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Sudden presentation of immune-mediated inner ear disease: characterization and acceptance of a cochleovestibular dysfunction

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Abstract

Since the McCabe report, growing indirect evidence has accumulated to indicate the implication of immune mechanisms in the pathogenesis of immune-mediated inner-ear disease (IMIED). A clinical study of a group of patients affected by this condition was performed in order to characterize the immune group, based on a recently reported profile, and compared with the vascular, viral and idiopathic aetiologies of sudden deafness. Patients affected by immune-mediated inner-ear disease had the best and the earliest recovery rate of hearing ($p = 0.0028$ and $p = 0.017$, respectively). However, this group of patients also had the higher rate of recurrence ($p = 0.034$), supporting the typical clinical course of the autoimmune disorders. On the basis of the results the criteria used in the diagnosis of the sudden presentation of the immune-mediated inner ear disease could be accepted leading to the characterization of this condition. Likewise, the role of the supporting cells in the pathogenesis of the IMIED is discussed.

Key words: Hearing Loss, Sensorineural; Immune System; Prednisolone; Treatment Outcome

Introduction

Immune-mediated inner-ear disease (IMIED) is one of the few causes of profound hearing loss in which prompt medical treatment can attempt to prevent progression toward greater impairment. The concept that the immune system may damage the inner ear was introduced in 1979 by McCabe,[1] who based his findings on clinical data, the presence of abnormal immunological tests and the response to immunosuppressive therapy.

Since the McCabe report,[1] growing indirect evidence, due to the fact that the human inner ear is not amenable to diagnostic biopsy, has accumulated to indicate the implication of immune mechanisms in the pathogenesis of IMIED. However, there is still a lack of clear criteria for diagnosing the different forms of presentation of IMIED: rapidly progressing bilateral sensorineural hearing loss, sudden deafness and bilateral immune-mediated Ménière's disease.[2]

Since sudden and rapidly progressive losses of hearing represent a true emergency in otology, a clinical study of a group of patients affected by this condition was performed in order to characterize the immune group, based on a recently reported profile,[3] and this was compared with the vascular, viral and idiopathic aetiologies of sudden deafness. The validation of the criteria used in the diagnosis of the immune-mediated group could facilitate the acceptance of this form of presentation of IMIED.

Patients and methods

Sixty-nine patients (46 men and 23 women; mean age 39.3 years; age range 15–73 years) with sudden sensorineural hearing loss (SSNHL) according to the criteria previously reported[4] were included.

Inclusion criteria

The following criteria were used for inclusion in the present study:[5,6] (1) stable, unilateral sensorineural hearing loss that occurred in less than 72 hours; (2) an interval of less than 14 days since the onset of hearing loss; (3) absence of retrocochlear disease; (4) average hearing levels from 250 to 4000 Hz at less than 30 dB in the affected ear; (5) average hearing in the affected ear at the initial examination of more than 30 dB; and (6) follow-up of more than one year.

Informed consent was obtained from all patients before withdrawal of the blood samples. They all underwent a protocol-guided study involving a

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routine history and physical examination, pure tone audiometry, syphilis tests, immunological and MR imaging studies.

The immunological work-up study included: erythrocyte sedimentation rate, antinuclear autoantibodies, serum immunoglobulins IgG, IgA and IgM, complement factors C3 and C4, heat shock protein 70 (OTOblot™, OTOimmune Diagnostics, IMMCO Diagnostics, Buffalo, NY) and phenotype of peripheral blood lymphocytes (by three-colour flow cytometry) in which patients were compared with 14 healthy controls subjects (nine men and five women; mean age, 36 years).

