ZIANE SELMANI

Otoneurological Work-up in Ménière’s Disease and Other Inner Ear Disorders

A Clinical Study

ACADEMIC DISSERTATION
To be presented, with the permission of the Faculty of Medicine of the University of Tampere, for public discussion in the auditorium of Finn-Medi 1, Biokatu 6, Tampere, on July 9th, 2004, at 12 o’clock.

Acta Universitatis Tamperensis 1021
ACADEMIC DISSERTATION
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Helsinki University Central Hospital
Finland

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Finland

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Printed dissertation
Acta Universitatis Tamperensis 1021
ISBN 951-44-6017-0
ISSN 1455-1616

Tampereen Yliopistopaino Oy – Juvenes Print
Tampere 2004

Electronic dissertation
Acta Electronica Universitatis Tamperensis 363
ISBN 951-44-6018-9
ISSN 1456-954X
http://acta.uta.fi
Acknowledgements

The present study was carried out at the Department of Oto-rhinolaryngology of the University Central Hospital of Helsinki and at the Department of Oto-rhinolaryngology of the University Central Hospital Tampere.

I wish to thank

Professor Ilmari Pyykkö, my supervisor for his constructive advises and criticisms which helped me in realising this work.

Timo Marttila, PhD, my second supervisor for creating a positive atmosphere for carrying out this work.

Professor Nureddin Ashammakhi for his encouraging support, assistance and for giving me the enthusiasm to achieve this work.

Hisayoshi Ishizaki for helping me to collect the data.

Docents Petri Mattila and Juha-Pekka Vasama, for their constructive suggestions and positives criticisms in the preparation of this manuscript.

My dear wife for her patience, support and understanding.

This work has been supported by grants from the Tampere University Hospital, Finnish audiological association and the culture centre of Satakunta, Pori.
To those who care most,
    To those who love best,
        To those who know and share their
            Wisdom
To those who need my
    care, my love, my knowledge, my life
        to all
            I present this work.
Abstract

**Background:** Various inner ear diseases present an indistinct entity because of their unclear aetiology, common clinical features, and unpredicted disease progression, which leads commonly to the handicap of hearing and/or balance function.

**Aim:** To investigate anatomical, electrophysiological, neuro-physiological, vascular changes and immunological manifestations secondary to viral infection which occur during various inner ear diseases, to differentiate between inner ear diseases and in particular to assist diagnosing MD.

**Materials and methods:** Otoneurological work-up consisted of A) low frequency stimulation in posturography (LFS) in 64 patients with various inner ear diseases; B) middle ear transtymapnic endoscopy performed to 265 patients who presented with vertigo, SNHL, with or without tinnitus; C) transtympanic ECoG with click stimuli performed to 249 patients having MD (n=131), tinnitus with or without vertigo (n=45), PSNHL of unknown aetiology (n=31), sudden deafness (n=11), otosclerosis (n=13) or other inner ear diseases (n=18); D) cochlear blood flow (CoBF) measurement with laser Doppler flowmeter (LDF) in 115 patients having either MD (n=69), PSNHL (n=38) or sudden deafness (n=8); E) Antiviral antibody and immunological assays evaluated in 159 patients with MD and the results of antiviral antibody assays compared with those from 26 patients operated upon because of vestibular schwannoma.

**Results:** LFS provoked unsteadiness in posturography in patients without PLF. At stimulation frequency of 25 Hz 25% and at frequencies of 50 and 63 Hz 30 % of patients with MD responded with increased vestibulospinal responses. In one patient, tympanoscopy revealed PLF in the round window. In seven cases the tympanic cavity could not be visualised because of adhesive tympanic membrane, abnormal anatomy or prominent exostoses of the external ear canal. The highest pathological SP to AP (SP/AP) ratio (0.40) was encountered in the
Ménière’s group. The mean amplitude was 2.6µV for the SP and 7.2µV for the AP while the CM characteristics remained unchanged or even increased in the Ménière group. Statistically significant correlation between the hearing level and CoBF amplitude was observed only in the SNHL group (r=-0.4, p<0.05). Vzos, AV, rotavirus, CVB5 and RSV titres were significantly elevated in patients with MD when compared with the control group. In MD, Vzos and AV titres were significantly higher in patients without EH than in patients with EH. The RSV titre correlated significantly with hearing loss at low frequencies (0.5–1 kHz).

One hundred and twenty-seven patients (80%) with MD had evidence of abnormal plasma IC.

**Conclusions:** The otoneurological work-up reveals that MD is characterised by:

1) partially pathological response to the LFS stimulation (Tullio phenomenon);
2) normal middle ear anatomy and absence of spontaneous PLF;
3) the inconsistent findings of EH in all patients;
4) the presence of EH either in MD and other inner ear diseases,
5) the characteristical hyperdepolarisation of the OHCs;
6) a normal vascular component and
7) High titres of IgG antibodies against Vzos, AV and RSV antigens, and elevation of immunological reactants during acute phase of MD.
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AAO-HNS</td>
<td>American Academy of Oto-laryngology, Head-Neck Surgery</td>
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<tr>
<td>AP</td>
<td>action potential</td>
</tr>
<tr>
<td>CM</td>
<td>cochlear microphonic</td>
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<td>CoBF</td>
<td>cochlear blood flow</td>
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<td>ECoG</td>
<td>electrocochleography</td>
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<tr>
<td>EH</td>
<td>endolymphatic hydrops</td>
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<td>ES</td>
<td>endolymphatic sac</td>
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<td>IHCs</td>
<td>inner hair cells</td>
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<td>LDF</td>
<td>laser doppler flowmetry</td>
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<td>LFS</td>
<td>low frequency sound</td>
</tr>
<tr>
<td>MD</td>
<td>Ménière’s disease</td>
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<td>OHCs</td>
<td>outer hair cells</td>
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<td>PLF</td>
<td>perilymphatic fistula</td>
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<td>SHL</td>
<td>sudden hearing loss</td>
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<td>PSNHL</td>
<td>progressive sensorineural hearing loss</td>
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<td>PTA</td>
<td>pure tone average</td>
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<tr>
<td>SNHL</td>
<td>sensorineural hearing loss</td>
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<td>SP</td>
<td>summating potential</td>
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<td>VS</td>
<td>vestibular shwannoma</td>
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</table>
List of original publications

This study is based on the original publications which will be referred in the text by their roman numbers (I-V)


Introduction

Ménière’s disease (MD) is a clinical disorder defined as idiopathic syndrome of endolymphatic hydrops (EH), resulting in fluctuating/or progressive hearing loss (PSNHL), tinnitus, sensation of fullness in the affected ear, and episodic rotational vertigo. Hydrops of the labyrinth is a pathophysiological condition in which dilatation of the endolymphatic system occurs. This condition is verified in man only by post-mortem histopathology of the temporal bone (Alford, 1972). On the basis of Ménière’s symptoms, the American Academy of Oto-laryngology, Head-Neck Surgery (AAO-HNS) has scaled MD into different categories or stages (AAO-HNS guidelines, 1995).

MD is still an obscure disease entity, although it was first defined more than 140 years ago, by Prosper Ménière who attributed the sudden onset of vertigo, tinnitus, hearing loss, nausea and vomiting to an abnormality within the inner ear. In 1923 Portmann demonstrated the role of the endolymphatic sac (ES). Early research focused on endolymph which is an inner ear fluid secreted by the stria vascularis and resorbed by the ES and endolymphatic duct. Patients with MD were found to have over distension of the inner ear chambers that contain the endolymph.

Unfortunately, symptoms of several inner ear disorders like sudden hearing loss (SHL), isolated PSNHL (Filipo et al., 1997), perilymphatic fistula (PLF) (Kohut et al., 1995), and some forms of otosclerosis (Liston et al., 1984) mimic MD and also each other. When evaluating only the clinical history of the patient, the proper diagnosis is often missed. Thereby, in addition to the clinical history, objective assessment is needed to distinguish MD from other inner ear diseases.

The function of the vestibular organ in Ménière’s disease can be evaluated on posturography by stimulating the inner ear with low frequency sound (LFS) at high intensity, which provokes unsteadiness and nystagmus (Young et al., 1977). This sound energy transmitted to the vestibular end organ through the
perilymph and endolymph reaches and stimulates the outer-hair cells of the crista ampullaris.

Ménière’s disease is not associated with PLF. To distinguish MD from PLF, otoendoscopy may be performed (Poe et al., 1992a). The oto-endoscopy was introduced by Nomura (1982) and Takahashi et al. (1990) for the inspection of the tympanic cavity.

To establish the presence of endolymphatic hydrops, electrocochleography (ECoG) has become an accurate method (Eggermont and Odenthal 1977, Gibson et al., 1977). ECoG can be used to evaluate the electrical function of the outer hair cells (OHCs) by cochlear microphonic (CM) measurements. ECoG measures also summation potential (SP) and action potential (AP), which give information on the function of the distal portion of the cochlear nerve. The SP/AP ratio gives information of endolymphatic hydrops (Durrant and Dallos, 1974, Asai and Mori, 1989, Arslan et al., 2000).

Changes in cochlear blood flow (CoBF) have been proposed to mediate pathogenesis of several inner ear diseases such as presbyacusis (Johnsson and Hawkins, 1972), noise induced hearing loss (Hawkins, 1971), sudden deafness (Jaffe 1975, Nakashima 1992), and MD (Kimura, 1974).

