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# P0 Antigen Detection in Sudden Hearing Loss and Ménière's Disease: A New Diagnostic Marker?

DESIDERIO PASSALI[1], VALERIO DAMIANI[1], RENZO MORA[2], FRANCESCO MARIA PASSALI[2], GIULIO CESARE PASSALI[1] and LUISA BELLUSSI[1]

*From the ENT Departments, [1]University of Siena Medical School, Siena, Italy and [2]University of Genoa Medical School, Genoa, Italy*

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**Objective**—To evaluate the presence of IgG autoantibodies against the P0 antigen in patients affected by sudden hearing loss and Ménière's disease (MD).

**Material and Methods**—All patients underwent a tonal audiometric evaluation, tympanometry, evaluation of the stapedial reflex threshold with decay time, determination of auditory brainstem responses and a complete vestibular assessment involving evaluation of spontaneous and positional nystagmus (Frenzel glasses), a head thrust test and a caloric test (Fitzgerald–Hallpike technique). Blood samples were drawn from all patients for the immunologic assessment of IgG antibodies against the P0 antigen (30-kDa protein) of guinea pig inner ear extracts using a Western blot assay.

**Results**—Ten patients affected by sudden hearing loss showed specific IgG antibodies against the P0 protein. Specifically, the P0 positive band was detectable in 5/45 patients with unilateral auditory impairment and in 5/5 of those with bilateral forms of auditory impairment. Among MD patients, the P0 positive band was detectable only in those with bilateral audiovestibular impairment ($n = 10$). Interestingly, in none of the 35 patients affected by monolateral MD were specific IgG antibodies against the P0 protein detectable.

**Conclusion**—The positive reactions to P0 in all bilateral MD and bilateral sudden hearing loss patients found in this study strongly indicate that these pathologies are the result of an ongoing autoimmune process directed against specific antigens of the inner ear. *Key words: autoimmunity, inner ear diseases, Ménière's disease, P0 antigen, sudden hearing loss.*

## INTRODUCTION

Sudden sensorineural hearing loss is a pathology characterized by the sudden onset of auditory impairment, without any previous otological pathology. It is usually monolateral (80% of cases), has an incidence of $\approx 1/10\,000$ new cases per year and mainly develops in males aged 30–60 years. It is possible to define the aetiology in $\approx 20\%$ of patients and many factors, such as viral infections, vascular diseases, trauma, ototoxic drugs and autoimmune disorders, can be involved in the pathogenesis of this disorder.

Ménière's disease (MD), first described by Prospero Ménière in 1861, is typically characterized by fluctuating hearing loss, episodic vertigo, tinnitus and a sensation of pressure in one ear. It is generally monolateral but in $\approx 20\%$ of patients bilateral forms can develop (1). The histopathological hallmarks of the disease, at the temporal bone level, are endolymphatic hydrops, atrophy and erosion of the endolymphatic sac.

Despite a rigorous pathological definition, the aetiology of MD, which is usually defined as idiopathic, should be ascribed to a variety of causes, such as endolymph production disorders, alterations in ionic homeostasis, vascular disorders, trauma, viral infections and immunologic disorders. In this context, many pieces of clinical and experimental data, accumulated in recent decades, have emphasized the key role played by immune-mediated injuries in the pathogenesis of sudden hearing loss and MD.

Bilateral sudden sensorineural hearing loss was first related to an autoimmune process against inner ear antigens by Lehnardt in 1958 (2). This hypothesis was confirmed by McCabe (3) in 1979, who highlighted the efficacy of corticosteroids and immunosuppressive therapy in the treatment of patients affected by sudden hearing loss.

In a retrospective analysis in 1983, Hughes et al. (4) showed that patients with a positive lymphocyte transformation test were affected by MD or presented symptoms of endolymphatic hydrops. Specifically, they found that patients with Cogan's syndrome (an immunomediated illness characterized by audio-vestibular dysfunction and ocular inflammation sensitive to steroid and immunosuppressant treatment) presented audio-vestibular alterations similar to those with MD. Considering that temporal bone histology in Cogan's syndrome often shows endolymphatic hydrops, the authors suggested that the two disorders may have a common autoimmune aetiopathogenesis.