Three-colour flow cytometry analysis

Peripheral blood mononuclear cells were obtained by use of Ficoll-Hypaque density gradient centrifugation (Lymphoprep, Nycomed, Oslo, Norway). Interphase cells were washed and resuspended in RPMI with 10 per cent fetal calf serum. The monoclonal antibodies used in this study were purchased from commercial manufacturers (Becton Dickinson & Co., Mountain View, Calif, and Caltag Laboratories, San Francisco, Calif). In three-colour flow cytometry analysis, saturation doses of fluorescein isothiocyanate, phycoerythrin, and perdinin chlorophyll or tricolour-conjugated antibodies were added to 0.1 ml of cell suspensions (0.5×10^6 cells), followed by 15 minutes of incubation at 4°C. After washing twice in phosphate-buffered saline solution, cells were resuspended in 0.2 ml of formaldehyde. Flow cytometry was performed by use of a fluorescence-activated cell sorter (FACScan, Becton Dickinson & Co) and the accompanying software for data acquisition (Lysis II, Becton Dickinson & Co) and processing (Paint-a-Gate, Becton Dickinson & Co.). The lymphocytes were electronically gated according to the forward and side light scatter pattern.

Therapy

These tests were carried out prior to the start of 'standard' therapy following the protocol outlined by Arellano *et al.*[7] Patients were hospitalized for three to six days and treated with 6-methylprednisolone (at a starting dose of 1 mg/kg body weight per day; this therapy was tapered during the next 21 days. In patients with profound hearing loss (>70 dB) a 500–1000 mg bolus of 6-methylprednisolone was administered. Steroid perfusion of 6-methylprednisolone to the round window was performed after failure of conventional therapy), 100 per cent O_2 inhalation, low molecular weight heparin (0.4 ml s.c./day) and nimodipine (30 ml i.v./8 h).

Audiological assessment

Pure tone audiometry was carried out either every day or every other day. Evaluation of hearing thresholds at 125–8000 Hz was performed. In relationship to the severity of the initial hearing loss, we included three groups: mild hearing loss (<50 dB pure-tone average), moderated hearing loss (51–89 dB) and profound hearing loss (>90 dB). According to the type of the audiogram three groups were observed: low frequency hearing loss, mid-frequency hearing loss and high frequency hearing loss.

The recovery rate of hearing (per cent) was calculated by dividing the hearing gain (change in decibels in the average of hearing levels at frequencies of 250 to 4000 Hz) by the difference between the initial hearing levels of the affected and unaffected sides. Patients were divided in two groups: those with a recovery rate >80 per cent and those with a recovery rate <80 per cent. The recovery rate was determined at the seventh day of treatment.

Immune-mediated group

On the basis of the immunological study we have recently proposed the profile of immune-mediated sudden sensorineural hearing loss:[3]

(1) Major criteria: bilateral affectation, presence of systemic autoimmune disease, high levels of antinuclear autoantibodies (ANA), reduced number of naive T cells (CD4RA) and recovery rate of hearing >80 per cent.
(2) Minor criteria: unilateral affectation, young/middle-aged patient, often female, serum reactivity against heat shock protein 70 and positive response to steroid treatment (recovery rate <80 per cent).

Three positive major criteria or two major criteria and more two minor criteria would support the suspect of immune-mediated disorder, taking into account that profound hearing losses (>90 dB) present a low percentage of recoveries, regardless of the aetiology.

Viral group

Patients with a recent history of an upper respiratory infection (<one month) with, or without, a significant rise (more than a fourfold increase) in the viral antibody titre and/or the presence of specific IgM when acute and convalescent serum samples were compared were considered as the viral group. Only this group underwent a microbiology study.[8]

Serological studies were carried out with commercially available kits according to the manufacturer's instructions. To detect IgM antibodies, the samples were treated with a sorbent (RF-Sorbotech) to avoid interference with rheumatoid factor and IgGs. The complement fixation assay (CFA) was utilized to study antibodies to *Mycoplasma*, respiratory syncytial virus (RSV), influenza A and B viruses, cytomegalovirus (CMV), varicella-zoster virus (VZV), herpes simplex virus (HSV), mumps virus and adenovirus in both serum samples. Only titres over 1/64 were assessed.