During the last few decades viral infections has been implicated in triggering a reactive immune response involving the inner ear in MD (Bergström et al., 1992). Such an infection and immune response in the vicinity of ES can alter the function of the sac and result in derangement of inner-ear fluid homeostasis (Linthicum and El-Rahman, 1980, Arenberg et al., 1991).
Review of literature

Anatomy of the cochlea and the vestibular system

Cochlea
The cochlea has a spiral form with 2.5 windings. Its largest dimension is 4 mm and its straight average length is 34 mm (Böhnke and Arnold, 1999). The cochlea consists of three lymph filled channels. The scala vestibuli and scala tympani are filled with perilymph, whereas the scala media is filled by endolymph. The perilymphatic compartment of the inner ear is directly connected with the cerebrospinal fluid in the posterior fossa through the cochlear aqueduct and several perineural and perivascular spaces (Rosing et al., 1998). Perilymph separates the membranous labyrinth from the internal layer of the labyrinthine capsule surrounding the various spaces. The origin of the perilymph is not known in detail, but it is assumed that it is formed by filtration from blood and possibly by diffusion of the cerebrospinal fluid (Kellerhals, 1979). The endolymph is secreted by the strial marginal cells in the cochlea and the dark cells in the vestibule organ (Lundquist et al., 1984). Its sodium and potassium content is completely different from perilymph. Potassium concentration is kept constant by the cellular network in the stria vascularis. The basis of the electrolyte exchange for the maintenance of a constant ion concentration is the cellular potassium-sodium exchange pump found in the stria vascularis, the utricule, and the saccule. Furthermore, there is passive diffusion between the endo- and perilymphatic spaces with potassium-sodium ion exchange in the saccus endolymphaticus (Becker et al. 1989).

The scala media is composed of the organ of Corti, the stereocilia and the tectorial membrane. The organ of Corti contains about 3000 inner hair cells (IHCs), which transform mechanical displacement into electric current, about 12
000 OHCs and a large number of mainly supporting cells. All these cells work together. However, the precise interaction of these types of cells is not completely understood (Böhnke and Arnold, 1999).

The blood supply of the human inner ear derives from the anterior inferior cerebellar artery, which gives rise to the internal auditory artery, which in turn divides into the common cochlear and anterior vestibular arteries (Axelsson, 1968). The common cochlear artery supplies the cochlea, and divides into radiating modiolar and vestibulocochlear arteries that divide further into the posterior vestibular artery and the cochlear branch. The radiating modiolar artery supplies the apical three-quarters of the cochlea, including the modiolus, whereas the cochlear branch supplies the basal quarter of the cochlea and adjacent modiolus. The radiating modiolar artery enters the central canal of the modiolus and gives off a few primary and numerous secondary arterioles. The main modiolar artery and the cochlear ramus divide further into radiating arterioles of scala vestibuli and laminae spirale. The branches of the radiating arterioles and the spiral arterioles of the scala vestibuli contribute to the capillary network of the stria vascularis (Bachor et al., 2001).

Vestibular system

The sensory apparatus of the peripheral vestibular system consists of the vestibular nerve, the semicircular canals and the otolith organs located within the labyrinth (Schuknecht, 1993). The vestibular nerve comprises neurones whose cell bodies form the vestibular or Scarpa ganglion within the internal auditory canal and whose peripheral processes innervate the hair cells of the otoliths and the semicircular canals. The vestibular hair cells consist of supporting cells and hair cells type I and II (Merchand, 1999) whose cilia are embedded in a gelatinous mass consisting of sulfomucopolysaccharides. On their surface lie the otoliths (or statoconia) which consist of rhomboid calcium carbonate crystals.

Vestibular stimulation is projected to a small area in the ventral post-central somatosensory region near the visual area of the cerebral cortex. This region is said to represent the primary vestibular cortical area.
Physiology of the cochlea and the vestibular system

Cochlea
The cochlea is the organ of sound processing and transduction. It provides a signal analysis in a spectro-temporal activity pattern. The coiled, fluid filled cochlea is divided lengthwise by the cochlear partition in two mechanical chambers. This partition contains the organ of Corti attached to the basilar membrane. The width and compliance of the basilar membrane increase with the distance from the oval window at the cochlear entrance. Vibration of the oval window creates pressure variations in the fluid provoking transverse vibrations of the cochlear partition called travelling waves. The travelling wave causes fluid displacement between the tectorial membrane and the basilar membrane at its point of maximal amplitude so that the cilia of the outer and inner hair cells are displaced at this point, responding then to the resulting vibrations of the basilar membrane and the signal is transduced from graded mechanoelectrical to an all-or-none electrical form that is conducted into the central auditory system (Ohlms et al., 1991). The OHCs' function is to amplify and transform the electrical activity to mechanical work and to assist hearing at low sound pressure intensities (Brownell 1990, Flock & Flock 2000).

For single frequency stimulation, a travelling wave propagates from the oval window toward the apex of the cochlea with displacement of the transverse basilar membrane, which builds up to a maximum at a fixed place and shows a rapid decline thereafter. The basilar membrane stiffness determines the location of the maximum shifts so that at higher signal frequency the maximum shift moves towards the oval window. In this way, the envelope of the transverse basilar membrane velocity represents the input signal, which converts frequency to information about position (Baumgarte, 1999).

The organ of Corti transduces the cochlear partition velocity into action potentials in the auditory nerve by means of IHCs, the acoustic sensory cells. Further to place of activity, the amount of activity in terms of the frequency of action potentials and the temporal envelope and fine structures of the impulse
pattern makes up the code signal information to each corresponding fibre of the auditory nerve (Baumgarte, 1999).

The OHCs support the adaptation of the input signal’s dynamic range to the limitations of the IHCs. It is agreed nowadays that small basilar membrane vibrations are mechanically amplified by OHCs (Kemp 1979, Brownell 1990, Flock and Flock 2000). Electrophysiologically, the stria vascularis plays a fundamental role in the homeostasis and functional integrity of the cochlea. Thus, it is responsible for the generation of the endocochlear potential as well as for the high concentration of $K^+$ in the endolymphatic space. This involves an active ion transport process which is mainly achieved by the operation of a $Na^+\cdotK^+$ pump in the cells of the marginal cell layer (Bibas et. al, 2000). In electrical terms this represents a battery.

Vestibular system

Human anatomic balance depends on four separate and interdependent systems. First, vestibular system senses angular and linear accelerating movement. Second, proprioceptive cues from joint position and muscle tone provide information concerning the relationship of the head to the part of the body. Third, vision gives perceptions of the position, speed, and orientation. Finally, all the senses are integrated within the central nervous system (Becker et al., 1989).

The basic elements of the postural control system are composed of myotatic segmental reflexes that are controlled by the projection of vestibulospinal and other myotatic or voluntary reflexes (Guidetti, 1992).

Pathophysiology of Ménière’s disease

The anatomical and functional changes of inner ear observed in MD are not totally resolved. One of the most commonly observed changes in MD is the presence of EH which leads to the assumption that the abnormality occurs either due to overproduction or hindered elimination of endolymph, possibly caused by the damage of the ES. ES is essential for maintaining normal fluid balance by
absorbing the endolymphatic fluid. Failure of ES can be secondary to fibrosis, infection, or an autoimmune or allergic process. Studies on temporal bones obtained at autopsy from patients with MD have reported variable degrees of atrophy of the stria vascularis in relation to extensive EH suggesting that stria vascularis has a role in the pathogenesis of MD (Kimura et al., 1976). It is also suggested that the symptoms of MD may result from the over-stimulation of the sensory cells by extreme distension of the inner ear structures caused by dysfunction of ES (Claes and Van De Heyning, 1997).

It has also been reported that MD could be caused by immunological reactions (Hughes et al., 1983). The underlying pathophysiological process is eventually a vasculitis, which disturbs the vascular function of the inner ear. This is supported by findings of inflammation in the ES in a patient with MD (Danckwardt-Lilliestrom et al., 1997). ES may have immune related functions that are not yet fully understood as ES has been reported to be able to phagocytose cell debris (Lundquist et al., 1984). Immune reactions in the inner ear may be triggered by viral infections (Bergström et al., 1992). Other infectious agents such as Borrelia have also been demonstrated to induce hearing loss and/or Ménière’s like disease (Peltomaa et al., 2000). In addition, trauma (Gussen, 1971) and allergy (Derebery, 1997) have been associated with MD. Based on different aetiologies for EH, it may be true that inner ear responses to varying type of injuries by producing EH, that may lead secondary to MD.

Symptoms and definition of Ménière’s disease

Classical symptoms of MD are hearing loss, tinnitus, sensation of pressure or fullness of the ear and vertigo. According to the degree of severity of these symptoms, the AAO-HNS (1995) has elaborated a diagnostic scale represented into clinical, audiological and vestibular evaluations (table 1, 2 and 3).

Table 1. Diagnosis scale for Ménière’s disease of the AAO-HNS (1995)

<table>
<thead>
<tr>
<th>Certain Ménière’s disease</th>
<th>Definite Ménière’s disease, plus histopathological confirmation.</th>
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<tr>
<td>Definite Ménière’s disease</td>
<td>Definite Ménière’s disease</td>
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</table>

21
Two or more definite spontaneous episodes of vertigo for 20 minutes or longer
Audiometrically documented hearing loss (frequencies 0.5, 1, 2 and 3 kHz) on at least one occasion.
Tinnitus or aural fullness in the treated ear.
Other causes excluded

Probable Ménière’s disease
One definite episode of vertigo.
Other criteria as for definite Ménière’s disease

Possible Ménière’s disease
Episodic vertigo of the Ménière type without documented hearing loss, or
Sensorineural hearing loss, fluctuating or fixed, with dysequilibrium, but without definite episodes
Other causes excluded

Table 2. The AAO-HNS (1995) guidelines for staging of definite and certain Ménière’s disease.

