

Sensorineural Hearing Loss in Patients with Mixed Connective Tissue Disease: Immunological Markers and Cytokine Levels

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ABSTRACT Objective. To investigate the frequency of sensorineural hearing loss (SNHL) in patients with mixed connective tissue disease (MCTD).

Methods. The study population consisted of 71 patients with MCTD (69 female; 2 male), with a mean age of 57.1 ± 7.9 years and a mean disease duration of 14.5 ± 8.0 years. All patients underwent audiological evaluation that included pure tone and speech audiometry. In addition, the systemic manifestations of the disease and drug therapy were recorded. All patients were tested for presence of autoantibodies. Fifty-one age-matched healthy subjects served as controls.

Results. SNHL was found in 33 (46.4%) of the 71 patients with MCTD. There was no correlation between SNHL and age and disease duration. An association was found between Raynaud's phenomenon ($p < 0.03$), secondary antiphospholipid syndrome (APS) ($p < 0.05$), and SNHL. MCTD patients with SNHL had higher serum levels of anti-U1RNP ($p < 0.05$), antiendothelial cell antibodies ($p < 0.001$), and IgG type anticardiolipin antibodies ($p < 0.0001$) than patients without SNHL. Serum levels of interferon- γ and tumor necrosis factor- α were increased in MCTD patients with SNHL compared to patients without SNHL. The absolute number of natural (CD4+CD25^{high}FoxP+) regulatory T cells (Treg) was lower compared to patients without SNHL.

Conclusion. In MCTD, SNHL is a specific organ manifestation and appears frequently. We have found that pathogenic autoantibodies, decreased levels of regulatory T cells, and overexpression of proinflammatory cytokines may play a role in the pathogenesis of immune mediated inner ear disorders in MCTD. (J Rheumatol First Release August 15 2009; doi:10.3899/jrheum.081314)

Key Indexing Terms:

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Audio-vestibular dysfunction is not unusual in autoimmune diseases. Sensorineural hearing loss (SNHL), which may occur as a distinct entity involving exclusively the inner ear, is more commonly part of a systemic autoimmune disorder¹⁻³. SNHL has been observed in many systemic autoimmune

diseases, in association with Wegener's granulomatosis⁴, polyarteritis nodosa⁵, systemic lupus erythematosus⁶, Sjögren's syndrome⁷, systemic sclerosis⁸, primary APS⁹, as well as relapsing polychondritis¹⁰. In these diseases the hearing loss is a part of the systemic autoimmune process, which involves the inner ear, causing auditory and vestibular dysfunction.

Contrary to other systemic autoimmune diseases, the association between mixed connective tissue disease (MCTD) and audio-vestibular impairment has not been described. MCTD is an autoimmune disorder, accompanied by chronic inflammation, which involves and damages several organs^{11,12}. The most common clinical features of MCTD are polyarthritis, Raynaud's phenomenon, esophageal hypomotility, and inflammatory myositis^{13,14}. Renal disease is less common in MCTD than in systemic lupus erythematosus (SLE). A high prevalence of pulmonary impairment is observed in patients with MCTD, of which the most severe is pulmonary arterial hypertension, a predominant cause of death in these patients¹⁵⁻¹⁸. The most relevant immune-laboratory marker of MCTD is the pres-

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ence of autoantibodies to nuclear ribonucleoprotein (U1RNP), however the patients' sera may contain autoantibodies against cardiolipin structures as well as endothelial cells.

The aim of our study was to determine the incidence of SNHL in patients with MCTD compared to healthy individuals, and to identify the association between auditory dysfunction and MCTD. Moreover we assessed the immunoserological and immune-regulatory characteristics of MCTD patients with SNHL.

MATERIALS AND METHODS

Patients. The study population consisted of 71 patients (2 men and 69 women) with MCTD and 51 age- and sex-matched healthy control individuals (2 men, 49 women). All patients were regularly followed up at the Autoimmune Outpatient Clinic of the 3rd Department of Medicine, Medical and Health Science Center, University of Debrecen between January 2006 and July 2006, and met the applied criteria established by Alarcon-Segovia and Villarreal for the diagnosis of MCTD¹⁹.

