice. This approach can be modified to maintain the perioperative postauricular contour by preserving the musculoperiosteal attachment to the cortex. The cortex and musculoperiostium can be elevated as an osteoplastic unit and 'hinged' anteriorly along the lateral margin of the external canal. Upon completion of the mastoid dissection, the cortex is restored to its anatomical position and the mastoidotomy closed. The application of the osteoplastic approach is not limited to sac surgery. It is especially well-suited as an adjunct to the translabyrinthine removal of acoustic neuromas where the bone flap maintains compression on the fat graft and buttresses the repair. This demonstration is consistent with the osteoplastic principle applied elsewhere in the head and neck.

We can all agree that the surgical approach to the endolymphatic sac and associated labyrinthine anatomy requires a cortical mastoidectomy. With few exceptions, this mastoid operation has remained unchanged since its inception. Modifications have employed the osteoplastic flap principle by maintaining the periosteal attachment to bone, which is temporarily displaced and subsequently replaced. Operations by which the canal wall is preserved and reconstructed by this method were described in the otolaryngology literature forty years ago[1,2] and revisited twenty years ago in the neurosurgical literature.[3] These procedures share the common goal of achieving adequate surgical exposure, while avoiding post-operative deformity.

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The concept of a cosmetic mastoidectomy is not revolutionary. Removal of the mastoid cortex as a unit is an alternative to conventional drilling but requires that the bone be re-fastened in place with miniplate fixation. We have developed a technique where the mastoid cortex can be preserved and replaced not as a free bone graft, but as a vascularized pedicle flap based on the mastoid soft tissue. Plating is unnecessary. Its application avoids the cosmetically undesirable postauricular sulcus, which may develop after cortical mastoidectomy. Considering the fastidious nature of today's patients, the procedure appeals to a population of consumers who expect conservative minimally invasive surgery. This is particularly the case with patients suffering from Ménière's disease.

2. Operative technique

Pre-existing conditions include a well-pneumatized mastoid that is free of pathology established by a preoperative CT scan. Based on CT evidence, the size and design of the cortical bone flap can be predicted. The periosteal incision is placed approximately one cm peripheral to the anticipated osteotomy (Fig. 1). The periosteum is not incised at the root of the zygoma or mastoid tip where the osteotomy is sub-periosteal. The musculoperiosteum is elevated to preserve a cuff of tissue along the margin of the osteotomy, which is achieved with a two-mm burr to a depth determined by the thickness of the cortex (Fig. 2). The cortical bone flap is developed with an osteotome introduced along the osteotomy in a plane that follows the approximate contour of the cortex (Fig. 3). The unit is upfractured with the osteotome and remains pedicled by the musculoperiosteum along the posterior meatal wall (Figs. 4 and 5). The rest of the mastoid is then
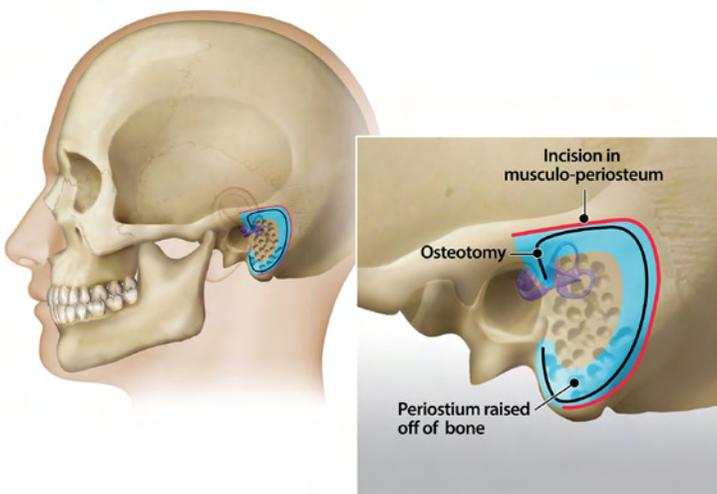
Fig. 1. Initial periosteal incision placed peripheral to anticipated osteotomy.
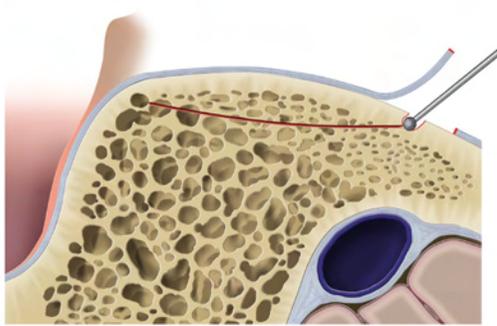
Fig. 2. Initial osteotomy performed with two-mm burr. Note location of periosteal incision and margins of elevation.
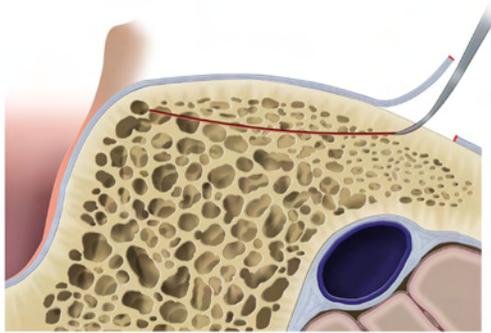
Fig. 3. Osteotomy completed with osteatome.
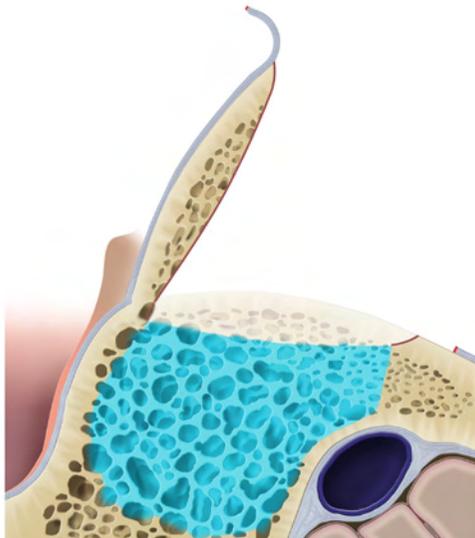
Fig. 4. Osteoplastic unit is up fractured and remains attached at anterior meatally based pedicle.
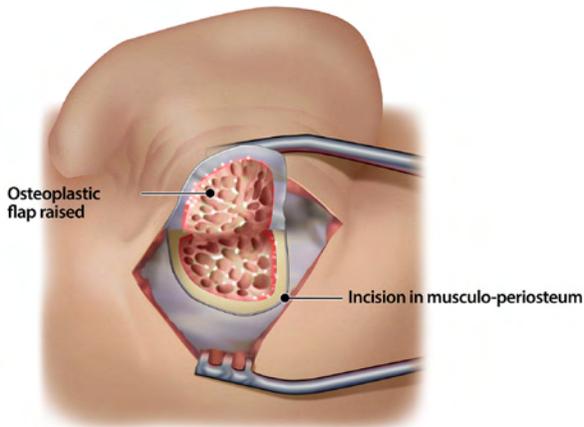
Fig. 5. Osteoplastic unit elevated exposing mastoid interior that will be removed with drill.
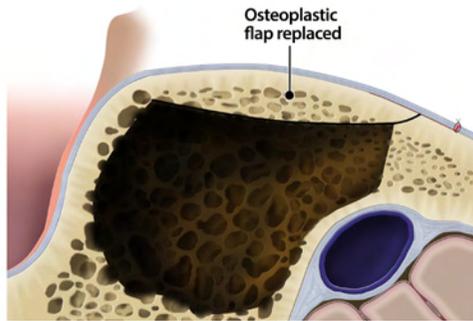
Fig. 6. Osteoplastic unit replaced and periostium reapproximated.