Audiological evaluation
Pure tone average (HT) of 0.5, 1, 2, 3 kHz

Staging of disease
Stage 1: HT ≤25 dB
Stage 2: HT 26-40 dB
Stage 3: HT 41-70 dB
Stage 4: HT >70 dB

Poorest hearing 6 months before and between 18 and 24 months after treatment:
Significant if HT ≥10 dB and /or word recognition (WR) change ≥10 dB.

Table 3. The AAO-HNS (1995) guidelines for certain and definite Ménière’s disease based on comparison of the frequency of vertigo (definite episodes per month for the previous 6 months) before and after treatment.

Vestibular evaluation
Frequency of vertigo (FV) (definite episodes per month for the previous 6 months)

Y: FV before therapy
X: FV 2 years after therapy

Results of therapy:

<table>
<thead>
<tr>
<th>(X/Y) x 100=</th>
<th>Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>A</td>
</tr>
<tr>
<td>1-40</td>
<td>B</td>
</tr>
<tr>
<td>41-80</td>
<td>C</td>
</tr>
<tr>
<td>81-120</td>
<td>D</td>
</tr>
<tr>
<td>&gt;120</td>
<td>E</td>
</tr>
<tr>
<td>Secondary treatment initiated due to disability from vertigo</td>
<td>F</td>
</tr>
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</table>
Other symptoms have been reported to be encountered in patients with MD, such as headache and sensation of paraesthesia of the face and occiput (Kentala, 1997). Eklund (1999) has reported a high frequency of headache (70%) in patients with MD. Headache was either persistent or temporary. It was also felt mainly in the forehead, the occiput region and around the neck.

**Diagnosis of Ménière’s disease and other inner ear disorders**

**Posturography**

The vestibulo-spinal function was first tested by Romberg in 1846 and Romberg’s test is still widely used. Terekhov et al. (1974) developed a system to measure the forces needed to maintain the upright stance on a force platform. During the last two decades, the force platform has been modified and used for quantification of body sway (Nashner et al., 1982, Aalto et al., 1988).

It has been reported that stimulation of the ear by sound at high intensity provokes unsteadiness and nystagmus. This sound energy is transmitted to the vestibular organ as sound waves reach and stimulate the OHCs of the crista ampullaris. To probe the effect of low frequency sound stimulation (LFS) on vestibular organ, posturography has been used (Young et al., 1977, Ishizaki et al. 1991, Pyykkö et al. 1992). This method is based on the principle of the Tullio phenomenon, which is a sensation of balance disturbance provoked by sound stimulation, and the Hennebert sign, which is a sense of dizziness with nystagmus following pressure changes in the external ear canal.

**Tympanoscopy**

During the last few decades endoscopic techniques have been adopted for diagnosis of various ear disorders. The middle ear can be visualised endoscopically by introducing a rigid endoscope with a small diameter through
the tympanic membrane (Poe et al. 1992b). The round and oval windows and mucosal membranes can be inspected to exclude perilymphatic leaks (Poe and Bottrill, 1994). Tympanoscopy is superior to the elevation of tympanomeatal flap, which may result in oozing of exudate in the middle ear and may lead to a false diagnosis of PLF (Poe et al., 1992b).

Transtympanic endoscopy has the advantage of being simple to carry out as an outpatient procedure. A myringotomy is carried out under local anaesthesia and the inspection of the middle ear anatomy is a very short procedure that can be accomplished in a few minutes. Compared to tympanotomy which takes about an hour, tympanoscopy is a fast yet effective method with reduced rate of morbidity. However, tympanoscopy may be associated with the generation of a permanent perforation of the ear drum.

Electrocochleography (ECoG)

Davis et al. (1958) demonstrated cochlear microphonics (CM) recording with the use of transtympanic ECoG and concluded that CM is essentially produced by OHCs that also generate summation potential (SP). The SP is thought to represent an asymmetry in the movement of the basilar membrane that results from pressure difference between the scala tympani and scala vestibuli during sound stimulation (Durrant and Dallos, 1974).

It is agreed that AP refers primarily to the activity of the auditory nerve and represents the sum response of several thousand individual nerve fibres that have fired synchronously (Levine et al., 1998). The response to click stimuli generally refers to the activation of distal part of the VIII nerve and suggests that the neural activity from the entire length of the basilar membrane is represented. The SP/AP ratio gives information of endolymphatic hydrops (Durrant and Dallos, 1974, Asai and Mori, 1989, Arslan et al., 2000). ECoG has been considered to be particularly useful in the assessment of EH (Portman and Aran 1971, Ruth et al. 1988) which is not restricted to MD.

ECoG is generally obtained from either transtympanic-or extratympanic electrodes. Each has advantages and disadvantages. The transtympanic electrode which is usually a shaped as thin steel- or a silver needle. This
electrode penetrates cautiously the eardrum before is placed with the tip on the promontory of the medial part of the middle ear. This technique has been solicited as it is in close proximity to the source of the electric activity, hence the signal-to-noise is greater, and thus larger amplitude waveforms can be recorded using fewer signal average. The major disadvantage to transtympanic ECoG is that the invasive nature of the procedure requires that active electrode has to be placed by an otologist (Hickey et al., 1990). Extratympanic electrode, which could be a silver ball, wire- or needle electrode placed on the skin of the ear canal close to the tympanic membrane. The primary advantage of extratympanic ECoG is that is non-invasive and does not require necessarily an otologist to place the active electrode (Coats & Dickey, 1970). The most commonly used acoustic stimuli for ECoG are clicks and tone burst stimulus. A click stimuli is produced by driving the sound transducer with a short square wave (50-100 µs): Depending upon the frequency characteristic of the transducer, it will give a short oscillatory acoustic signal (close to the impulse response) which, if measured in frequency domain, will have a broad frequency spectrum capable of stimulating most of the basilar membrane in the inner ear. Tone burst is a sinusoid signal which, during its rise time it increases in amplitude until it reaches its full level - the plateau - and then decreases to zero during decay time. The advantage of tone burst stimuli is of appropriate spectral content, and at levels typically below 60 dB. Tone burst elicits responses originating selectively from that part of the cochlear partition tuned to the frequency of the stimulus (Eggermont et al., 1976).

Laser Doppler flowmetry (LDF)
Vascular pathology of the inner ear is considered to be behind a number of clinical disorders of the inner ear (Johnsson and Hawkins, 1972). Schuknecht (1974) demonstrated in temporal bone studies loss an atrophy of the cochlear vessels which support the vascular basis of some forms of presbyacusis. Duvall et al. (1974) have indicated a potential role of vascular changes in noise-induced hearing loss. Hydropic cochlea may induce atrophia of stria vascularis and cause a reduction in a blood flow in the lateral wall. Accordingly, decreased blood flow in the hydropic ears may be detected with laser Doppler flowmeter
placed on the lateral wall of the cochlea (Yazawa et al., 1998). Yamamoto et al. (1991) measured the blood flow in a unilateral hydropic ear of a guinea pig and showed that the decrease in CoBF was more prominent in the operated (hydropic) than in the intact ear.

Cochlear vascular bed investigations has been of a great interest to delineate the vascular disease entity of inner ear disorders, hence CoBF measurements have been adopted by otologists and hearing scientists. In the beginning, different techniques have been used to quantify changes in CoBF like intravital fluorescence microscopy (Nuttal, 1987), microsphere techniques (Ohlsen et al., 1994). These techniques are invasive and can be used only in animal studies. Scheibe et al. (1990) reported that CoBF could be measured in humans by placing the tip of a laser-Doppler probe on the promontory through a perforation existing in the tympanic membrane or during middle-ear surgery. Laser Doppler flowmetry (LDF) is considered to be a non-invasive method and provides direct and continuous information about CoBF changes in cochlear vessels over time. LDF technique permits study of cochlear blood circulation through intact wall of the cochlea. A scattered light penetrates various tissues and by the Doppler phenomenon it evaluates the flux, i.e. the number of the flowing red blood cells through a defined point per unit of time (velocity). The depth of penetration varies and it depends on the size of the laser beam. Some believe that LDF indicates the blood flow of only limited area of 1-2 mm of the inner ear and mucosa due to the reflection and penetration of laser beam (Salerud et al., 1983).

Since the laser Doppler output should be considered as a scale of relative change, the response of laser Doppler output to various agents should be examined in order to investigate the responsiveness of cochlear blood vessels in different inner ear diseases. In clinical investigations, Laser Doppler measurements were performed to study the variation of CoBF during EH (Miller et al, 1995), and in patients with sudden deafness (Nakashima, 1992). Other experimental studies have demonstrated an increase or decrease in inner ear blood flow in animals with noise-induced hearing loss (Prazma et al. 1983, Thorne and Nuttal 1987). Laser Doppler measurements were also consistent with some histologic studies which showed that alterations in cochlear blood
vessels were generally associated with decreased circulation, such as in endothelial cellular oedema, constriction of capillaries and unusual spacing of red blood cells (Hawkins 1971, Axelsson et al. 1981).

