Cogan’s Syndrome: Anti-Hsp70 Antibodies are a Serological Marker in the Typical Form

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ABSTRACT: Background: Cogan’s syndrome (CS) is a rare autoimmune vasculitis characterized by ocular inflammation and sensorineural hearing loss. CS is divided into a “typical” form with non-syphilitic interstitial keratitis and audiovestibular symptoms, and an “atypical” form with ocular involvement affecting structures other than the cornea. Anti-Hsp70 antibodies were found at variable levels in patients presenting with various forms of autoimmune sensorineural hearing loss (ASNHL).

Objectives: To assess the correlation between anti-Hsp70 antibodies and specific ASNHL subgroups.

Methods: We divided 112 subjects into four groups: 14 subjects with typical CS, 24 with atypical CS, 55 with ASNHL, and 19 control subjects (healthy subjects and patients with systemic autoimmune diseases but no sensorineural hearing or audiovestibular alterations). Patients were tested for serological autoimmunity markers including anti-Hsp70.

Results: Positivity of the anti-Hsp70 antibody test was highest in the typical CS group (92.9%) and lowest in the control group (5.2%). The test was positive in 52.7% of patients in the ASNHL group and 16.6% in the atypical CS group. The paired comparison analysis between groups showed that sensitivity of anti-Hsp70 in the typical CS group was significantly higher, as compared to the other three study groups.

Conclusions: Anti-Hsp70 antibodies can be considered a serological marker of “typical” CS. “Atypical” CS is conceivably a sort of “melting pot” of different forms of autoimmune diseases still characterized by ocular inflammation and sensorineural hearing loss but whose antigenic characteristics need to be further defined.

KEY WORDS: Cogan’s syndrome (CS), heat shock protein (Hsp), anti-Hsp70 antibodies, autoimmune inner ear diseases

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Cogan’s syndrome is named after American ophthalmologist David G. Cogan who, in 1945, described four patients with “non-syphilitic interstitial keratitis and audiovestibular symptoms” [1]. The disease was probably present long before this formal identification, and a prominent example is the celebrated composer Ludwig von Beethoven [2]. CS is a rare autoimmune vasculitis characterized by ocular inflammation and sensorineural hearing loss; 10% of patients have abdominal aneurysms. General symptoms of the acute inflammatory phase are often non-specific and may include headache, fever, dizziness, arthralgia and abdominal pain. As a result, patients may first be referred to a neurologist for vertigo, or to a surgeon for abdominal symptoms [3]. In 1980, Haynes and co-authors [4] proposed a distinction between “typical” CS, as originally defined by Cogan, and “atypical” CS in which the ocular involvement does not affect the cornea and is responsible for chronic or recurrent conjunctivitis, scleritis, uveitis, optic disk edema, and retinal vasculitis.

Laboratory tests commonly used to detect autoimmune diseases are rarely positive for CS. A negative result does not exclude the possibility of the disease. Therefore, diagnosis remains essentially clinical and chiefly involves ophthalmologists and otolaryngologists. If not promptly identified, CS may progress to deafness, severe visual impairment, and disability. With deafness, patients may benefit from a cochlear implant. However, this procedure is expensive and represents, especially in young people, a life-long commitment to the device. On the other hand, the majority of CS cases can be well controlled with medical treatment. This consists of systemic immunosuppressants (steroids, cyclosporine, cyclophosphamide, methotrexate) and biological agents used alone or in combination [5]. Since 1945 approximately 400 articles on this topic have been published, which include more than 450 patients, mainly young adults, with no gender predilection (personal literature review). Previous studies showed that antibodies against a 68 kDa protein isolated from the bovine inner ear were detected in patients with sensorineural hearing loss [6,7]. This 68 kDa
antigen was then known to be ubiquitous and not specific to the inner ear. The 68 kDa antigen has been identified by purification studies as "heat shock protein 70" [8,9]. In patients with autoimmune sensorineural hearing loss, Hsp70 is thought to be the target of the identified antibody, or of a group of antibodies. Assaying these antibodies in serum could be a serological marker for this autoimmune disorder [8,10].

Sensitivity and specificity of anti-Hsp70 antibodies were analyzed in different series of ASNHL patients [11-13]. The present study involved a large cohort of patients subdivided into "typical" CS, "atypical" CS, ASNHL (idiopathic and/or associated with systemic autoimmune diseases other than CS), and controls. This aimed to confirm the specificity of anti-Hsp70 antibodies for sensorineural hearing involvement and, eventually, to show a significant association with a peculiar subgroup.