drilled in the standard fashion to include sac decompression or other labrynthine surgery. Upon completion, the flap is replaced and stabilized by reapproximation of the musculoperiosteum (Fig. 6). Alternatively, the flap may be placed over fat graft to buttress a dural repair.

3. Comment

There are limitations which require attention to patient selection. The mastoid must be well-pneumatized and free of pathology. There are line-of-sight restrictions imposed by what may be considered 'keyhole' surgery. In most cases, however, we have found the exposure to be adequate for endolymphatic sac decompression, labyrinthectomy, acoustic neuroma surgery, and facial nerve

decompression. Should the exposure be less-than optimal, the procedure is easily modified to conventional cortical mastoidectomy.

In addition to providing the necessary exposure, the osteoplastic cortical mastoidectomy maintains profile and avoids the need for miniplate fixation. Perhaps most important from the patient perspective, it satisfies their definition of minimally invasive or cosmetic surgery.

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COCHLEAR AND VESTIBULAR IMPLANT

VESTIBULAR IMPLANT FOR SENSORY RESTORATION – CANDIDACY AND EPIDEMIOLOGY

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Abstract

Loss of bilateral vestibular function causes disabling illusory drift of the visual fields known as oscillopsia, chronic disequilibrium, and postural instability from failure of the vestibulo-ocular and vestibulo-spinal reflexes. There is no adequate treatment for many individuals disabled by this loss despite performance of vestibular rehabilitation exercises after cessation of all vestibular-suppressant medications. Recent epidemiologic data from a national survey of United States adults reveal that more than 64,000 US adults (28/100,000) suffer a constellation of symptoms consistent with chronic, profound bilateral loss of vestibular sensation. By current global population estimates, this predicts that as many as two million individuals worldwide may be affected. Affected individuals report reduction or cessation of driving due to their symptoms (44%), reduced participation in social activity (56%), and a 31-fold increase in fall risk in comparison to the age-adjusted nationwide average.[1] Individuals with bilateral vestibular dysfunction (BVD) also report significantly reduced quality of life, increased health care expenses, and decreased productivity due to dizziness-related workplace absenteeism.[2] Several treatments are under development that may be beneficial for patients with severe to profound BVD. An implantable prosthesis, for instance, that senses head rotation and electrically stimulates selective semicircular canals to partially restore the vestibular-ocular reflex could significantly improve quality of life for these individuals. This communication will review data on the prevalence and impact of BVD and the cost-effectiveness of its treatment.

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1. Background

A primary function of the vestibular system is maintenance of steady gaze during head movements. Vestibular nerve afferents discharge at a spontaneous rate, such that afferents of both ears may be temporarily either excited or inhibited above or below this rate. Through complementary pairs of semicircular canals, the vestibular system responds to head rotations with excitation of semicircular canal afferents ipsilateral to the direction of rotation and simultaneous inhibition of afferents on the contralateral side. This functional arrangement across the two labyrinths mitigates the effect of losing vestibular sensation on one side, because compensatory mechanisms allow individuals with unilateral loss of vestibular function to maintain relatively stable gaze, except for during fast head movements toward the deficient side.

Bilateral loss of vestibular function, however, poses a unique challenge for an individual, in that it renders one incapable of maintaining steady gaze during quick head movements in any direction. Although other vision-stabilizing systems such as smooth pursuit, optokinetic responses and anticipatory saccades can maintain a clear image for relatively low speed, low frequency, predictable movements, the majority of head movements encountered during daily activity exceed the functional range for these other systems, leading to image slip on the retinae and the clinical symptom *oscillopsia*.

An excellent description of this symptom is reported in one physician's experience of bilateral vestibular loss after treatment of a knee infection with the vestibulotoxic medication streptomycin:

> Imagine the results of a sequence taken by pointing the camera straight ahead, holding it against the chest and walking at a normal pace down a city street. In a sequence thus taken and viewed on the screen, the street seems to career crazily in all directions, faces of approaching persons become blurred and unrecognizable and the viewer may even experience a feeling of dizziness or nausea.[3]

In his account, the physician J.C. goes on to report his struggles of learning to walk again and navigating the world using the non-vestibular components of balance: vision and proprioception. In individuals like J.C. with severe or total loss of vestibular function, these other two components of balance become critical for maintaining posture. Limiting either remaining component, for example by standing on a thick carpet, swimming, or navigating in darkness makes staying upright and orienting nearly impossible, and it can lead to potentially life-threatening trauma.

2. Treatment of bilateral vestibular deficiency (BVD)

Effective treatments for bilateral loss of vestibular function are needed. Physical rehabilitation with a therapist trained to perform vestibular physical therapy

can drive central nervous system compensatory circuits to optimize use of re-
sidual vestibular function and encourage substitution of other sensory cues for
the missing vestibular input. Rehabilitation is the current standard of care for
bilateral loss, and it is effective in some patients;[4] however, neither rehabilita-
tion exercises nor any other widely available therapy definitively addresses the
underlying pathophysiology.

Fortunately, several treatments are currently in development, addressing new
compensatory strategies,[5-7] cellular regeneration of the vestibular end organ,[8] and
electrical restoration of the vestibulo-ocular reflex (VOR).[9-12] While promising,
these treatments are likely to be costly, so research is needed not only to better
define the population most likely to receive benefit but also to understand the
cost-effectiveness of such therapies.

3. Prevalence of BVD

BVD is a clinical description of individuals with bilateral loss of labyrinthine
function, and does not reflect a common underlying etiology. The causes of
BVD are myriad. Known causes include systemic use of vestibulotoxic antibi-
otics like gentamicin or streptomycin;[13] bilateral Ménière's disease; syndromes
such as neurofibromatosis type 2 or cerebellar ataxia, neuropathy, vestibular
areflexia syndrome (CANVAS);[14] congenital mutations such as COCH/DFNA9;[15]
and theoretical clinical conditions such as vestibular atelectasis.[16] Furthermore,
common chronic conditions such as diabetes[17] and age-related vestibular loss
(presbystasis)[18] may lead to BVD, as well. Despite existence of numerous eti-
ologies, the cause of BVD remains unknown in approximately half of cases.