Yoo et al. (5) found higher levels of antibodies directed against type II collagen in MD patients compared to normal-hearing controls. In 2001, Yoo et al. (6) evaluated, using an ELISA, the presence of antibodies against several inner ear antigens (ryw H collagen, C-Raf, tubulin, type IX and XI collagens, CB 11 peptide) in the serum of 108 patients with MD. They found that 91% of their patients presented antibodies against one of more of these antigens,

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confirming the importance of immune disorders in the pathogenesis of this disease.

Concerning sudden hearing loss, Veldmann et al. (7), using swine as an antigenic source, reported the presence of several specific antigens with different molecular weights (27, 45, 59 and 80 kDa) in the sera of their patients. In addition, Harris and Sharp (8), using Western blot techniques, showed the presence of a 68-kDa protein in 32% of patients affected by bilateral sudden hearing loss.

It has been suggested in recent studies (9, 10) that heat shock protein-70 (HSP-70) could represent a reliable marker of autoimmune alterations of inner ear disorders; it has a specificity of 90% but a sensitivity of only 42%. In this context, major problems concerning the use of autoantibody detection as a marker of inner ear autoimmune pathologies are the heterogeneity of the tested samples (often including both unilateral and bilateral forms) and the multiple aetiopathogenetic mechanisms of sudden hearing loss and MD, which can act as confounding factors in the analysis of the immunological test results.

From a general point of view, the best test to use is the one with high sensitivity and specificity and an acceptable positive predictive value. The positive predictive value of a positive test derives from comparison of true positives (patients affected by the disease who react positively to the test) and the total number of positive results, namely true and false (patients who show positive results but do not have the pathology) positives. However, if a laboratory test is performed on a population with a high prevalence of the analysed disease (or with a high risk of positivity for the tested parameter) a positive result is more likely to be a true positive. In contrast, if the same test is applied to a population with a low prevalence (or low risk) of the pathology, a positive result is more likely to be a false positive. This means that, in the specific field of the identification of autoimmune inner ear disease, if we perform a laboratory test on an unselected population of patients affected by sudden hearing loss and MD, without pre-selecting those at higher risk of immunological deregulation at the inner ear level, our results will be dramatically biased. In this study, based on our previous positive experience (11) with the definition of autoimmune markers in patients affected by sudden loss of vestibular function, we tested the presence of serum IgGs against P0 protein in patients affected by bilateral sudden hearing loss or MD (i.e. those at higher risk of having an immunological disease) and in those affected by unilateral forms of the same diseases (probably due to vascular problems, trauma, ototoxic drugs, etc.), with the aim of evaluating its applicability as a diagnostic test.

## MATERIAL AND METHODS

Between January 2000 and December 2002 we enrolled 95 patients suffering from inner ear disorders. Specifically, 50 of them were affected by sudden sensorineural hearing loss—both monolateral (45 patients; 24 males, 21 females; age range 18–64 years; mean age 44 years) and bilateral (5 patients; 3 males, 2 females; age range 18–60 years; mean age 39 years)—and the remaining 45 fitted the diagnostic criteria for MD proposed by the Committee on Hearing and Equilibrium (1) (Table I). In addition, 30 healthy subjects with a negative history of labyrinthine, acoustic or neurological pathologies (15 males, 15 females; age range 18–62 years, mean age 40 years) were also enrolled. All patients gave their informed written consent to participate in the study.

After obtaining an accurate clinical history, all patients underwent a complete objective ENT evaluation, a tonal audiometric evaluation, tympanometry, evaluation of the stapedial reflex threshold with decay time, determination of auditory brainstem responses and a complete vestibular assessment involving evaluation of spontaneous and positional nystagmus (Frenzel glasses), a head thrust test and a caloric test (Fitzgerald–Hallpike technique).