Enzyme immunoassay (EIA: Wampole, Captia Biotech, Immunowell) was employed to examine the presence of IgG antibodies against HSV, VZV, Epstein-Barr virus (EBV), CMV and parainfluenza virus in all the samples, and that of IgM antibodies

against *Mycoplasma pneumoniae*, mumps, parainfluenza and measles in the samples obtained during the acute phase.

The detection of IgM antibodies to CMV was performed by means of enzyme-linked fluorescent assay (ELFA; Vidas, bioMerieux). Indirect immunofluorescence was employed to study the presence of IgM antibodies against the viral capsid antigens of EBV (EBV VCA RIFA, MRL Diagnostics).

Vascular group

Patients with vascular risk factors (diabetes mellitus, hypertension, thromboembolic disease, atherosclerotic disease, hypercoagulation states, migraine, high levels of total cholesterol, triglycerides, high density lipoprotein-cholesterol -HDL, low density lipoprotein-cholesterol -LDL) and intracranial haemorrhage, infarction and vertebrobasilar insufficiency showed by MRI and/or computerized tomographic (CT) scanning studies were included in this group.

Idiopathic group

Once the multiple presumed aetiology has been eliminated, patients were considered as idiopathic.

Statistical analysis

For statistical analysis, the Student's t test and the Mann-Whitney nonparametric test were used in two-groups comparisons, whereas the ANOVA (analysis of variance) and the Student Newman-Keuls for multiple group comparisons were applied for three or more groups. The results were expressed as a mean ± standard deviation (SD). The comparison between categorical variables was evaluated by the χ^2 test or the continuity correction chi-square.

To identify the factors that might be of independent significance influencing a recovery rate <80 per cent, a logistic regression model was fitted. Variables included in model were: vertigo (presence *vs* absence), IMIED (presence *vs* absence), high-frequency losses *vs* low-frequency hearing loss and initial level of the hearing loss >90 dB. For each variable was performed the odds ratio (OR) and the confidence intervals of 95 per cent (CI 95 per cent).

All P values were two-sided and values of 0.05 or less were considered to indicate statistical significance. The SPSS v.10.0 software package was used for all statistical analysis.

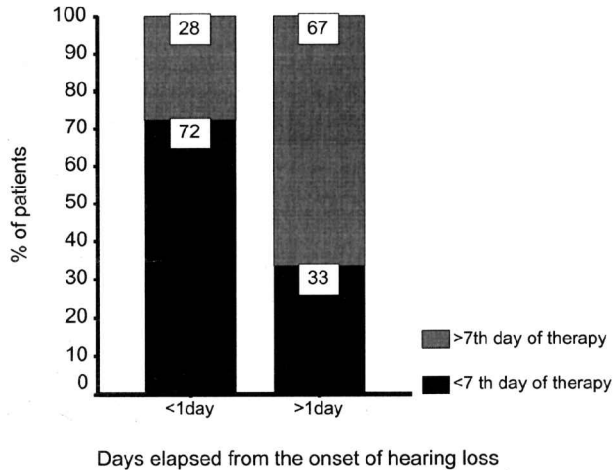

FIG. 1

Hearing recovery time-related. The majority of patients who were assisted within one day (72 per cent) had a recovery of hearing >80 per cent before the seventh day of treatment.

Results

The right and left ears were affected in 33 and 36 patients respectively. Twenty-five patients complained of rotatory vertigo (36.8 per cent). Time elapsed between the onset of the hearing loss and the start of treatment was 4.1 days. Patients were divided in two groups according to the time elapsed from the onset of the hearing loss: 39 patients (54.2 per cent) who were assisted in less than 24 hours and 30 patients (45.8 per cent) who were assisted in two to seven days.

After the application of the panel, 26 patients (37.6 per cent) were considered as the immune group, 16 patients (23.1 per cent) presented as the viral group, 13 patients (18.8 per cent) were considered as the vascular group and 14 patients (20.2 per cent) were included in the idiopathic group. The mean recovery rate of hearing was 61.2 dB (33.8). Patients from the immune group showed a significantly better response to standard therapy than the other groups ($p = 0.028$). A high percentage of IMIED patients showed a hearing recovery before the seventh day of treatment ($p = 0.017$) (Figure 1). However, this group of patients also had a higher rate of recurrence ($p = 0.034$), supporting the typical clinical course of the autoimmune disorders (Table I).