Association of infections and immune abnormalities in MD

1. Detection of microbes in the inner ear, post mortem and surgical samples.
   - Virus isolation (culture): Schuknecht (1978) reported that inner ear lesions induced by viruses in childhood are considered to be one of the causes of delayed EH. In this sense many studies using various techniques have been performed in attempt to confirm this assumption, like histological examination of the temporal bones of patients with delayed EH (Schuknecht et al., 1990), ultrastructural analysis of ES biopsies (Arenberg et al., 1991). In addition, other studies have been done on culture of HSV and CMV from Scarpa ganglia obtained from patients with MD (Palva et al., 1978). Direct isolation and culture of viruses from the inner ear is difficult, and not routinely used, except in certain circumstances like surgery (Welling et al., 1994), or during post-mortem examination (Kumagami, 1996).
   - Viral antigen detection, PCR, immunohistochemistry, in situ hybridisation: The most important test of any antigen preparation is the titration assay as it determines not only the quantity of material but weather it is functionally as well. One of the titration assay used is the test of ELISA (Markoulatos et al., 1997). Polymerase chain reaction (PCR) technique has also been used for detecting the presence of neurotropic viruses in a portion of the ES removed at the time of surgery (Welling et al., 1994). Immunohistochemical examination has been performed for identification of viral antigens in the ES (Sata et al., 1988) and DNA examination by post-mortem in situ hybridisation of ES (Kumagami, 1996).

2. Antibodies anti-microbes in sera of MD patients: Several serologic studies by Williams et al. (1987) have been used to confirm the viral aetiology of MD like titration of IgG and IgM antiviral assays. Dornhoffer et al. (1993) showed that IgG deposits could be detected in the endolymphatic sac (ES) of 40% of patients undergoing shunt surgery.
3. Serum autoantibodies: It has been reported that in addition to its role in resorption of the endolymphatic fluid, the ES has an immunological role (Altermatt, 1990). Autoantibodies to the ES in MD have also already been demonstrated by Alleman et al. (1997). Arnold et al. (1984) have demonstrated the presence of immunoglobulins in the human ES.

The mechanism by which the inner ear is involved during infection is considered to be either systemic, local or both. Local immune response occurring in the vicinity of ES can alter the function of the sac and may result at later stages in derangement of homeostasis of the inner-ear fluid (Linthicum and El-Rahman 1980, Arenberg et al. 1991). This mechanism may result in prolonged immune response with consequent clinical picture of recurrent fluctuating hearing loss, attacks of vertigo and tinnitus as first described by McCabe (1979). Systemic or local inner ear immunological disorders may play a significant role in various types of inner ear disorders, such as progressive bilateral sensorineural hearing loss (SNHL) (Moscicki et al., 1994), MD (Gottschlich et al., 1995) or delayed EH (Harris and Aframian, 1994).

Treatment of Ménière’s disease

Treatment modalities of MD are based on current understanding of the function of the inner ear and the pathophysiology of the disease. Treatment alternatives comprise either medical, surgical, or both. Today, the mainstay of MD treatment remains non-surgical. The cornerstone of treatment is a strict low salt diet (maximum <1500 mg/day) (Devaiah and Ator, 2000), eliminating caffeine and alcohol from the diet is also recommended (Knox and Mc Pherson, 1997).

Medical treatment consists of various drugs for the short-term (acute phase) or long-term management. Among the most commonly used medicines during the acute attack are neuroleptic and anticholinergic drugs besides central muscular relaxants, which have a vestibular sedative activity. However, these drugs are not without adverse effects, and some of them may provoke extrapyramidal symptoms or glaucoma (Claes and Van de Heyning, 1997). Betahistine has been widely used either during the acute attack or for long-term treatment but its mechanism of action is still unclear. It has been demonstrated
that betahistine produces cerebral and peripheral vasodilatation inducing an improvement in the microcirculation of the internal auditory, cochlear and vestibular systems (Laurikainen et al., 1998). Diuretics have also been recommended in order to have an effect on the changes of extracellular osmolarity, but their use has diminished during past years (Van Deelen and Huizing, 1986). Since MD has also been linked to an autoimmune aetiology, steroids have been tried as oral or intra-tympanical preparations (Hirvonen et al., 2000). To eliminate signals from a dysfunctional vestibular organ, chemical labyrinthectomy may be performed by intratympanical application of aminoglycosides (Eklund, 1999).

Table 4. Various treatment modalities used for treatment of Ménière’s disease.

<table>
<thead>
<tr>
<th>Medical treatment</th>
<th>Surgical treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>During acute attack</strong></td>
<td><strong>Long term treatment</strong></td>
</tr>
<tr>
<td>Strict low salt diet</td>
<td>Strict low salt diet</td>
</tr>
<tr>
<td>Eliminating of alcohol and Caffeine</td>
<td>Eliminating of alcohol and Caffeine</td>
</tr>
<tr>
<td>Betahistine</td>
<td>Betahistine</td>
</tr>
<tr>
<td>Neuroleptic drugs</td>
<td>Diuretics</td>
</tr>
<tr>
<td>Anticholinergic drugs</td>
<td>Steroids</td>
</tr>
<tr>
<td>Antihistamines</td>
<td>Intra-tympanical injection of aminoglycosides</td>
</tr>
<tr>
<td>Antiemetics</td>
<td></td>
</tr>
</tbody>
</table>

Surgical treatment of MD is indicated when a patient’s symptoms become resistant to maximal medical therapy. Traditional operations for MD can be classified into hearing-conserving and hearing-sacrificing procedures. Hearing-conserving procedures are recommended when serviceable hearing is present and they include vestibular neurectomy or decompression with or without drainage of the ES. Surgical labyrinthectomy sacrifices any residual hearing but offers excellent control of vertigo.
Other treatment modalities have also been reported such as the application of repeated excess pressure to the middle ear (maximum 2 kP via a ventilating tube (Andrews and Strelioff, 1995). This treatment may reduce the frequency and intensity of vertigo, dizziness, aural pressure and tinnitus in patients with active MD (Odkvist et al., 2000).
Purpose of the study

The aim of this study was to investigate electrophysiological, neurophysiological, and vascular changes occurring in various inner ear diseases in order to differentiate between these diseases and in particular to help diagnose MD and possible infectious aetiology of the MD.

The specific aims of the study were:

To investigate findings in posturography of LFS stimulation for diagnosis of the possible presence of altered sound sensitivity in patients suffering from vertigo and SNHL.

To evaluate the use of tympanoscopy in establishing the diagnosis of PLF and in defining it’s incidence in patients with SNHL and/or vertigo and tinnitus.

To determine electrophysiological changes in inner ear diseases by transtympanic ECoG measurements.

To identify possible inner ear vascular changes in patients with MD and in patients with other inner ear diseases by measuring CoBF.

To examine whether MD is a consequence of pathological immune reaction triggered by viral infection and whether such findings correlate with EH or hearing loss.
Materials and Methods

Patients

The inclusion criteria of the patients to the study were vertigo, PSNHL and tinnitus either combined or isolated. (Table 5).

Study I.

Sixty four patients (age range 15 to 80 years, mean age 48 years) with different inner ear disorders were assessed by LFS stimulation posturography.

Study II

Two hundred sixty five patients (range 22 to 80 years, mean age 48 years) were included in the study. Patients were suffering from vertigo, tinnitus, sudden, or PSNHL. Most of the patients had a typical MD

Hearing was considered to fluctuate if the difference between the best and worst PTA (the mean of 0.5, 1, 2 and 4k Hz) was at least 10 dB. Patients with vertigo (n=53) had episodes of isolated vertigo without hearing loss or tinnitus. The third group comprised patients (n=32) with PSNHL with or without tinnitus of unknown aetiology. The fourth group consisted of 14 patients with SHL. The patients with otosclerosis (n= 16) were selected on the basis of their continuous tinnitus with or without sensation of vertigo. Moreover, their hearing loss had become sensorineural after the correction of the air-bone gap by stapedoplasty. Unfortunately, despite the success of surgery, although, the evolution of otosclerosis process has continued to involve other parts of the inner ear and causing then a failure on cochlea function.
Table 5. Work-up procedures and number of subjects investigated as well as their disease or presenting condition.

<table>
<thead>
<tr>
<th>Study</th>
<th>Tests</th>
<th>Disease</th>
<th>n</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Low frequency stimulation in posturography</td>
<td>Ménière’s disease</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Possible perilymphatic fistula</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stapes prosthesis</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Others (sudden deafness, tinnitus and vertigo)</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>Middle ear endoscopy</td>
<td>Ménière’s disease</td>
<td>149</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vertigo</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PSNHL</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sudden hearing loss</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Otosclerosis</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Perilymphatic fistula</td>
<td>1</td>
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<tr>
<td></td>
<td></td>
<td>Total</td>
<td>265</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>Transtympanic electrocochleography</td>
<td>Ménière’s disease</td>
<td>131</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tinnitus with or without Vertigo</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Progressive sensorineural hearing loss of unknown aetiology</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sudden hearing loss</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Otosclerosis</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Others</td>
<td>18</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Total</td>
<td>249</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>Cochlear blood flow measurement with laser Doppler flowmeter</td>
<td>Ménière’s disease</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Progressive sensorineural hearing loss of unknown aetiology</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sudden hearing loss</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>115</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>Anti-viral antibody and immune assays</td>
<td>Ménière’s disease</td>
<td>159</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vestibular schwannoma</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>185</td>
<td></td>
</tr>
</tbody>
</table>
Study III
Two hundred forty nine patients (range 15 to 88 years, mean age 53 years) with sensorineural hearing loss of different aetiologies were studied. Most of the patients had MD (n= 131). The tinnitus patients with or without vertigo (n= 45), PSNHL of unknown aetiology (n= 31), 11 patients with sudden hearing loss, 13 patients had otosclerosis. Eighteen patients with sensorineural hearing loss were allocated to the same group (11 patients with confirmed and 4 with suspected borreliosis, two had an autoimmune disease and one with Cogan syndrome).