Blood samples were drawn from all patients for the immunologic assessment of IgG antibodies against the P0 antigen (30-kDa protein) of guinea pig inner ear extracts using a Western blot assay (Marblot Strip Test System Kit; Arnika s.rl.). Serological specimens were collected aseptically, stored at 4°C and analysed successively over a period of 4–7 days. Patient serum

Table I. *Diagnostic criteria for MD*

| |
|---|
| Certain MD: |
| Definite MD, plus histopathologic confirmation |
| Definite MD: |
| Two or more definitive spontaneous episodes of vertigo lasting $\geq 20$ min |
| Audiometrically documented hearing loss on at least one occasion |
| Tinnitus or aural fullness in the treated ear |
| Other causes excluded |
| Probable MD: |
| One definitive episode of vertigo |
| Audiometrically documented hearing loss on at least one occasion |
| Tinnitus or aural fullness in the treated ear |
| Other causes excluded |
| Possible MD: |
| Episodic vertigo of the Ménière type without documented hearing loss, or sensorineural hearing loss, fluctuating or fixed, with dysequilibrium but without definitive episodes |
| Other causes excluded |

was diluted and incubated for 15 min with individual Marblot strips. After a reaction time of 4–12 min the presence of specific antibodies was shown by their binding to corresponding antigen bands. The unbound serum was washed from the strips, and the bound antibodies were then incubated with alkaline phosphatase conjugated with anti-human IgG. Reaction of the antibody-reacted antigen bands with a colour developing solution that produced a purple precipitate clearly showed the presence of the specific band.

Statistical analysis was performed using the $\chi^2$ test.

## RESULTS

A total of 45/50 patients with sudden hearing loss were affected by a monolateral pathology, while the remaining 5 experienced a bilateral disturbance. In all patients, the hearing deficit was a stable loss of at least 30 dB, as determined by 3 audiometric tests performed on 3 consecutive days. Specifically, we noted mild ( $<40$ dB of hearing loss) or moderate (40–60 dB of hearing loss) impairment of auditory function in 46.6% and 28.9% of patients, respectively. Moreover, 24.5% of patients were affected by a severe unilateral hearing loss ( $>60$ dB).

Concerning the five patients affected by bilateral sudden hearing loss, in two of them we noticed a symmetric bilateral mild auditory deficit, while in the remaining three the hearing loss was asymmetric, being mild in one ear and moderate in the other.

Focusing on patients affected by MD, we noticed a monolateral audiovestibular deficit in 35 and a bilateral impairment in 10. In these patients, we defined the severity of audiologic deficits according to the four-step staging system proposed in 1995 by the Committee on Hearing and Equilibrium (1). According to this classification, of 35 patients with monolateral auditory deficit, 18 and 12 were affected by stage 2 and stage 3 hearing loss, respectively, the remaining 5 showing a stage 4 pathology. Moreover, in the 10 patients affected by bilateral MD, the hearing loss was classifiable as stage 4 in 2 cases, stage 3 in 5 and stage 2 in 3 (Table II). Bedside examinations and caloric binaural stimulation (Fitzgerald–Hallpike test) clearly demonstrated monolateral ($n = 35$) or bilateral ($n = 10$) impairment in all these patients.

Ten patients affected by sudden hearing loss showed specific IgG antibodies against the P0 protein. Specifically, the P0 positive band was detectable in 5/45 patients with unilateral auditory impairment and in 5/5 of those with bilateral forms of auditory impairment. Among patients affected by MD, the P0 positive band was detectable only in those subjects with bilateral audiovestibular impairment ($n = 10$) (Table III). It is interesting that specific IgGs against the P0 protein were detectable in none of the 35 patients affected by monolateral MD. Finally, only 5/30 healthy subjects gave a positive result to P0 antigen detection.