Demographic and clinical characteristics of patients are summarized in Table 1. Seventeen patients received nonsteroidal antiinflammatory drugs (NSAID), while 15 patients were administered 10–20 mg per day prednisolone. Thirty-nine patients were treated with combination prednisolone and cytotoxic drugs: 28 patients received 10 mg per week methotrexate and 10 mg per day prednisolone, and 11 patients 100 mg per day cyclophosphamide and 10 mg per day prednisolone.

Fifty-one healthy female volunteers (mean age: 55.3 ± 7.9 yrs) constituted the control group. Patients and controls did not have a history of otologic surgery, cranial trauma, exposure to occupational or recreational noise, any previous ototoxic drug use, or any acute or chronic otologic disease. The study was approved by the ethics committee of the University of Debrecen.

Otolaryngologic evaluation. All patients and controls underwent complete otolaryngologic examination and audiologic evaluations, performed by the same physician in all subjects. Tympanic membranes were investigated by otomicroscopy (OPMI 9; Carl Zeiss, Germany). All patients and controls had intact tympanic membranes. Tympanometry was used to test the condition of the middle ear, mobility of the tympanic membrane and conduction bones. The inclusion criterion in our study for both patients and controls was an intact middle ear.

Audiologic tests, including the assessment of pure tone air and bone conduction thresholds, were conducted in a soundproof chamber with a Clinical Audiometer (GSI61, Sonic Innovations, Salt Lake City, UT, USA). Pure tone air and bone conduction threshold at 125 Hz to 8000 Hz were cal-

Table 1. Demographic data and clinical features of patients with mixed connective tissue disease (N = 71). Values are number (%) unless otherwise indicated.

Characteristic	
Mean age ± SD, yrs (range)	57.1 ± 7.9 (40–86)
Mean followup ± SD, yrs (range)	14.5 ± 8.0 (3–30)
Female/male	69/2
Polyarthritis	63 (88.7)
Raynaud's phenomenon	39 (54.9)
Myositis	20 (28.1)
Esophageal hypomotility	35 (49.2)
Interstitial lung disease	44 (61.9)
Pulmonary hypertension	11 (15.4)
Serositis	20 (28.1)
Secondary Sjögren's syndrome	17 (23.9)
Secondary antiphospholipid syndrome	16 (22.5)

culated for each ear. The average value of the low- (125, 250 Hz), speech- (500, 1000, 2000 Hz), and high- (4000, 8000 Hz) frequency threshold levels were determined in each patient. The level of hearing impairment was classified as normal hearing (< 25 dB), mild (26–40 dB), moderate (41–60 dB), and severe hearing loss (61–80 dB).

Immunoserological assessment. Antinuclear antibodies were detected on Hep-2 cells by indirect immunofluorescence method. IgM rheumatoid factor was assessed by nephelometry, values > 50 U/l were considered positive.

Serum concentrations of autoantibodies were analyzed by ELISA (anti-U1RNP, Pharmacia and Upjohn, Freiburg, Germany; anti-SSA, anti-SSB, anti-Jo1, anti-Scl70, anti-dsDNA, Cogent Diagnostics, Edinburgh, UK) according to the manufacturer's instructions. Serum anticardiolipin (aCL) and anti-β₂-glycoprotein I (anti-β₂-GPI; IgG, IgM and IgA) antibodies were measured by ELISA (Orgentec Diagnostica GmbH, Mainz, Germany). Values higher than +3 SD of the mean normal cutoff point were considered positive. aCL positive patients were retested 12 weeks subsequently, in order to evaluate their continuous persistence and coexistence with anti-β₂-GPI antibodies.

Plasma levels of antiendothelial cell autoantibodies (AECA) were determined by a cellular ELISA with cultured human umbilical vein endothelial cells according to our described method²⁰.