The population prevalence of BVD has until recently also been unknown. Prior
prevalence estimates had been based on data representing patients in dizziness
and balance disorder specialty clinics. Using data from a nationwide survey
of adults in the United States, we estimated the prevalence of BVD to be 28
per 100,000.[1] This estimate approximates the prevalence of Ménière's disease
(ranges from 15-515/100,000[19]) and suggests that over 64,000 individuals in the
US alone were affected by BVD in 2008. Given that the use of the systemic
antibiotic gentamicin is the most identified common cause of acquired BVD,
BVD prevalence may vary depending on country-specific usage rates. Assum-
ing similar demographics and medical care practices between the US and the
European Union, one can estimate that as many as 142,000 European adults
may currently have BVD.

4. Functional impact of BVD

Functional impairment as a result of BVD may depend on the degree of re-
sidual vestibular function;[20] nevertheless, it is clear that many individuals with

BVD experience severe functional limitations.[2,21] In the US adult population, among individuals with BVD, 44% reported a change in their driving habits, 56% reported limiting social activities, and 90% reported a fall within the last five years, with a 31-times increased odds of falling compared to the nation-wide average.[1] Perhaps unsurprisingly given these findings, those with BVD were more likely to be disabled or unemployed and to suffer from depression.[1] Furthermore, they saw an average of 5.6 (SD 2.9) health professionals over the preceding 12 months, and remarkably only 25% reported receiving any benefit with respect to their balance from a health care professional.

In a follow-up study using the Health Utilities Index (HUI), which is a well-validated quality of life instrument, patients with BVD experienced substantial decrements to overall health-related quality of life compared to both patients with unilateral vestibular loss and normal controls, with several BVD individuals reporting negative (*i.e.*, 'worse than death') health utility estimates in specific domains.[2] In that study, healthcare utilization and employment impact were carefully assessed, allowing estimates of societal cost. Patients with unilateral vestibular deficiency (UVD) each incurred a calculated mean annual economic burden of $ 3531 (€ 3124) in 2011 values and those with BVD incurred a mean burden of $ 13,019 (€11,521). These are likely conservative estimates, as they do not account for costs associated with either therapy such as medications and rehabilitation or fall-related injuries due to BVD.

Quality-adjusted life years (QALYs) are often calculated in assessing the cost-utility of an intervention and have been used to quantify cost-utility of other interventions such as cochlear implants in order to justify whether the projected intervention's cost to society is warranted.[22] In the United States, interventions costing below ~ $ 50,000 (~ € 44,000) per QALY are considered highly cost-effective. As a reference, total knee replacement, one of the most common orthopedic surgeries performed in the US, costs an average of $ 59K (€ 52K)/QALY.[22] A prospective cost-utility analysis was performed for a vestibular implant system currently under development.[11] This analysis revealed both that the system's implantation and use incurs costs similar to cochlear implantation and that if the system restores VOR function sufficient to improve an implant recipient's HUI score by at least 50% of the difference between mean BVD and UVD patient scores, then vestibular implantation would on average be at least as cost-effective as a total knee replacement. Studies assaying the safety and efficacy of that continuously-stimulating system are expected to start soon, and studies of transient stimulation using similar devices in humans are already underway,[9,12,23] with promising results.

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COCHLEAR IMPLANT AND MÉNIÈRE'S DISEASE: VESTIBULAR AND AUDITORY FUNCTION

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1. Introduction

For the last three decades, cochlear implantation is the rehabilitation treatment for patients with profound sensorineural hearing loss (SNHL). Along these years, cochlear implant (CI) indications are growing due to the outstanding results achieved. Therefore, CI is a novel indication for patients with asymmetric hearing loss. In such, patients with Ménière's disease (MD) can benefit for CI in case a profound SNHL is developed in one or both ears. MD is an inner ear disease characterized by cochlear and vestibular symptoms including tinnitus, vertigo and progressive sensorineural hearing loss.[1] The clinical course of MD varies among patients. Hearing loss may initially fluctuate. However, given the disease process, hearing loss progresses to a severe-profound degree in the many patients. In such cases, cochlear implantation is thought to be the rehabilitation therapy. Few studies have analyzed the auditory benefit achieved after cochlear implantation in patients with MD. Moreover, few studies have analyzed consequences of cochlear implantation in the vestibule.

The aim of this study is to measure auditory and vestibular outcomes in MD patients who have undergone CI.

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2. Material and methods

A prospective study design was performed in a tertiary hospital. Twenty-one patients, CI users with profound SHNL due to uni- or bilateral MD, were included. Patients underwent auditory and vestibular evaluation before and after CI.

Auditory evaluation based on pure tone audiometry (PTA), disyllabic (65 dB SPL in quiet) with and without the CI was recorded before and at least two years after CI. Results were compared to a control group (n = 29) (otosclerosis etiology). Besides, a comparison regarding surgical approach (cochleostomy vs round window) was made within the MD group.

Vestibular function was measured based on video head impulse test (VHIT) and vibratory ocular and cervical vestibular evoked myogenic potentials (VEMPs) before and after CI. A Dizziness Handicap Inventory (DHI) test was performed after CI. The vestibular ocular reflex (VOR) was evaluated with the head-impulse test (HIT) in order to register and measure head and eye velocity during the head impulse (GN Otometrics, Denmark). All six semicircular canals were measured (lateral, posterior and anterior plane).Vestibular evoked myogenic potentials (VEMP) were performed with Fz 500 Hz vibration delivered with a Bruel & Kjaer minishaker in order to quantify otolithic function. Disability and handicap assessment was based on the DHI, a validated tool of subjective dizziness handicap that consists of a 25-item three-point questionnaire which assesses self-care skills, psychosocial behaviors and physical activity.

Statistical analysis with the IBM SPSS Statistics was used. Parametric comparisons with Student's t test were performed. If p value is lower or equal to 0.05 a significant statistical difference is considered.

3. Results

A total of 21 patients diagnosed with MD and CI users were included. The mean age at implantation was 59 years (SD 12 years). Mean age of hearing loss was 48 years (SD 16 years). Of those 38% were female and 62% were male. Surgery was accomplished via a round-window approach in 13 cases and via a promontorial cochleostomy in 11 cases.

Auditory results are summarized in Figure 1. Preoperative PTA thresholds are 99 dB and 122.5 dB for the MD group and the control group respectively. No statistical significant differences are seen. Postoperative PTA thresholds without CI are 127.5 dB for the MD group. Mean threshold difference pre-postoperative for the MD group is 28.5 dB. Postoperative PTA thresholds with CI are 34.25 dB and 38 dB for the MD and control group respectively. No statistical significant differences are seen.