The existence of vertigo ($p = 0.025$), profound losses (>90 dB) ($p = 0.04$) and high frequency hearing losses ($p = 0.019$) were correlated with a poor and late recovery rate of hearing (RR), according with the classic topics with regard to the bad

TABLE I

COMPARATIVE ANALYSIS OF THE DIFFERENT GROUPS OF PATIENTS AFFECTED BY SUDDEN SENSORINEURAL HEARING LOSS. IMIED-IMMUNE-MEDIATED INNER EAR DISEASE. SD – STANDARD DEVIATION

Diagnosis	IMIED	Viral	Vascular	Idiopathic	*p* value
Number of patients (%)	26 (37.6)	16 (23.1)	13 (18.8)	14 (20.2)	
Age (SD)	39.5 (14.7)	31.7 (11.4)	50.6 (16.8)*	36.6 (12.2)	$p<0.05$
Vertigo	8 (32)	4 (25)	5 (38.5)	8 (57.1)	
Recovery rate of hearing >80% (%)	16 (61.5)*	6 (37.5)	3 (23.1)	4 (28.6)	$p = 0.028$
Complete recovery at seventh day (%)	15 (65.2)*	8 (50)	5 (50)	2 (16.7)	$p = 0.017$
Recurrence (%)	7 (26.9)*	0	1 (7.7)	2 (14.3)	$p = 0.034$

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TABLE II
PROGNOSTIC FACTORS INVOLVED IN THE HEARING RECOVERY OF THE PATIENTS INCLUDED IN THE PRESENT STUDY *STATISTICALLY SIGNIFICANT DIFFERENCE

	Recovery rate		Recovery rate	
Prognostic factors	<80%	>80%	7th day	>7th day
Initial hearing loss (dB)				
<50	10 (50%)	10 (50%)	16 (84.2%)* $p = 0.001$	3 (15.8%)
>90	17 (77.3%) $p = 0.027$	(22.7%)	5 (29.4%)	12 (70.6%)* $p = 0.001$
High frequency hearing loss	19 (79.2%) $p<0.05$	5 (20.8)		
Vertigo	21 (77.8%)	6 (22.2%)	6 (28.6%)	15 (71.4%)*
Time elapsed from hearing loss (<1 day)			22 (64.7%) $p = 0.008$	12 (35.3%)
Immune-mediated inner ear disease group (IMIED)	10 (38.5%)	16 (61.5%)* $p = 0.028$	15 (65.2%)* $p = 0.017$	8 (34.8%)

prognosis factors of sudden deafness (Table II). It was observed that 77.8 per cent of patients were affected by vertigo, 79.2 per cent of patients with high frequency hearing losses ($p<0.05$) and 77.3 per cent of patients with an initial hearing loss >90 dB ($p = 0.027$) showed a RR<80 per cent.

- **This is an article looking at the laboratory parameters and the clinical features of patients presenting with presumed immune mediated inner ear disease**
- **The findings in these patients is contrasted with a group presenting with sudden hearing loss due to vascular, viral and idiopathic aetiologies**
- **The authors present evidence to suggest that the immune mediated losses respond better to systemic steroids with better recovery than the other patient groups albeit that they also tend to relapse more readily**

Regression lines were obtained for the relationship between factors that affect prognosis and recovery rate. Recovery rates were higher in patients with mild hearing loss (<50 dB), in those who were assisted within one day from the onset of hearing loss and patients with IMIED. A statistically significant number (84 per cent) of patients with mild hearing loss and 64.7 per cent of patients assisted before one day from the onset of hearing loss had a RR>80 per cent at seventh day of treatment ($p = 0.001$ and $p = 0.008$, respectively).