PATIENTS AND METHODS

Subjects diagnosed with unilateral or bilateral ASNHL, idiopathic, or associated with a systemic autoimmune disease, were examined at the Ocular Autoimmune and Inflammatory Diseases Service of the University Hospital of Parma (Italy) to assess for possible concomitant ocular inflammation. All patients underwent blood samples to test for autoimmunity markers, including anti-Hsp70 antibodies. The protocol was approved by the hospital ethics committee, and informed consent was obtained according to the tenets of the Helsinki Declaration. Autoimmunity blood tests were also performed for the control group subjects, who included healthy volunteers and patients with systemic autoimmune disease but with no sensorineural hearing alterations.

For each patient, a complete medical history was obtained on general health and possible concomitant autoimmune disease. Diagnosis of ASNHL was made by an otolaryngologist and excluded syphilis, noise trauma, drug toxicity, head trauma, and hereditary hearing impairment. A brain magnetic resonance imaging scan with gadolinium was performed to exclude acoustic neurinoma, metastatic disease, lymphoma, and multiple sclerosis.

The subjects included in this study were divided into four groups:
- Typical CS (i.e., with interstitial keratitis)
- Atypical CS (i.e., with autoimmune ocular inflammation other than interstitial keratitis)
- ASNHL (including idiopathic and associated with systemic autoimmune disease)
- Control (healthy subjects and patients with systemic autoimmune disease without sensorineural hearing or audiovestibular alterations).

Affected patients were followed for a minimum of 3 years. On the basis of the activity, severity, and potential progression of their inflammatory symptoms, patients were possibly treated according to a previously described immunosuppressive systemic protocol [14].

LABORATORY TESTS

The following serological autoimmunity tests were performed at enrollment: antinuclear antibodies, anti-extractable nuclear antibodies, anti-double-stranded DNA antibodies, anticardiolipin antibodies, anti-neutrophil cytoplasmic antibodies, anti-mitochondrial antibodies, and anti-smooth muscle antibodies. A "positive" result was recorded in the following cases:
- a value higher than the reference interval for dsDNA, ENA, or ACL antibodies.

Serum Hsp70 antibodies were detected with the western blotting method using purified recombinant inducible Hsp70 antigen extracted and purified from bovine kidney (IMMCO Diagnostic, Buffalo, USA). The western blotting test contains strips with two proteins: one 70 kDa protein corresponding to the Hsp70 protein, and one 116 kDa protein that serves as a molecular weight alignment marker. The test was considered positive when a 70 kDa blue-violet color could be detected. Each test was compared to a positive, lot-specific, control card included in the kit. Serological evaluation was repeated in affected subjects (i.e., excluding the healthy volunteers) twice a year on average for the entire follow-up period. Positivity was assumed for at least one positive result during the follow-up. Pearson’s chi-square test was used to compare differences among groups. A $P$ value < 0.05 was considered significant.

RESULTS

A total of 112 subjects (57% women, age range 8–76 years) were assigned to one of four study groups: typical CS (n=14), atypical CS (n=24), ASNHL (n=55), and controls (n=19: 11 healthy volunteers and 8 subjects with systemic autoimmune disease without sensorineural hearing disorders such as Behçet’s disease, Sjögren syndrome, lupus erythematosus, rheumatoid arthritis, and sarcoidosis). Demographics for each group are detailed in Table 1. No significant differences were found between the groups.

| ANA = antinuclear antibodies |
| ANCA = anti-neutrophil cytoplasmic antibodies |
| AMA = anti-mitochondrial antibodies |
| ASMA = anti-smooth muscle antibodies |
| dsDNA = anti-double-stranded DNA antibodies |
| ENA = anti-extractable nuclear antibodies |
| ACL = anticardiolipin antibodies |

Hsp70 = heat shock protein 70
ASNHL = autoimmune sensorineural hearing loss
AUTOANTIBODY SPECIFICITY AND SENSITIVITY

The anti-Hsp70 antibody test had the highest positivity in the typical CS group (93%) and the lowest positivity rate in the control group (5%). Anti-Hsp70 was positive in about half of ASNHL patients, and in only 4 of 24 subjects (17%) presenting with atypical CS [Table 2].

Of the other autoantibodies tested (i.e., different from anti-Hsp70), the overall positivity never exceeded 40%, with the highest value in the control group (which included patients with systemic autoimmune disease). The second group with the highest positivity was ASNHL patients (27%) with a predominance of the ANCA profile.

A paired comparison analysis of the positivity rate of anti-Hsp70 antibody was performed between groups. The pairs showing significant differences are shown in Table 3. Sensitivity of the typical CS group was highly significant compared to the other three study groups. A significant P value was also found for ASNHL patients versus atypical CS.