PTA thresholds regarding the approach show no statistical differences between the round window and cochleostomy group. Disyllabic testing after CI shows no statistical differences (p = 0.092) when comparing round window approach (median 82.18 dB) versus cochleostomy approach (median 78.4 dB).
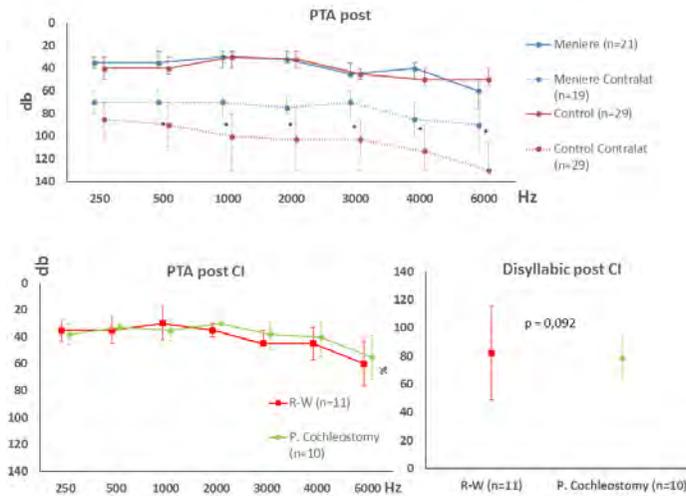
Fig 1. Auditory performance. Upper row: mean thresholds for MD group vs control group after two years of follow-up. Lower row: round window and cochleostomy group mean thresholds for each frequency and speech discrimination.

VHIT is performed before and after cochlear implantation (Fig. 2). The mean follow-up is nine months (range 0 to 22 months). The mean gain for the lateral plane is 0.73 pre- and 0.73 postoperatively (p = 1; CI 95% (-0.18; 0.18)). Mean gain for posterior plane is 0.65 pre- and 0.62 postoperatively (p = 0.76; CI 95% (-0.21; 0.27)). Mean gain for anterior plane is 0.52 pre- and 0.56 postoperatively (p = 0.96; CI 95% (-0.2; 0.2)).

Also, considering postoperative results (n = 14) a comparison is made considering surgical approach. Mean gain for the lateral plane is 0.7 for the round window (RW) group and 0.57 for the promontory group (p = 0.37; CI 95% (-0.43; 0.17)). Mean gain for the posterior plane is 0.69 for the RW group and 0.51 for the promontory group (p = 0.11; CI 95% (-0.4; 0.048)). Mean gain for the anterior plane is 0.62 for the RW group and 0.57 for the promontory group (p = 0.69; CI 95% (0.3; 0.83)).

Vestibular evoked myogenic potentials are performed in the same basis (Fig. 3). Ocular VEMPS before and after implantation is analyzed (n = 11). Asymmetry is 44% pre- and 51.84% postoperatively (p = 0.7; CI 95% (-61; 45.8)). Amplitude is 0.48 µV pre- and 6.03 postoperatively (p = 0.44; CI 95% (-20.8; 9.79)). N10 latency is 14.87 mseg pre- and 10.36 postoperatively (p = 0.139; CI 95% (-1.7; 10.74)). Regarding postoperative results with this test, asymmetry is 31.82 for RW and 75.87% for the promontory group (p = 0.016; CI 95% (10.25; 77.55)). Amplitude is 7.68 µV for RW and 4.06 µV for the promontory group (p = 0.55; CI 95% (-17.08; 9.83)). N10 latency is 11.68 mseg for the RW group and 8.78 mseg for the promontory group (p=0.21; CI 95% (-7.8; 2.04)).

Cervical VEMPS before and after implantation (n = 12) show the following: asymmetry is 34.9% pre- and 58.4% postoperatively (p = 0.36; CI 95% (-77;
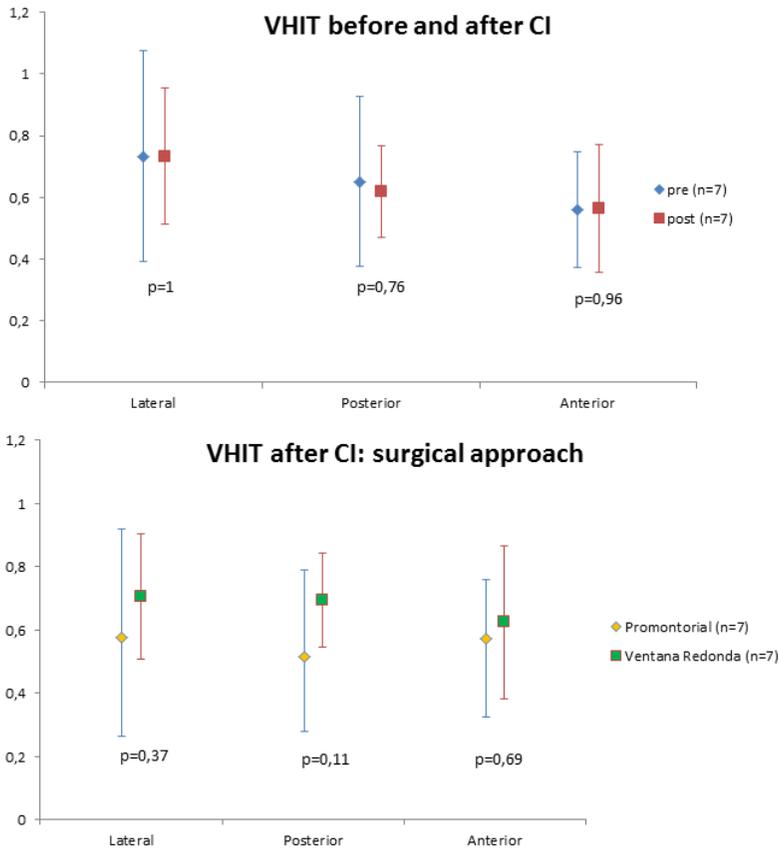
Fig 2. Head impulse test mean gains for each semicircular canal. Upper graph shows a comparison before and after CI. Lower graph shows a comparison considering surgical approach. No statistical significant differences are seen (p values shown).

30.17)). Amplitude is 14.73 µV pre- and 31.21 postoperatively (p = 0.21; CI 95% (-43.6; 10.64)). N16 latency is 13.43 mseg pre and 16.83 postoperatively (p = 0.05; CI 95% (-6.87; 0.072)). Regarding postoperative results with this test, asymmetry is 49.96 for RW and 66.84% for the promontory group (p = 0.43; CI 95% (-29.3; 63.09)). Amplitude is 37.08 µV for RW and 25 µV for the promontory group (p = 0.29; CI 95% (35.2; 11.7)). N16 latency is 17.11 mseg for the RW group and 16.55 for the promontory group (p = 0.66; CI 95% (-3.33; 2.22)).