Study IV
The study comprised 69 patients with MD, (range from 15 to 80 years, mean age 48 years), 38 patients with PSNHL (range 28 to 71 years, mean age 50 years), and 8 patients with SHL (range 31 to 71, mean age 51 years).
Only the affected side was studied for CoBF. In some cases where the disease was bilateral, only the most recently affected side was studied. Four patients with MD had bilateral disease, in 31 patients the disease was in the left ear, and in 32 patients the disease was in the right ear. Thirteen patients with PSNHL had left-sided, 24 had right-sided, and one patient had bilateral disease. Three patients with SHL had left-sided, four had right-sided, and one patient had bilateral disease

Study V
One hundred and fifty-nine patients (age range 22 to 81 years, mean age 48 years) had MD (102 right-sided, 53 left-sided and 4 bilateral). Twenty six patients had vestibular shwannoma (age range 30 to 72 years, mean 54 years).
Diagnostic tests

Audiometric evaluation

Hearing threshold with air conduction was examined at the frequencies from 0.25 to 8 kHz and bone-conducted threshold was examined at the frequencies from 0.25 to 6 kHz with Madsen OB-822 diagnostic audiometers employing TDH-39 headphones. The audiometers were calibrated according to ISO standards. Pure-tone average (PTA) was used as a variable for hearing loss (0.5-4 kHz). All patients were tested in an electrically and acoustically shielded room.

Low Frequency Sound stimulation on posturography (Study I)

Posturography is platform applied in otoneurology as an examination technique for evaluation of postural stability. The postural stability is under the influence of vestibulospinal reflex on balance in the standing position (Norré, 1990). Standing is achieved by a process of active body movements around the centre point of gravity, called postural sway (Roberts, 1978). This sway is recorded and measured by the posturographic methods. The sway can be also evaluated under other situations like during different perturbations (visual, proprioceptive and movement of the platform) which provoke unsteadiness, especially when there is a failure in the vestibular system.

In our study, the postural stability was evaluated by a force platform, which is constructed with the strain gauge principle, and the system evaluates changes in the centre point of force on the platform surface. The signal is fed into a computer (Hewlett packard 900, series 3000), which analyses and evaluates the displacements of body position during quiet stance (non-stimulus time) and during perturbation of the posture with LFS.

LFS was generated with a piston type air sampling pump (Mine Safety Appliances CO (MSA), Pennsylvania, USA), which has an adjustable stroke frequency ranging from 6 to 80 Hz. Each ear was stimulated separately with sound pressure levels of 130 dB at the frequency of 25 Hz, and 132 dB at the
frequencies of 50 and 63 Hz. Posturography was performed with a force platform on which the patient was standing without shoes, heels together, feet in a 30 degree angle and with arms crossed over the chest. Vertical forces over the platform were measured in reference to antero-posterior displacements and lateral directions (Enbom et al., 1988). The patient was instructed not to bend her/his knees to avoid the generally protective stance. After the initial 20-second sway stabilisation, the quiescent stance was recorded during a 30-second period. Measurements were made under visual control (eyes open) and under non-visual control (eyes closed). A microcomputer (Hewlett Packard 75 C) controlled the stimulator unit by adjusting the intensity of the stimulus in three stimulation sequences, which were arranged in ascending order, giving the greatest stimulus intensity at the end of the test. This was achieved through a programmable power supply (Hewlett-Packard 50501A and 6200B). Each stimulus period lasted for 30 seconds, followed by a 20-second non-stimulation period. Each stimulus frequency was delivered once to each patient and the vibration was delivered with rotating shakers, giving at all frequencies a 0.4 mm peak-to-peak amplitude (tested when the vibrator was hanging freely).

The primary variable used in the analysis of the force platform signal is the position of the centre point of force (CPF) on the platform surface. The movement of this point (body sway) is analysed to characterise postural stability. Movement of the CPF contains different kinds of information at different frequency ranges. The static (low frequency) part described the projection of the centre point of body mass on the platform surface and the dynamic part (frequencies above 0.5 Hz) described the forces used to correct body position.

We characterise the movement of the CPF by calculating its (average) velocity (sway velocity) during given test periods. In the following equation, velocity is derived by dividing the length by the duration as follows;

\[
\text{Sway velocity} = \frac{1}{n} \sum_{i=1}^{n-1} \sqrt{(x_i - x_{i+1})^2 + (y_i - y_{i+1})^2}
\]

where \( T \) is the duration, \( x_i \) and \( y_i \) are the \( i \)th coordinate pairs, and \( n \) is the last sample point of the time period. By using this parameter as an indicator of
postural instability, direct comparison of postural stability between tests at different times is possible.

The posturography test lasted 180 seconds. The antero-posterior displacements and the lateral body sway signals were processed in a microcomputer (Hewlett Packard 9000 series 300). Data were sampled at 33.3 Hz frequency, and to eliminate artefacts, filtered with a non-linear 3 point median filter and smoothened with linearly moving average filters of 3 and 5 points to remove random noise (Enbom et al., 1988). The program calculates sway velocity (SV).

The Romberg quotient was calculated. In our laboratory the average Romberg quotient in the normal population between 30 and 60 years of age, is 1.43 (Hytönen et al., 1989). The proprioceptive index, the ratio between the SV during vibration perturbation at 80 Hz and the baseline SV (eyes closed) was also calculated. The proprioceptive index of normal persons is 1.32 (Hytönen et al., 1989). Patients were compared with normal controls from an earlier study (Hytönen et al., 1989).

Sway velocity at baseline was used as reference value to indicate undisturbed postural control activity.

Tympanoscopy (Study II)

During tympanoscopy, the patient was placed under the operation microscope in Trendelenburg position. Part of the tympanic membrane (an area about 1 mm wide and extending from the annulus to the umbo) was anaesthetised with 90% phenol solution. After a radial paracenthesis incision of 5 mm in length, a bloodless view into the posterior tympanic cavity was achieved. The endoscope with a 5-degree angle was inserted into the middle ear. The anatomy of the tympanic cavity was examined. The 25-degree rigid tympanoscope (Wolf endoscope, Germany) was then introduced, and the area of the oval window was examined while special attention was paid to the fissula ante fenestram.

In order to enhance the chance for possible perilymph leakage, a Valsalva manoeuvre and light compression of the ipsilateral jugular vein were performed to increase the intracranial pressure when viewing the round window with the 5 degree optics and the oval window are with the 25 degree
optics. Valsalva manoeuvre was conducted by instructing the patient to take a
deep breath, hold her/his nose and mouth closed and attempt to blow air out.
During this manoeuvre the contraction of thoraco-abdominal muscles results in
increased venous pressure that causes the veins of the head and neck to
protrude.

Transtympanic ECoG (Study III)
The recording electrode, a silver ball, was introduced under microscopic
visualisation through the same myringotomy as the endoscopes and placed
against the round window niche. The response in ECoG was tested first, and
when good response was achieved, the right position of the electrode was
maintained manually and the final measurement was carried out.

The reference and ground electrodes were placed on the ipsilateral
mastoid tip and forehead. We used response analyser (Medelec MS60, England)
equipment to record ECoG. An external loudspeaker (Sony SP6, Sony
electronics, Japan) was mounted on the stand of the microscope and kept at a
distance of 70 cm from the tested ear. Alternating clicks were used as the sound
stimuli. Square wave pulses of 100µs duration were presented at a rate of 5.5 per
second, and the time window was 10ms. The cut-off frequencies for the low and
high pass filters were of 3 and 3000Hz. The sound intensity level was 100 dB
hearing level.

ECoG responses were obtained by alternating the click polarity to
suppress the CM and enhance the identification of SP and AP. The amplitude
(µV) of the SP was measured from the baseline (onset of response) to the notch
on the descending limb of the response waveform. The AP amplitude was
measured from the baseline to the maximum negative deflection of the N1 peak
(fig 2). The recordings were interpreted according to the guidelines given by
Densert et al. (1994). CM was measured as positive and negative amplitudes
before initiating N1 waveform measurement. The SP/AP ratio was calculated by
dividing the amplitude of SP by that of AP. To determine the AP latency
differences to rarefaction and condensation clicks. When rarefaction is applied,
the first part of the stimulus will give an outward movement of the tympanic membrane and thereby an upward movement of the basilar membrane in the cochlea. When the condensation stimulus is used, the first part of the stimulus will force the eardrum to move inward and the basilar membrane will move downward. The responses were measured separately with the same stimulus pattern as in alternating mode. This procedure permitted us to evaluate the CM amplitude from the peak-to-peak oscillation that precedes the AP.

**Figure 2 showing the trace of ECoG to alternating stimulation.**

![Figure 2 showing the trace of ECoG to alternating stimulation.]

Laser Doppler flowmetry (Study IV)
The LDF flowmeter system was composed of a 2-W laser Doppler with a diameter of 1.0 mm (Periflow 2-B flowmeter, Perimed, Stockholm, Sweden). The Doppler-shifted light and the reference light which are conducted to two photodetectors via two optical fibres of 200 µm diameter. The ensemble is covered by a steel probe.