## DISCUSSION

Since the introduction of the theory of an autoimmune pathogenesis for inner ear diseases the attention and efforts of clinicians and researchers have focused on the detection of the specific antigen involved in these immunological processes.

In our opinion, the presence of IgG autoantibodies against a 30-kDa inner ear protein in 10/50 patients with sudden hearing loss and 10/45 patients affected by MD suggests that in some cases these pathologies may have an autoimmune origin, as these autoantibodies are directed against specific inner ear antigens. In our sample, the presence of the specific IgG against P0 antigen was detectable in 20% of patients affected by sudden hearing loss (both bilateral and unilateral), 22.3% of those affected by MD and 16.6% of healthy subjects. Statistical analysis revealed no significant differences in P0 antigen positivity between these three groups ($p = 0.8$).

Our data are comparable with those of Cao et al. (12) who, in 1996, using guinea pig inner ear tissue as an antigenic source, found that 35% of serum samples of patients with MD and idiopathic progressive sensorineural hearing loss reacted with the 30-kDa

Table II. *Staging of hearing loss in patients with MD*

| Stage | All patients | Monolateral MD | Bilateral MD |
|---|---|---|---|
| 1 | 0 | 0 | 0 |
| 2 | 20 | 18 | 3 |
| 3 | 15 | 12 | 5 |
| 4 | 10 | 5 | 2 |

Table III. *Determination of P0 antigen*

| Pathology | P0-positive | P0-negative | Total |
|---|---|---|---|
| Sudden hearing loss | 10 | 40 | 50 |
| Monolateral | 5 | 40 | 45 |
| Bilateral | 5 | 0 | 5 |
| MD | 10 | 35 | 45 |
| Monolateral | 0 | 35 | 35 |
| Bilateral | 10 | 10 | 10 |
| Healthy subjects | 5 | 25 | 30 |

immunoblots. As already discussed, these data are not surprising and mainly relate to the lack of selection of the study population.

If we consider bilateral and unilateral forms of hearing loss separately, we found that, in our sample, 5/5 (100%) patients affected with bilateral sudden hearing loss had IgG autoantibodies against P0 antigen ($p < 0.0001$ versus healthy subjects). In contrast, only 5/45 subjects with monolateral forms of hearing loss gave a positive result to this test (11%), with no statistically significant difference compared with the control group ($p = 0.4$). Moreover, none of the patients with monolateral MD were positive for P0 antigen. In contrast, positivity to P0 was 100% in bilateral MD patients ($p < 0.0001$ versus controls). In other words, although the detection of specific IgG autoantibodies against P0 antigen has a sensitivity of $\approx 21\%$ when used in an unselected population of patients affected by sudden hearing loss or MD, its specific sensitivity is 100% when it is applied to patients affected by bilateral forms. Moreover, if used in an unselected population, the positive predictive value of this test is $\approx 80\%$.

In our opinion, positive reactions to P0 in all bilateral MD and bilateral sudden hearing loss patients, together with the data from our previous study on sudden loss of vestibular function, strongly indicate that these three pathologies are the result of an ongoing autoimmune process directed against specific antigens of the inner ear.

In conclusion, at the moment we cannot recommend the detection of specific IgGs against P0 antigen as a diagnostic test for all forms of sudden hearing loss or MD, because of its low sensitivity in unselected patients. However, considering that the specificity of the test (83.4%) and its positive predictive value (80%) are comparable with corresponding values for commonly used diagnostic tests, and that the sensitivity increases dramatically after selection of the population, our results certainly open new perspectives in the field of the diagnosis of immunological inner ear pathologies. Of course, further researches are needed (for example, our results have to be integrated with those obtained with the use of other markers, such as the heat shock proteins) but an interesting route has certainly been found.

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Address for correspondence:
Desiderio Passàli
ENT Department
University of Siena Medical School
Policlinico "Le Scotte"
Viale Bracci
IT-53 100 Siena
Italy
Tel.: +39 577 585470
Fax: +39 577 47940
E-mail: passali@unisi.it