And last, DHI mean score is 34.5 (SD 16.97). A comparison with moderate incapacity shows no statistical significant differences (p = 0.921).
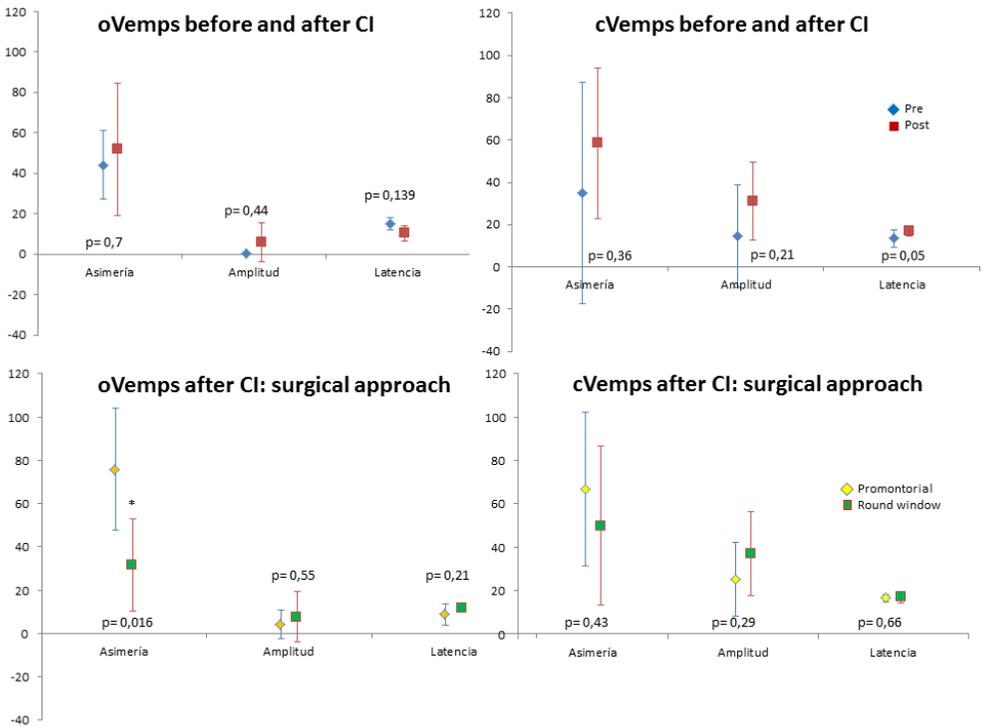
Fig 3. Vestibular evoked myogenic potentials values are represented before and after surgery in the upper row. No statistical significant differences are seen. In the lower row a comparison considering surgical approach is shown (p values described). Note p value < 0.05 for the utricle asymmetry.

4. Discussion

Severe to profound SNHL in patients with end-stage MD may occur. For such condition, CI is the treatment offered. Our results suggest that an improvement in speech discrimination can be achieved. Similar findings are described by Mick et al.[2] Their data supports the use of CI in patients with MD who are audiologic and surgical candidates for CI. An improvement in speech understanding and tinnitus is demonstrated. Such results are not significantly different from age, sex and device.

Although auditory rehabilitation with prosthesis show a limited result with hearing aids for moderate SHNL in MD patients compared to other etiologies, an improvement similar to a control group is seen for severe to profound SNHL with CI. Similarly, Vermeire et al.[3] demonstrate that CI improves speech understanding. Also, despite different surgical approaches (round window vs cochleostomy), no significant statistically differences are seen regarding pure tone audiometry nor speech discrimination. However, McRackan et al.[4] conclude that hearing outcomes in a sample of 21 MD patients seem to be worse than

general CI population. Better performance is described in patients with active MD and those with history of ablative intervention. Further research with larger samples is needed.

Cochlear implantation may cause trauma by insertion of the electrode into the ear. Theoretically, surgical maneuvers and electrode array may alter inner ear fluid homeostasis, provoke trauma of inner ear structures or inflammation and fibrosis. Thus, hearing preservation may not be achieved. Our results show a mean threshold difference of 28.5 dB suggesting that trauma during surgical procedure may damage the inner ear in this condition. On the other hand, whether CI may damage the otolith organ and/or the semicircular canals function is controversial. Our results suggest no deterioration of otolithic function or semicircular function before/after CI. Buchman *et al.*[5] found that significant adverse effects on the vestibular system were uncommon. In fact, several studies have indicated that the rate of spontaneous complete or partial resolution of vertigo over the long term in MD is 70% leaving about 30 % of subjects with chronic dizziness.[6] Thus, vestibular symptoms after CI in MD patients may be the ongoing disease itself.

Regarding surgical approach, our results suggest that there are not significant statistical differences concerning semicircular function. Whereas promontorial approach is more prompt to damage the otolithic function at the level of the utricle. Similar findings have been reported by Batuecas-Caletrio *et al.*[7] The round window approach is safer and less traumatic than a cochleostomy for CI.

5. Conclusions

CI is an appropriate treatment for severe to profound SNHL in MD patients. Auditory performance is improved and similar to general CI population.

No dysfunction of the otolithic or the semicircular canal function has been demonstrated after CI surgery. Round window approach should be considered in order to minimize trauma in the utricle.

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COCHLEAR IMPLANT IN MENIERE'S PATIENTS WITH ASYMMETRIC HEARING LOSS

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1. Introduction

Ménière's disease (MD) is characterized by complex cochlear and vestibular symptoms including vertigo, roaring tinnitus, fluctuating aural fullness and progressive sensorineural hearing loss.[1] In most cases, MD affects only one ear with an incidence that ranges from 4.3-15.3 cases per 100,000 per year.[2] Less data are available on the bilateral variant of the disease. Bilateral MD can have a strong negative impact on a patient's quality of life because of possible incapacitating vertigo and bilateral hearing loss. Patients with end-stage MD who develop bilateral profound hearing loss are now usually treated with cochlear implantation (CI).

The management of asymmetric hearing loss in bilateral MD is more controversial especially in the paradigmatic case of a deaf ear with a moderate or moderate-to-severe hearing loss in the contralateral ear. Amplification, included Bi-Cros configuration, is sometime ineffective in these cases because of high distortion or frequent hearing threshold fluctuation in the 'best' ear.

As a part of a more general clinical study on the 'new' indications we implanted in the last years an increasing number of MD patients with asymmetric hearing loss. The purpose of this report is to analyze the post-operative hearing results in patients suffering from MD and asymmetric hearing loss.

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2. Methods

2.1 Study design

This is a retrospective observational nonrandomized study. All subjects signed an informed consent. The study design and the recruitment of subjects were accomplished according to the local ethical requirements.