In the regression model the presence of vertigo, absence of IMIED and a high-frequency hearing loss influenced negatively the probability of achieving a recovery rate >80 per cent. The results of the logistic regression analysis were: the presence of vertigo (OR = 3.5 95 per cent CI: 1.05 to 11.8) $p = 0.041$, the absence of IMIED (OR = 4.5, 95 per cent CI: 1.4 to 14.3) $p = 0.011$ and high-tone hearing losses (OR = 5.4 95 per cent CI: 1.2 to 23.3) $p = 0.024$.

Discussion

Since the clinical manifestations (hearing loss, tinnitus and vertigo) are shared with entities of different aetiologies (i.e. vascular, toxic, metabolic, genetic, traumatic or idiopathic), the diagnosis of IMIED represents a challenge for the clinician, particularly in those isolated forms in which there are no systemic manifestations supporting the diagnosis.[9]

Numerous attempts in order to characterize the different forms of presentation of IMIED have been performed.[6,10–12] The search for a specific diagnostic marker have led to the identification of diverse cochlear proteins as immune targets by means of experimental animal models of immune-mediated labyrinthitis.[13–18] However, contradictory results have not clarified the fundamental mechanisms involved in the pathophysiology of IMIED.

These limitations have encouraged the search for diagnostic profiles to characterize the IMIED. Criteria for the diagnosis of immune-mediated sudden sensorineural hearing loss have been recently reported[3] supported by a comprehensive analysis of the immunopathology of the inner ear. These criteria have allowed to define and characterize the patients affected by IMIED and a comparison with patients affected by other aetiologies of sudden

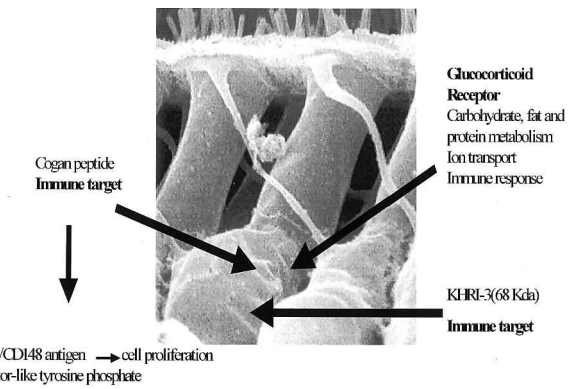

FIG. 2
Scanning microscopy imaging showing the immune targets location in the supporting cell.

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deafness. The comparative study of the groups showed that patients with IMIED have a different behaviour with a better and earlier recovery rate of hearing than the rest of the patients. Likewise, IMIED patients have higher rate of recurrences, similarly to the clinical evolution of the systemic autoimmune diseases. The validity of the study is also supported by the confirmation of the topics involved in the prognosis of sudden hearing loss: the existence of vertigo, profound hearing loss and high frequency losses are statistically related to a poor recovery of hearing.

However, the early recovery of hearing showed by IMIED patients suggests reversible damage to the cellular components of the organ of Corti. The affectation of the stria vascularis and spiral ligament could represent the first step in the immune damage to the inner ear. Metabolic changes induced by the immune system on the lateral wall structures (endothelial cells and fibrocyte II dysfunction leading to impaired diffusion of K+ through marginal cells to the endolymph fluid) could affect the supporting cells of the organ of Corti preceding the late effect of the hair cells.[19] This new theory has been raised by the presence of Cogan's peptide and the KHRI-3 cochlear protein (68 kDa) in the supporting cells,[17,20] making them vulnerable to the immune attack. Likewise, the existence of a great number of glucocorticoid receptors in the stria vascularis and the supporting cells suggest their role as immune targets of the inner ear (Figure 2).[21] This finding could justify the early diagnosis and treatment of the IMIED in order to avoid the generation of a fibro-osseous matrix after the initial immune cells infiltration.[22,23]

Further research on the precise role of the inner ear immune targets could determine the pathophysiological mechanisms of IMIED and the involvement of these targets in other inner ear disorders.

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