DISCUSSION

Multiple studies support an autoimmune pathogenesis for CS. They indicated various antibodies directed against inner ear, corneal and endothelial antigens as possible etiology and serological markers of the disease [15,16]; in particular, antibodies against a peptide antigen (Cogan peptide) which shares sequence homology with CD148 and connexin 26, ANCA, and anti-Hsp70 antibodies. In the present study, the rate of CS patients (typical and atypical forms) who tested positive for anti-Hsp70 antibodies at least once during the follow-up was similar to that found in a prior pilot study (45% vs. 50%) [13]. This previous series already observed a prevalence of positive anti-Hsp 70 antibody in patients affected by Typical CS, compared to those with Atypical CS (66.7% vs. 37.5%, respectively). The larger cohort in the present study allowed statistical confirmation of this tendency (P < 0.001). The prevalence of anti-Hsp70 in typical CS was also significant, as compared to the two other study groups (i.e., ASNHL and controls). The only subject with typical CS and negative anti-Hsp70 was the youngest in the series (8 years old). This could be explained by the uncompleted development of “immunity competence,” as proposed by other authors [7,8]. This finding also supports the usefulness of test repetition in patients with suspected typical CS and pediatric onset until the age of complete immune competence. Among CS subjects, no patients with a negative initial anti-Hsp70 profile converted during the follow-up. Alternatively, 64% of typical CS patients and 5% of atypical CS patients converted from positive to negative during the follow-up. All of these patients had undergone immunosuppressive treatment with substantial improvement in their clinical condition. The control of active disease was likely to be responsible for the decreased anti-Hsp70 titer.

The second highest positive rate for anti-Hsp70 antibody (52.7%) was found in subjects affected by ASNHL. This confirms data from the prior smaller cohort [13].

Tested autoantibody classes other than anti-Hsp 70 had a low positive rate in all three study groups, with subjects affected by sensorineural hearing involvement. A remarkable positivity rate for ACL and beta2 glycoprotein antibodies found in other ASHNL series was not observed in this study [17,18].

CONCLUSIONS

The anti-Hsp70 antibody test confirms a significant relationship with autoimmune sensorineural hearing disorders. This evidence is further reinforced by the control group, in which subjects had autoimmune diseases that do not involve sensorineural and/or audiovestibular structures. Among the various categories of autoimmune sensorineural hearing disorders, anti-Hsp70 antibody testing has an extremely high sensitivity for the typical form of CS. Atypical CS, with sensorineural hearing loss associated with any kind of ocular inflammation
other than interstitial keratitis, is likely to involve quite different autoimmune entities whose characteristics need to be defined [19]. The test should be performed at the beginning of the diagnostic course, before any immunosuppressive systemic therapy. If the anti-Hsp70 test is negative in the absence of immunosuppressive treatment, the diagnosis of typical CS may be reasonably excluded. The test does not need to be repeated except in rare cases of pediatric onset.

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A new transcriptional role for matrix metalloproteinase-12 in antiviral immunity

Interferon-alpha (IFNα) is essential for antiviral immunity, but in the absence of matrix metalloproteinase-12 (MMP-12) or kBα (encoded by NFKBIA) Marchant et al. show that IFNα is retained in the cytosol of virus-infected cells and is not secreted. These findings suggest that activated kBα mediates the export of IFNα from virus-infected cells and that the inability of cells in Mmp12−/− but not wild-type mice to express kBα and thus export IFNα makes coxsackievirus type B3 infection lethal and renders respiratory syncytial virus more pathogenic. The authors show that after macrophage secretion, MMP-12 is transported into virus-infected cells. In HeLa cells MMP-12 is also translocated to the nucleus, where it binds to the NFκBIA promoter, driving transcription. They also identified dual-regulated substrates that are repressed both by MMP-12 binding to the substrate’s gene exons and by MMP-12-mediated cleavage of the substrate protein itself. Whereas intracellular MMP-12 mediates NFκBIA transcription, leading to IFNα secretion and host protection, extracellular MMP-12 cleaves off the IFNα receptor 2 binding site of systemic IFNα, preventing an unchecked immune response. Consistent with an unexpected role for MMP-12 in clearing systemic IFNα, treatment of coxsackievirus type B3-infected wild-type mice with a membrane-impermeable MMP-12 inhibitor elevates systemic IFNα levels and reduces viral replication in pancreas while sparing intracellular MMP-12. These findings suggest that inhibiting extracellular MMP-12 could be a new avenue for the development of antiviral treatments.

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“Manners are a sensitive awareness of the feelings of others. If you have that awareness, you have good manners, no matter what fork you use”

Emily Post (1872-1960), American author and columnist famous for writing on etiquette