2.2. Subjects

Only patients who clearly met the AAO-HNS guidelines[1] for diagnosis of Ménière's disease before implantation were included in the study group. Fifteen subjects (ten males and five females) with bilateral MD who received CI were included in the study. All patients were recruited from the CI program at 'Guglielmo da Saliceto' Hospital in Piacenza. In all cases the poorer hearing ear was implanted, based on the standard audiological criteria (speech recognition performance with well-fit, powerful hearing aids) for implantation. The mean age at CI activation was 58.8 years (SD ± 11.5; range: 41-74). No subject has ever discontinued the device use. Nine subjects were implanted with a Nucleus multichannel device (Cochlear LTD, Sydney, Australia), three patients with an Advanced Bionics multichannel device (Advanced Bionics AG, Stäfa, Switzerland) and the remaining three with a Med-El device (Med- El Gmbh, Innsbruck, Austria). Nine subjects wore a CI on one ear and a hearing aid on the opposite side ('bimodal stimulation'). The remaining six recipients wore a unilateral CI.

The pre-operative PTA (500-4000 Hz) was 121.6 dB HL (SD ± 0.8) on the CI ear and 78.4 dB HL (SD ± 3.9) on the contralateral ear. At time of study, the CI experience at testing was 31.4 months (SD ± 29.4), ranging from one to 84 months.

Other clinical data were also extracted from the database like recurrence of vertigo spells and device use.

2.3. Audiologic testing and questionnaires

Speech perception in noise was measured in a sound field under best-aided conditions. Tests were conducted in a sound-treated chamber via two loudspeakers positioned one meter away from the subject's head, with an angle of +45° and -45°. The two presentation setups, $S_{+45°}N_{-45°}$ and $S_{-45°}N_{+45°}$, were used for testing speech perception in background noise. Depending on the implanted ear, the presentation setups are reported as configurations $S_{ci}N$ (speech from the implanted ear/noise from the un-implanted ear), and SN_{ci} (speech from the un-implanted ear/noise from the implanted side). Speech perception in noise was assessed using taped disyllabic word presented at 70 dB SPL with a fixed signal-to-noise ratio (SNR) of +5 dB (cocktail party noise at 65 dB SPL). Two lists of 50 words were used. Speech perception was scored as percentage correct

word score. All speech lists were randomized between subjects; no one received two of the same lists during any test session.

In the present study we reported pre-operative and last available follow-up score.

The Tinnitus Handicap Inventory (THI)[3] was administered pre and post-operatively such as the Speech, Spatial and Qualities of hearing scale (SSQ).[4] The THI is a 25-item questionnaire. Each item has three potential answers with 'yes' assigned four points, 'sometimes' two points, and 'no' zero points. The total score can range from zero indicating no tinnitus handicap and 100 the worst patients' annoyance. The SSQ questionnaire has three sections and assesses speech understanding, spatial hearing, and hearing quality with a scoring system of zero to 10 for each item, in which zero represents unable to hear and ten means hears perfectly.

3. Results and discussion

No intra-operative complications occurred. Only one subject presented vertigo in the post-operative period with a resolution after three weeks. All patients wore their devices throughout the waking day with a mean of 13 hours.

3.1. Speech perception in noise

Pre- and post-operattive speech perception in noise (SNR +5dB) are shown in Figure 1. Mean pre-operative score was 2% when speech was presented from the 'worst' side ($S_{ci}N$); after CI use a mean score of 60% was observed. Scores improvement range from 20% to 90%. This difference was statistically significant ($p < .0001$). When speech was presented from the 'best' side (SN_{ci}) the mean speech perception score was 26.6%. Also in this case a significant improvement with use of the CI was found over time (mean score 56%; $p < 0.05$).

The improvements in speech perception in noise after CI are not unexpected. In fact, a significant degeneration of the spiral ganglion is rare and the severity of hearing loss does not always correlate to neuron survival in MD subjects as demonstrated by histopathologic studies.[5] The other series of patients with MD treated with CI reported improvements in words and sentences recognition score in quiet[6-7] and in noise[8] after the CI activation, but all series comprises subjects with bilateral severe to profound hearing loss at MD's end stage.

3.2. Tinnitus

Pre- and post-operative THI scores are shown in Figure 2. The mean preoperative score was 62.2 (SD ± 28.5) ranging from 18% to 100%. At the last post-operative evaluation the mean THI score was 44.5 (SD ± 32.2). The THI score's reduction was highly significant ($p < 0.002$). Therefore, a positive effect of CI stimulation on tinnitus annoyance was found. In all cases but one the CI use
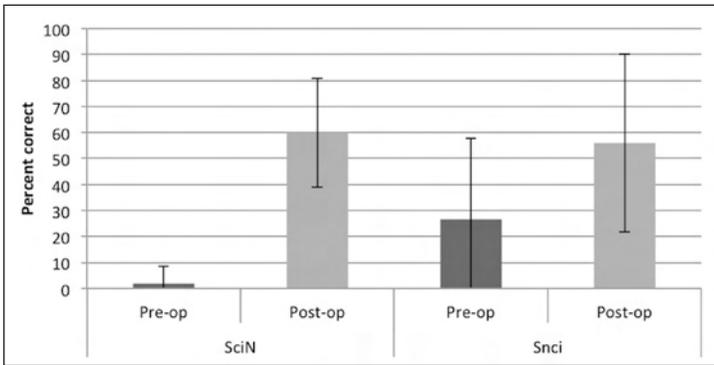
Fig. 1. Pre-operative and post-operative speech-in-noise perception scores in patients with Me-
niere Disease suffering from asymmetric hearing loss. Test is performed at +5 dB SNR. Signal
and noise were presented separately from two loudspeakers. The configuration $S_{ci}N$ means the
speech was presented from the loudspeaker near the not-implanted side and the noise was pre-
sented from the loudspeaker near the implanted side. SN_{ci} denotes the opposite configuration.
Speech perception score after CI use is significantly improved for both configuration SN_{ci} ($p <$
0.05) and $S_{ci}N$ ($p < 0.0001$).
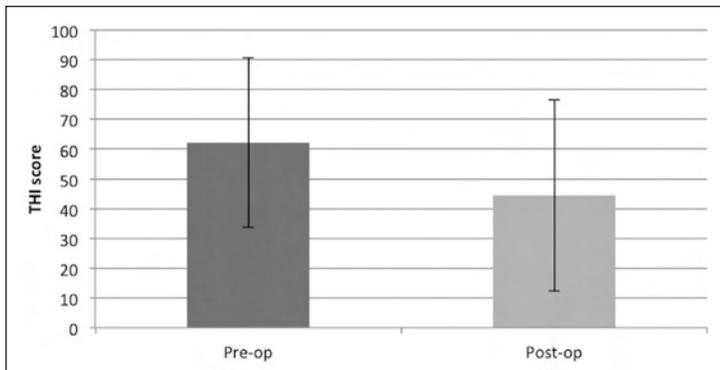
Fig. 2. Pre-operative and post-operative Tinnitus Handicap Inventory scores. After CI use patients
showed a significant reduction of THI score ($p < 0.002$).

reduced tinnitus severity. Subjects reported tinnitus relief especially when the
device was turned on. This effect remained constant over time.