The probe was positioned through the myringotomy perforation 2-3 mm anterior and superior to the oval window. This position permitted us to measure the cochlear circulation from the lateral wall. The signals were fed into a microcomputer through an analogue digital converter board.
The data were expressed as microvolts without an absolute reference point. Flow-up of the LDF was measured in an attempt to evaluate the rise of systolic CoBF, reflecting the ability of the cochlear blood system to respond to heart patterns, which may be deranged during certain inner ear disorders. This state may occur in cochlear vascular resistance. A flattened response reflects poor circulation due to high peripheral resistance (Ren et al., 1994).

Antiviral antibody and immune assays (Study V)

The IgG against neurotrophic viruses was assessed by protein-denaturing ELISA employing endpoint titration of IgG (Hedman K et al. 1993). Serum samples from patients with MD and from patients with vestibular schwannoma were studied to detect antibodies against various viruses. IgG antibodies against herpes simplex 1 (HSV1), herpes simplex 2 (HSV2), Varicella zoster (Vzos), adenovirus (AV), mycoplasma (Myp), rotavirus, enterovirus (Evir), Coxsackie virus B5 (CVB5), Echo 22 (Ec22), cytomegalovirus (CMV), Epstein-Barr virus (EBV), influenza A (InflA), influenza B (InflB), pikorna virus 1 (Pin1), respiratory syncytial virus (RSV) and chlamydia (Cla) were measured. In addition, IgG and IgM antibodies against Borrelia were evaluated. IgG antibodies were analysed with indirect enzyme-linked immnosorbent assays according to the manufacturer’s instructions (Gull Laboratories, Salt Lake City, Utah, USA). Serum samples were stored at \(-20\, ^\circ C\) before analysis.

The basic immune assay in our study was the titration of status measurements of immunoglobulins A, G and M, complement components C3 and C4, rheumatoid factors (RF) and antinuclear antibody (ANA).

The immunoglobulins IgA, IgG and IgM were evaluated by immunoturbidimetric technique. Complement function was measured by gel-hemolysis assay according to Meri (1987) and antinuclear antibody (ANA) was measured by immunofluorescence (Tan, 1982). Serum proteins were analysed by total protein assay and electrophoresis on cellulose-acetate membrane resulting in per cent values for albumin, $\alpha_1$, $\alpha_2$, $\beta_1$, $\beta_2$ and $\gamma$ globulin. The complements were analysed by the immunoturbometry method and by CH100. The antibodies to nuclear antigens (ANA) were analysed by indirect immuno-
fluorescence techniques, CRP were analysed by the immunoturbidimetric, rheumatoid factor was tested by latex agglutination test, anti-streptolysin- by sheep red cell hemolysis assay (Cabau and Badin, 1965). Direct Coombs’test was performed on patients’ red cells by agglutination with polyvalent anti-serum-immunoglobulin and anti-serum complement (C3b/C3d) (Petz, 1986). Since Cold agglutinin cause red blood cells to clump only at temperature lower than 37 °C, the test was performed by cooling the tube to where the blood of the patient was collected in ice water for 30-60 seconds, and evaluate for clumped red blood cells with a microscope.

Statistical evaluation

Significance of differences between different groups of diseases were tested with one way analysis of variance (ANOVA, Bonferoni test). (Study I)

Pearson’s bivariate correlation coefficients were calculated between the PTA and SP/AP ratios. The statistical significance of the correlation was tested with Bonferroni test. (Study III).

The data were analysed with a non-parametric test for independent samples (Mann-Whitney U test) to assess the differences in the arithmetic mean flow amplitudes of the LDF between the groups, and Pearson’s bivariate correlation rate with a two tailed test of significance was also used. (Study IV).

Correlation between hearing loss, EH, symptoms of MD, and circulating IC and antiviral titres were examined by using Spearman’s matrix. Elevated titters of antibodies against neuroviruses in patients with MD were compared with those of controls by means of analysis of variance (ANOVA). Findings were considered to be statistically significant when p was less than 0.05. The calculations were performed with SPSS 8.0 statistics program (Study V).
Results and comments

Evaluation by LFS stimulation on posturography in patients with different inner ear diseases (Study I)

Patients with possible PLF were particularly prevalent to respond pathologically to LFS stimulation either at all stimulation frequencies. The pathological rate of response to LFS stimulation was equal between patients with MD and patients with stapes surgery. (table 6)

Table 6: Effect of low frequency sound stimulation on ear.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>25 Hz</th>
<th>50 Hz</th>
<th>63 Hz</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ménière’s disease</td>
<td>25</td>
<td>25</td>
<td>30</td>
<td>36</td>
</tr>
<tr>
<td>Possible perilymphatic fistula</td>
<td>75</td>
<td>100</td>
<td>100</td>
<td>5</td>
</tr>
<tr>
<td>Stapes prosthesis</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 6 shows the outcome of the LFS test at different stimulation frequencies. At lower frequencies 25% and at higher frequencies 30% of patients with MD responded with increased vestibulospinal responses. Patients with suspected PLF had more vigorous responses to sound stimulation. Twenty five percent of patients with stapes prosthesis responded with same manner at the different frequencies of sound stimulation.

Comments

The results of our study indicate that besides patients with possible PLF, also other patients responded pathologically to LFS stimulation. Shepard et al. (1992) used posturography in a pressure loading test, and they evaluated the outcome
of the test in surgery conducted by two teams. The sensitivity of LFS in posturography for the verification of PLF reported by the teams varied from 53% to 100% and the specificity from 56% to 89%. Black et al. (1987) used posturography and reported a high hit rate of positive fistula tests (97%) in the vestibulospinal responses of 64 patients. Compared with the results of our study, the high positive fistula test results reported by Black et al. (1987) may be due to the selection of patients with a high suspicion of having a PLF, whereas our study was designed to identify possible “spontaneous PLF” in patients suffering from different inner ear diseases without any clear evidence of circumstances in which PLF could occur.

Tympanoscopic inspection to evaluate middle ear pathologies in patients with vestibulo-cochleopathy (Study II).

Tympanic cavity could be visualised in all but 7 out of 265 patients. A secondary membrane in the round window that occluded direct viewing of the window membrane was observed in 143 patients (53.9%). In 92 patients (34.7%) a part of the round window membrane could be identified. Only in 23 patients (8.6%) the round window membrane could be visualised. In 7 patients the tympanic cavity could not be visualised because of adhesive tympanic membrane, abnormal anatomy or prominent exostosis of the external ear canal.

We found only one definite PLF (diving accident) in the round window that was covered with a fibrinous membrane. One patient had an abnormal mucosal shining suggesting possible PLF in the round window niche or oval window area. In three patients there was abnormal shining around the round window, probably due to mucosal oedema resulting from irritation by the tympanoscope. However, a leak from a fistula might have also caused this abnormal shining.
Comments

The symptoms and signs of PLF can vary, there are no characteristic diagnostic signs or symptoms, nor are there any reliable diagnostic tests available (Podoshin et al., 1994). Kohut et al. (1995) examined serial sections of cadaver post-mortem temporal bone with microscopy and defined objective criteria based on such symptoms of PLF as sudden or fluctuating hearing loss or slowly progressive sensorineural hearing loss and vestibular symptoms equivalent to positional vertigo or constant dysequilibrium. All the patients of this study presented at least one sign that could be encountered in PLF.

In this study only one traumatic PLF was detected. This result is in line with a general consensus that trauma to the ear, either explosive or implosive, is the most important cause of PLF. It has been debated whether a disease called “idiopathic or spontaneous PLF “ actually exists (Meyerhof, 1993). For example, Shea (1992) could not verify idiopathic PLF in his large series of patients.

These patients in whom the leakage in the area of the round window was visualised were first suspected to be PLF, but with closer examination no oozing occurred during the provocation, and PLF was excluded. The slow accumulation of fluid in the area close to the round window was thought to be due to mechanical irritation of the middle ear mucosa by the tympanoscope.

Electrophysiological changes in Ménière’s disease and other inner ear disorders assessed by ECoG (Study III).

Among the patients with MD the prevalence of EH was high, 46.4%. There was no correlation between hearing level and EH as assessed by ECoG (Table 7).
Table 7. SP-AP ratios in relation to PTA among the MD patients. (r = Pearson correlation coefficient), n.s.= non-significant, SD= standard deviation).

<table>
<thead>
<tr>
<th>PTA (dB HL)</th>
<th>SP/AP ratio</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>SD</td>
<td>&lt;0.30</td>
<td>0.30-0.34</td>
<td>&gt;0.35</td>
<td>Total</td>
<td>Pearson Correlation Coefficient</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>R</td>
</tr>
<tr>
<td>0-19</td>
<td>0.41</td>
<td>0.05</td>
<td>13</td>
<td>9.9</td>
<td>9</td>
<td>6.9</td>
<td>17</td>
</tr>
<tr>
<td>20-39</td>
<td>0.38</td>
<td>0.04</td>
<td>21</td>
<td>16.0</td>
<td>4</td>
<td>3.1</td>
<td>24</td>
</tr>
<tr>
<td>40-59</td>
<td>0.38</td>
<td>0.04</td>
<td>10</td>
<td>7.6</td>
<td>5</td>
<td>3.8</td>
<td>12</td>
</tr>
<tr>
<td>≥60</td>
<td>0.44</td>
<td>0.05</td>
<td>3</td>
<td>2.3</td>
<td>5</td>
<td>3.8</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>0.39</td>
<td>0.02</td>
<td>47</td>
<td>35.8</td>
<td>23</td>
<td>17.6</td>
<td>61</td>
</tr>
</tbody>
</table>

Table 8 shows that the MD patients had, on average, higher SP/AP ratios (over 0.40) than the other groups. The abnormal SP/AP ratio in MD patients tended to be the result of an increased SP (2.6µV) and a decreased AP (7.2µV). The MD patients also had the highest CM (2.7µV), whereas in patients with sudden deafness the CM was the lowest (1.5µV).

Comments

In this study patients with MD had increased SP values. Interestingly, Kitahara et al. (1981) reported that MD patients with a long-term duration of symptoms had a tendency to show abnormal click-induced SP. Mechanical displacement of the basilar membrane toward the scala tympani causes a non-linear movement as a result of the presumed abnormal volume/pressure condition, and this may result in a large endolymphatic potential and enhanced negative SP. Schmidt et al. (1974) have also found larger SP values in patients with MD than in patients with SNHL.
Table. 8. The CM, SP, AP and the SP/AP ratios for different inner ear disorders.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>CM µV</th>
<th>SP µV</th>
<th>AP µV</th>
<th>SP/AP Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Menière's disease</td>
<td>2.7</td>
<td>0.4</td>
<td>2.6</td>
<td>0.2</td>
</tr>
<tr>
<td>Tinnitus + vertigo</td>
<td>2.5</td>
<td>0.4</td>
<td>2.1</td>
<td>0.4</td>
</tr>
<tr>
<td>PSNHL</td>
<td>2.4</td>
<td>0.4</td>
<td>1.6</td>
<td>0.3</td>
</tr>
<tr>
<td>Sudden deafness</td>
<td>1.5</td>
<td>0.2</td>
<td>0.6</td>
<td>0.2</td>
</tr>
<tr>
<td>Otosclerosis</td>
<td>2.6</td>
<td>0.6</td>
<td>2.3</td>
<td>0.7</td>
</tr>
<tr>
<td>Others: (Borreliosis, suspected borreliosis, immune disease &amp; Cogan syndrome)</td>
<td>1.8</td>
<td>0.2</td>
<td>1.1</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>2.5</td>
<td>0.3</td>
<td>2.2</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Conlon and Gibson (2000) found that MD patients have a mean SP/AP ratio of approximately 40% whereas “Control” ears have a mean SP/AP ratio of approximately 25%. A high SP/AP ratio is considered to be a result of an enhanced SP during the early stage of MD or of a decreased AP in an advanced stage of the disease. Mori et al. (1987) found that the AP decreased as hearing impairment increased, whereas the SP did not vary systematically as a function of hearing threshold. The SP may increase in MD as the volume and electrophysiological conditions change in the intralabyrinthine fluid during the disease process (Sass et al., 1998). In early stage of the disease a deterioration of hearing may be due to uncoupling of OHCs’ electrical membrane and in later stage probably the degeneration of the OHCs will also have an impact on AP. In our study the mean CM amplitude in MD patients was higher than in other patient groups. This finding may reflect abnormally increased excitability of the hair cells, as it is apparent in hyperacusis.

In our study hearing loss did not correlate with EH, and the result is in concordance with the findings reported by Horner et al. (1989). They found that
hearing thresholds were not directly related to the endolymphatic volume in the scala tympani. Moreover, Levine et al. (1998) found no association between the hearing threshold level, ECoG’s findings and the different stages of MD. Konrádsson et al. (1999) found no significant correlation between the hearing levels, speech discrimination, and the SP amplitude.

Relatively high SP/AP ratio was also found in patients with sudden deafness and otosclerosis. EH has been detected in certain patients with otosclerosis and sudden deafness (Shea et al., 1994). Temporal bone dissection studies have also provided further evidence of the association between otosclerosis and MD (Liston et al. 1984, Johnsson et al. 1995).

Cochlear blood flow assessed by laser doppler flowmetry (Study IV).

The mean LDF amplitude was 0.70 mV (SD 0.25) for the patients with MD, 0.66 mV (SD 0.21) for the SNHL patients, and 0.69 mV (SD 0.23) for the sudden deafness patients. There were no statistically significant differences between the patient groups. A statistically significant correlation was observed between the flow amplitudes and hearing losses among patients with SNHL (r = -0.40, p<0.05).

Comments

The present study showed no differences in CoBF between MD and sudden deafness. In MD, the EH does not seem to have an impact on the cochlear blood circulation. This is in line with CoBF studies in animals. For example, Baldwin et al. (1992) found no differences between the hydropic and normal ears of guinea pigs after the infusion of mannitol, urea or glycerol. Moreover, no changes were found in the regional and total CoBF of hydropic ears of guinea pigs (Larsen et al., 1988).

An impairment of CoBF has been proposed as one of the most likely aetiologies in sudden deafness (Nakashima, 1992). In our study, the CoBF measurements did not show any statistical difference between the sudden
deafness and other groups of inner ear diseases. This finding indicates that the vascular pathology may not be the primary cause for sudden deafness.

There appeared to an association between cochlea blood flow and hearing loss among patients with PSNHL. Attanasio et al. (2001) reported a reduction of CoBF of Mongolian gerbils during the first 4 days of exposure to sound, which was then followed by a progressive correction of CoBF. This suggests that excessive exposure to noise may be associated with CoBF. This is intriguing as among patients with PSNHL exposure to noise may be an etiological factor.

The finding that there were no statistical differences between the CoBF measurements of the different inner ear disease groups indicates that there was no vascular aetiology and that most of the patients were in an advanced stage of disease, where the vascular component is no longer involved.

Evaluation of antiviral antibodies, autoantibodies, and other serum proteins in Ménière’s disease (Study V)

Patients with MD had significantly higher IgG titres against Varicella zoster, adeno, rota, Coxackie B5 and respiratory syncytial viruses when compared with patients with VS (table 9).

Table 9. Mean values and standard deviations of IgG titres of antibodies against different microbes in patients with MD and VS. Statistically significant difference between the groups are indicated with an asterix. The different viruses presented are herpes simplex 1 (HSV1), herpes simplex 2 (HSV2), Varicella zoster (Vzos), adenovirus (AV), mycoplasma (Myp), rotavirus, enterovirus (Evir), Coxsackie virus B5 (CVB5), Echo 22 (Ec22), cytomegalovirus (CMV), Epstein-Barr virus (EBV), influenza A (InflA), influenza B (InflB), pikornavir virus 1 (Pin1), respiratory syncytial virus (RSV) and chlamydia (Cla) were measured
<table>
<thead>
<tr>
<th>Virus</th>
<th>MEAN (SD)</th>
<th>MEAN (SD)</th>
<th>MEAN (SD)</th>
<th>MEAN (SD)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV1</td>
<td>50.80 (32.20)</td>
<td>45.75 (26.06)</td>
<td>0.77</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HSV2</td>
<td>38.59 (19.28)</td>
<td>38.42 (16.14)</td>
<td>0.97</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vzos</td>
<td>59.34 (22.40)</td>
<td>24.50 (17.08)</td>
<td>0.01*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AdV</td>
<td>61.55 (36.32)</td>
<td>40.15 (26.95)</td>
<td>0.02*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myp</td>
<td>38.27 (22.70)</td>
<td>31.26 (21.53)</td>
<td>0.40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rota</td>
<td>47.30 (34.50)</td>
<td>33.92 (23.09)</td>
<td>0.00*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Evir</td>
<td>71.91 (23.19)</td>
<td>29.80 (19.50)</td>
<td>0.53</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CVB5</td>
<td>59.45 (32.84)</td>
<td>40.73 (29.08)</td>
<td>0.01*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The antibodies against viruses herpes simplex 1 (HSV1), herpes simplex 2 (HSV2), Varicella zoster (Vzos), adenovirus (AV), mycoplasma (Myp), rotavirus, enterovirus (Evir), Coxsackie virus B5 (CVB5), Echo 22 (Ec22), cytomegalovirus (CMV), Epstein-Barr virus (EBV), influenza A (InflA), influenza B (InflB), pikorna virus 1 (Pin1), respiratory syncytial virus (RSV) and chlamydia (Cla) were measured and compared in patients with MD based on EH. Those MD patients with EH had lower IgG titres against varicella zoster (P<0.01) and adeno viruses (P<0.05) than those MD patients without EH (Figure 4).

One hundred and twenty-seven patients with MD had abnormal levels of serum proteins. Abnormal levels of serum α2-globulin was observed in 34.3% of the MD patients. Also, abnormal levels of β1-globulin, β2-globulin, γ-globulin, IgM, IgA, serum haemolytic complement (CH100,) and anti nuclear antibodies were observed among 54.5%, 26.5%, 17.3%, 14.1%, 13.1%, 36.4%, and 43.4% of MD patients.
Figure 4: IgG titres (mean ± SD) in the sera of patients with MD, with and without EH. IgG antibody titres against varicella zoster (Vzos), adenovirus (ADEN), rotavirus (ROTA), Coxackie B5 virus (COB5) and Respiratory syncytial virus (RSV) are given.

Levels of \( \beta_1 \)-globulin correlated also significantly with the SP/AP ratio (\( r = -0.31, \ p<0.05 \)) in patients with MD. When the SP/AP ratio was high (sign of EH), the \( \beta_1 \)-globulin level was normal and when the SP/AP ratio was normal or low the \( \beta_1 \)-globulin titre was high (fig. 6). (\( p<0.05 \)) (fig. 6).
Figure 5. Association of high serum β1-globulin concentration and hearing level (HL) at different frequencies (0.25 kHz, 0.5 kHz, 1kHz, 2 kHz, 4 kHz and at 8 kHz) in patients with Ménière’s disease. Mean ± and errors of mean values are shown.