3.3. Hearing disability

The SSQ subscales scores are shown in Figure 3. For the 'Speech' section the
mean score was 2.8 (SD ± 1.4) pre-operatively and 5.5 (SD ± 2.3) at the last
follow-up visit. The difference was statistically significant ($p < 0.005$) and denotes
a reduction in perceived disability. A similar trend was found for the 'Spatial'
dimension of the hearing disability; MD patients scored 2.8 (SD ± 1.9) before
and 4.7 (SD ± 2.4) after CI ($p < 0.01$). A significant improvement in perceived
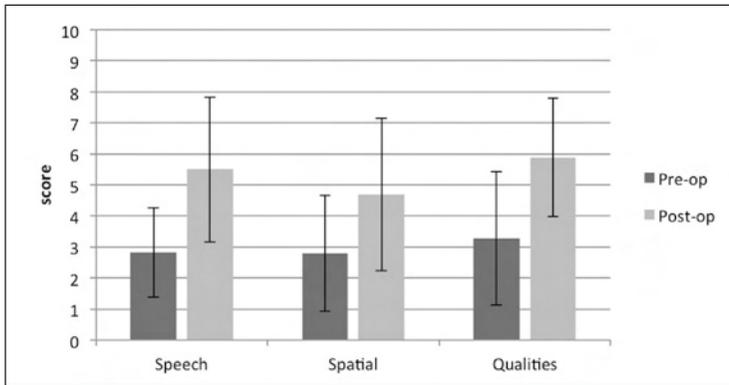
Fig. 3. Pre-operative and post-operative scores at different subscales of SSQ questionnaire. A significant score improvement (reduced disability) is found with CI use over time for speech section (p < 0.005), hearing spatial section (p < 0.01) and quality section (p < 0.01).

disability was also found in the 'Quality' domain (pre = 3.3; post = 5.9; p < 0.01). The SSQ data revealed an overall benefit of CI use in the MD patients.

3.4. Vertigo

Five patients (33.3%) had vertiginous spells at the time of CI surgery; they were here defined as the 'active' MD subgroup. There was no statistically significant difference in pre-operative speech perception in noise between the active and non-active MD subjects. Only one patient with 'active' MD reported a long-lasting vertigo relief after surgery. The remaining four patients had no subjective improvement after CI. In general terms patients with active MD continued to have vestibular attacks after implantation with a similar pattern than before. In fact, no patients reported any increase in spells recurrence or in severity of vertigo's attack.

   Some patients experienced alterations in their implant performance in association with fluctuations in vestibular symptoms. It was impossible anyway to describe a specific trend; some experienced a less clear perception before of vertigo attack, some other during or after resolution.

4. Conclusion

This study adds some evidence of benefit in a specific area of the so-called CI 'new indications'. The use of CI seems to produce over time significant benefit in MD patients with asymmetric hearing loss.

   The benefit covers different domains. Speech perception in noise is significantly improved at +5 dB SNR when speech and noise source are separated. This is a clear demonstration of some binaural effect due to head shadow and

possibly to squelch effect although the study design is not specifically addressed to differential contributes of different acoustic cues processing.

The significant score improvement in speech, spatial and qualities hearing disability dimensions denotes an overall benefit on patients' quality of life.

Finally, an important result is the marked reduction of tinnitus annoyance. This has a profound impact on quality of life and it is especially perceived when the CI is turned on as a result of an effective masking. Benefit is also perceived when CI is turned off probably as the result of some habituation.

The natural history of vertigo spells in this patient cohort seems relatively unaffected. This evidence and the referred hearing fluctuation related in some way with vertigo spells needs to be validated with high quality prospective observational studies.

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SPATIAL HEARING IMPROVEMENT AND LONG-TERM SUPPRESSIVE EFFECT ON TINNITUS AFTER COCHLEAR IMPLANTATION IN SINGLE-SIDED-DEAF PATIENTS WITH AND WITHOUT MÉNIÈRE'S DISEASE

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1. Introduction

Tinnitus is often associated with hearing loss. In patients with unilateral profound hearing loss (UHL) the effect of a Cochlear Implant (CI) is under scientific attention.[1] The first study that treated incapacitating tinnitus in UHL subjects was conducted by Van de Heyning *et al*.[2] CI was found to have the potential to significantly reduce tinnitus in single-sided-deaf (SSD) patients and to restore binaural hearing.

The current study presents the long-term evaluation of the auditory and tinnitus outcomes up to ten years with specific interest to Ménière's disease (MD).

2. Materials and methods

A VAS (Visual Analogue Scale) was used to assess subjective tinnitus loudness, pre-operatively, one, three, six, 12 and 36 months post-operatively and at the long-term test interval.[3]

The Tinnitus Questionnaire (TQ) was used to quantify tinnitus complaints pre-operatively, one, three, six, 12 and 36 months post-operatively and at the long-term test interval. This widely used questionnaire was developed by Hallam *et al*.[4] and modified by Goebel and Hiller.[5]

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Given the relative small sample size, quantitative data are presented as median and range (minimum and maximum) and nonparametric statistics were used.

3. Results

Long-term evaluation was derived from four MD and 19 non-MD patients suffering from SSD and accompanying incapacitating tinnitus. The latter was defined as tinnitus loudness visual analogue scale (VAS) score exceeding six out of ten. Patients had normal hearing or mild to moderate hearing loss on the contralateral ear. They received the CI at a median age of 55 years (ranging 22-71 years) and had eight years (ranging three to ten years) of experience with their CI.

All patients wore their CI seven days a week. It appeared that in all but one, CI switch-on is the first act when rising and CI switch-off is the last act before bedtime. In the majority of the patients (*i.e.*, 70%) the tinnitus reduction started within one minute and the residual inhibition after CI switch-off was less than a minute (in 65% of cases). The long-term tinnitus VAS scores indicate a significant improvement between the CI_{OFF} condition (7.95/10) and the CI_{ON} condition (2.85/10).

The TQ also showed a significant tinnitus relief that remained stable over eight years. A significant decline ($p < 0.01$) and thus improvement was found one month after the first fitting. The median TQ score preoperatively was 55 (27-78) and was 41.5 (4-64) one month after the first fitting. The degree of perceived tinnitus reduction remained significantly stable with the CI up to eight (three to ten) years.

Speech perception in noise and sound localization improved significantly after cochlear implantation.

No statistically significant differences were observed between MD patients and the other patients.

4. Discussion

Appropriate patient selection is essential. The incapacitating tinnitus must result from ipsilateral sensorineural deafness as it is expected that tinnitus arises from deafferentation. Furthermore, the tinnitus has to be present for at least two years and stable during the past year, so that spontaneous improvement of the tinnitus is highly unlikely. The subjects have to be non-responsive to existing conventional treatments.

This study aimed to do a long-term analysis of the tinnitus reduction in the UHL study cohort of Van de Heyning *et al.*[2] ten years after CI. The results demonstrate that a CI in UHL subjects is a durable treatment for incapacitating tinnitus. The study of Kleine Punte *et al.*[6] found that VAS loudness scores and TQ scores reduced significantly after CI up to three months after first fitting.

The present study demonstrated that this tinnitus reduction is significantly stable after ten years.

The initial idea was to restore the normal sensory input in the UHL subjects and in that way to reduce their incapacitating tinnitus caused by auditory deprivation. The hypothesis was suggested that providing a chronic tinnitus sufferer with electrical stimulation via a CI might result in cortical reorganization that suppresses the tinnitus and restores an acceptable degree of quality of life for these individuals. The current study refutes this assumption, since the residual inhibition of tinnitus after CI switch-off is less than one minute in the majority of the subjects.

5. Conclusion

CI can significantly improve speech perception in noise and restore binaural hearing in SSD patients. Several years of CI use are necessary to fully take advantage of binaural cues available from the CI. The accompanied tinnitus relief appears to be stable over ten years of follow-up, even in MD patients.

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VESTIBULAR FUNCTION BEFORE AND AFTER COCHLEAR IMPLANTATION IN PATIENTS WITH POST-LINGUAL DEAFNESS: A PROSPECTIVE, OBSERVATIONAL STUDY

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1. Introduction

There is no consensus in the literature with regard to the effects of cochlear implantation (CI) on vestibular function and balance in patients with deafness. Some authors believe that CI negatively influences vestibular function,[1] some believe that it has no such effect,[2] while others have suggested that CI may improve balance.[3]

An association between the auditory and vestibular pathways has been documented in studies using electrophysiological investigations,[4] imaging techniques[5] and electron microscopy.[6] This association is responsible for integration between the two systems and the effects of CI-generated stimuli on balance. The influence of CI on balance is also determined by anatomic factors, individual predispositions to the stimulus pattern produced by CI, and the plasticity of the neurological system of each individual.[7]

This study aimed to evaluate vestibular function before and after CI in patients with post-lingual deafness by assessing the influence of new auditory stimuli on the maintenance of posture over the course of one year after CI.

2. Methods

This prospective observational study was conducted in patients between 12-65 years (mean age, 42 years). A total of 24 patients (ten females, 14 males) were

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assessed between February 2008 and September 2011. All patients completed a questionnaire on the presence and manifestations of vertigo and underwent caloric tests, rotary chair (RC) testing, and Computerized dynamic posturography (CDP). The tests were performed sequentially before and 60, 120, 180, and 365 days after CI surgery. The significance level was set at 5% ($\alpha = 0.05$) for all results.

3. Results

Thirteen patients (54.2%) reported dizziness before CI. At the end of the study, dizziness remained unchanged in one (7.7%) patient, ameliorated in 11 (84.6%), and worsened in one (7.7%). Overall, 20.8% patients in our sample reported immediate postoperative dizziness, which resolved within a month after CI. Baseline caloric tests identified 29.2% patients with normal reflexes, 33.3% with unilateral areflexia or hyporeflexia, 12.5% with bilateral hyporeflexia, and 25% with bilateral vestibular loss (BVL). Most patients exhibited objective improvements in postural stability. At 365 days, the CDP condition, particularly C5 and composite scores (CS) were higher for caloric tests responders at baseline than for those with BVL at baseline (Table 1).

4. Discussion

The auditory information provided by the cochlear implant was a determining factor in triggering plasticity, which integrated the neural networks involved in postural stability. The development of a new afferent auditory pathway produces changes in the neural network in charge of balance and consequent reorganization. This structural change in the central vestibular system was documented by our findings of improved in caloric tests responses during the postoperative period, not only in the implanted ears, but also in the contralateral non implanted ears. Patients with deafness exhibit cross-modal rewiring of the auditory cortex to visual representation, a condition that determines the auditory prognosis of patients who undergo CI.[8] Therefore, the auditory experience, both deprivation thereof and the subsequent stimulation provided by the cochlear implant, may affect both the auditory and visual systems in these patients.[9] In our sample, patients with BVL were able to integrate their new auditory afference into the visual abilities developed throughout their lives and were able to use this visual information for postural stabilization (condition 4), with repercussions on the CS. Finally, patients with residual vestibular function at baseline and patients with BVL showed significant differences in CS, representing improved performance in postural recovery strategies. However, we found that improvements in CS were substantially greater among patients with residual vestibular function at baseline ($P = 0.005$) than in those with BVL ($P = 0.018$). This finding is easily explained by the fact that patients with BVL could only achieve improvement

Table 1. P values for C1, C2, C3, C4, C5 and C6 scores and composite scores (CS) at day zero (before CI) and day 365 (after CI) in patients with bilateral vestibular loss (BVL) and those who responded to caloric tests (CT) at baseline (before CI).

	CT	BVL	CT RESPONSE	p
	Day zero			0,048*
Condition 1	Day 365			0,292
	p	0,176	0,356	–
	Day zero			0,172
Condition 2	Day 365			0,324
	p	0,080	0,477	–
	Day zero			0,324
Condition 3	Day 365			0,848
	p	0,028*	0,794	–
	Day zero			0,061
Condition 4	Day 365			0,086
	p	0,018*	0,001*	–
	Day zero			0,008*
Condition 5	Day 365			0,007*
	p	0,18	0,008*	–
	Day zero			0,008*
Condition 6	Day 365			0,029*
	p	0,180	0,033*	–
	Day zero			0,004*
CS	Day 365			0,024*
	p	0,018*	0,005*	–

CTs: caloric tests. BVL: bilateral vestibular loss. CT response: patients with normal reflexes, unilateral hyporeflexia or areflexia and bilateral hyporeflexia. C1, C2, C3: conditions with platform fixed. C4, C5, C6: conditions with platform in motion (sway-referenced). C1 and C4 with eyes open. C2 and C5 with eyes closed. C3 and C6 with sway-referenced visual surround.
* Denotes statistically significant difference.

in C4 scores, whereas caloric tests responders at baseline achieved improvement in scores for all test conditions involving a moving support platform.

5. Conclusion

Overall, 20.8% patients in our sample reported immediate postoperative dizziness, which resolved within a month after CI. Electrical stimulation affected both ears and interfered with the progression of postural recovery after cochlear implant activation, which led to a significant improvement in CDP scores over the year after CI. Improvements in balance followed the time frame proposed in the literature for central compensation mechanisms. The presence or absence of

caloric test response was a decisive determinant of balance outcomes over the year after surgery. Patients with BVL were able to use the visual information for postural stabilization (condition 4), with improvement in the CS. Finally, it is essential that vestibular assessment findings be documented before CI surgery because a patient's prognosis in terms of learning skills and postural recovery over time depends on this information.

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INDEX OF AUTHORS