Comments
The result of our study on viral antibody assays showed that in MD, IgG titres against Adenovirus, Varicella zoster virus, respiratory syncytial virus and Coxsackie virus B5 viruses were significantly higher than among patients with vestibular schwannoma. Virus infection has been advocated to be associated MD by many authors (Williams 1987, Cotter et al. 1994). The ES is suspected to be a site of inflammation in addition to being a site of resorption of endolymphatic fluid (Altermatt 1990, Arenberg et al., 1991). Kumagami (1996) examined virus antigens of HSV1, HSV2, mumps and CMV using immunohistochemical methods and DNA examination by in situ hybridisation in the ES of 14 fresh cases having no known pre-mortem diseases of the middle or inner ear. HSV antigen and DNA were observed in 9 of the 14 cases studied.
The author concluded that the viruses invade the ES but are impeded by an immune defence mechanism under normal conditions. In a study of 21 specimens of randomly obtained temporal bone samples, Arbusow et al. (2001) demonstrated that HSV-1 DNA was present in the labyrinths in 48% of cases, with 62% in vestibular ganglion cells and 57% in the geniculate ganglion.

Figure. 6. Relationship between β1-globulin (low and high concentrations) and the level of SP/AP ratio in patients with Ménière’s disease (N=159). Mean ± errors of mean values are shown.

The assumption that MD is associated with an inner ear viral infection has not been without controversy. Welling et al. (1994) used PCR to detect the presence of neurotropic viruses in a portion of the ES removed at the time of surgery from 22 patients with MD and from 11 patients with shwannomas. HSV DNA was detected in 2 of the 22 extracts from the ES obtained from the patients with MD. There was no evidence of a positive relationship for any of the other viral DNA when the PCR was performed on control tissue extracts or other
tissue extracts of patients with MD. The authors concluded that their results did not show a significant difference and did not statistically support the postulation that ongoing viral infection in the ES is a frequent factor in the development of MD.

We observed that antibodies against adeno and Varicella zoster viruses were associated with EH among patients with MD. Also association was found between acute phase beta-1-globulin response and the hearing loss. When the hearing loss was mild, beta-1-globulin levels were high and when the hearing loss was moderate to severe, the levels were low. Hearing loss in MD usually involves low frequencies. The RSV titre was found to correlate with hearing loss at low and middle frequencies (0.25–1 kHz). In our study, the assumption that RSV not only causes middle ear infection, but may also cause inner ear infection, is not new (Sataloff and Vassalo, 1968).

In our study, immune assay showed that patients with MD presented an elevation of circulating IC with varying levels. Elevated β1-globulin levels were found in more than half the cases (54.5%). Furthermore, β1-globulin was the only abnormality which correlated significantly with hearing loss at all frequencies and with the SP/AP ratio.

Our results on the presence of immune abnormalities in MD are in concordance with previous observations of Brooks (1986) and Derebery et al. (1991). Derebery et al. (1991) demonstrated significantly elevated levels of circulating IC among 30 patients with MD when compared with 20 control subjects. Brooks (1985) found IC in 55% of patients with MD and in only 3% of control subjects. Dornhofer et al. (1993) showed that IgG deposits could be detected in the ES of 40% of patients undergoing shunt surgery. In animal experiments, Tomiyama et al. (1994) demonstrated that severe inflammatory cellular infiltration occurred in the sac from 8h to day 3 after a secondary keyhole limpet hemocyanin (KLH) challenge to the sac. These authors demonstrated that increased vascular permeability occurred on day 2 after the secondary KLH challenge. Histology revealed significant staining of IgG and C3 on the sensory epithelium of the vestibule. An increase in hearing threshold was confirmed by ECoG. The hearing threshold was significantly increased in association with elevation of perilymphatic antibody levels in the early phase of
the secondary immune reaction in the ES, indicating that the elevation of hearing threshold was dependent on the intensity of a type III immune reaction.

Consequently, an elevation of perilymph antibody levels in the early phase suggests that there is a large quantity of harmful immune products, enough to disturb the normal function of the inner ear sensory organs, and that elevation of circulating ICs is linked to the immune response.
General discussion

Inner ear diseases are considered to be an indistinct disease entity regarding their various aetiologies, shared symptoms and the similar course of development. The establishment of early diagnosis of MD is still considered to be one of the most challenging problems facing the otolaryngologist today. Hence, the clinical progress in this field of inner ear and particularly in MD has been hindered by methodological flaws including the diversity of reporting methods. Among these methods are clinical approaches using a set of scales for different symptoms (AAOHNS, 1995), and audiological methods; performing ECoG in assessing the endolymph hydrops when SP/AP is found to be high (Dornhoefer 1998). From otoneurological standpoint as non-specific signs for MD, it has been reported that patients with MD may respond positively to LFS test on posturography (Pyykkö et al., 1995), as was confirmed in the present study. It was reported that some patients with MD have radiological changes in the inner ear imaging (Yazawa and Kitahara, 1994), and in laboratory screening (Bergström et al. 1992, Boulassel et al. (2001)): These studies has been performed in attempt to delineate the disease entity of MD. On the other hand, the evolution and the course of the disease are highly variable and this partly hampers the therapeutical management (Kaasinen et al., 1998).

By performing a detailed otoneurological and laboratory work-up, we attempted to elucidate from different methodological approaches the pathophysiology of different inner ear diseases and to establish the appropriate diagnosis. Regarding the methodological complexity of this work-up, the tests were partly invasive but well tolerated and it was possible to perform them in clinical setting and patients were discharged immediately after the procedure. However, some problems occurred either during the course of the procedure or later. There were no unexpected adverse effects in tympanoscopy, as discomfort and dizziness due to the thermal heating of the lateral semicircular canal by the endoscope are common (Bottrill et al. 1996). Infection as a complication may also occur during any of the investigations, since the ECoG’s silver ball
electrode, the laser Doppler probe and the endoscopes are all introduced through the same myringotomy hole and contamination of the external canal wall may introduce infection.

There may also be irritation of the chorda tympani or permanent perforation of the eardrum in association with the otoneurological work-up. Thus far, no ossicular chain luxation has been reported. In our series the rate of postoperative infection was 2.2%, and all the infections were treated successfully with antibiotics. The incidence of complications can be reduced if care is taken with the procedure, for example by keeping the endoscope, the ECoG electrode or the laser Doppler probe from touching the wall of the ear canal. Patients with a history of chronic middle ear infection or with a thin secondary tympanic membrane were more prone to infection in our study. Permanent perforations may occur in cases in which the infection persists longer. However, no case of permanent perforation was recorded in our series.

In the present study five patients had a temporary reduction in taste (1.8%). This result was probably related to the use of phenol in the anaesthesia of the eardrum in cases where the chorda was too much in contact with the eardrum. Alternative topical anaesthetics that can be used include tetracaine-based powder dissolved in isopropyl alcohol (Silverstein and Call, 1969), racemized lidocaine-prilocaine emulsion (EMLA cream), or iontophoresis with a 4% lidocaine solution with 1:1000 epinephrine (Poe et al., 1992 (a)). However, these anaesthetics do not provide as good bloodless entry to the tympanic cavity as phenol. Furthermore, by using phenol, only the paracenthesis streak is anaesthetised, not the entire eardrum, as with other anaesthetics. Therefore, we have preferred the use of phenol in this study.

Transtympanic investigations could not be performed in cases of prominent exostosis, retracted eardrums (otitis media adhesiva) or when visibility could not be established.

This work would be more conclusive if a control group of healthy subjects had been included. However, this otoneurological work-up was not without risks. Furthermore, as it was supposed to be invasive, it would not have been ethically acceptable to perform it on healthy subjects.
Conclusions

1. Findings of LFS stimulation in posturography showed that patients without PLF, such as patients having MD, responded pathologically to LFS.

2. In tympanoscopy, we were not able to detect more than one perilymphatic fistula in patients with SNHL and/or vertigo and tinnitus. This may be due to the low probability of eventual presence of spontaneous PLF in these patients.

3. By using transtympanic ECoG, we were able to determine the electrophysiological changes of the inner ear in especially MD, such as the elevation of the CM, enhanced SP, decrease in the amplitude AP; and the findings indicate the presence of EH (SP/AP) in MD as well as others inner ear diseases.

4. LDF showed that there was no difference in blood flow changes between patients with MD and patients with others inner ear disorders. In PSNHL the hearing loss correlated with reduction of CoBF. The finding indicates that there was no gross changes in the cochlear blood flow in patients with MD.

5. Elevation of IgG antibodies against Vzos, RSV and adenovirus in patients with MD might be an indication that MD is caused by viral infection of the inner ear, which subsequently triggers an IC reaction.

It can be hypothesised that a part of patients with MD may have as an etiological factor a local or systemic reaction triggered by viral infection, which involves either the cochlea or the vestibule as well. The disease is characterised by variable symptomatology with normal middle ear anatomy. EH is not a pathognomonic sign of MD and can be present in other inner ear diseases, such as sudden deafness and otosclerosis. The vascular component is not involved anymore during the late stage of the disease. On the basis of the results of this study, it is strongly recommended to perform investigations during the acute stage of the disease when the anatomical and electro-physiological manifestations are more apparent.
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