Determination of serum soluble cytokines. Serum interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α) interleukin 10 (IL-10), and IL-4 levels were measured by BD OptEIA ELISA Sets (Becton Dickinson Pharmingen, San Diego, CA, USA) according to manufacturer's instructions. The intraassay and interassay coefficients of variation, respectively, were 1.8% and 2.3% for IFN-γ, 0.9% and 1.6% for TNF-α, 2.8% and 5.1% for IL-10, and 0.9% and 1.8% for IL-4.

Evaluation of intracellular cytokines by flow cytometry. Briefly, 1 ml heparinized blood was diluted 2-fold in RPMI 1640 (GIBCO, BRL)²¹. Cells were stimulated using 25 ng/ml phorbol 12-myristate 13-acetate (PMA) (Sigma Aldrich Corp, St. Louis, MO, USA), and 1 ng/ml ionomycin (Sigma Aldrich Corp) for 4 h in the presence of 10 μg/ml brefeldin-A (BFA) (Sigma Aldrich Corp). The incubator was set at 37°C under a 5% CO₂ environment. Unstimulated cells containing BFA served as controls. After stimulation cells were surface stained with anti-human CD4-PC5 or CD8-PC5 antibody (Immunotech) for 30 min at room temperature and washed, fixation and permeabilization were performed using IntraPrep™ Permeabilization Reagent according to the manufacturer's instructions (Beckmann Coulter, Fullerton, CA, USA). Samples were washed in phosphate buffered saline and were further incubated for 30 min in darkness with specific monoclonal antibodies: PE-conjugated anti-human IL-10 (Caltag Laboratories, Burlingame, CA, USA) or with their proper isotype controls (anti-mouse IgG1-FITC or IgG1-PE isotype antibodies). The cells were fixed using 1% paraformaldehyde and analyzed within 6 hours by a Coulter FC500 flow cytometer (Beckmann Coulter). At least 10,000 CD4+ cells were counted and analyzed using the CXP Analysis Software (Beckmann Coulter).

Analysis of CD4+CD25^{high}FoxP3+ T cells by Flow Cytometry. FoxP3 staining was carried out by following the instructions of the manufacturer (eBioscience, San Diego, CA, USA). Briefly, peripheral blood mononuclear cells were separated by Ficoll/Hystopaque gradient centrifugation; then 100 μl of prepared cells was added to each tube (1 × 10⁶ cell/ml). After cell surface staining by CD4, CD25 monoclonal antibody cells were washed in cold flow cytometry staining buffer (eBioscience). Freshly prepared 1 ml fixation/permeabilization working solution was added to each sample. Cells were incubated at 4°C for 30–60 min in darkness, washed by adding 2 ml of 1x permeabilization buffer (eBioscience), and blocked with 2% (2 μl) normal rat serum in 1x permeabilization buffer in 100 μl at 4°C for 15 min. After blocking, 20 μl anti-human FoxP3-PE antibody was added and cells were incubated at 4°C for at least 30 min in darkness. Finally, cells were washed with 2 ml 1x permeabilization buffer and resuspended in Flow Cytometry Staining Buffer (eBioscience).

Lymphocytes were gated on the basis of their forward and side scatter

properties. Ten thousand gated events (lymphocytes) were collected in each sample on a FACSCalibur equipment (Becton Dickinson, Heidelberg, Germany). Data were analyzed using CellQuest software (Becton Dickinson, Heidelberg, Germany). FoxP3 positivity of CD4+CD25^{high} suppressor T cells was verified by FoxP3 staining. The mean fluorescence intensity (MFI) of FoxP3 was significantly higher in CD4+CD25^{high} suppressor T cells compared to the CD4+CD25^{low} or CD4+CD25⁻ cells ($p < 0.01$). The following reagents were used: Ficoll and CD4-FITC monoclonal antibody (Sigma-Aldrich Corp., St. Louis, MO, USA), CD25-PC5 (Immunotech, Marseilles, France), Foxp3-PE and intracellular staining kit (eBioscience, San Diego, CA, USA).

Statistical analysis. Statistical analyses were performed by using SPSS software, version 15.0. Data are presented as mean value \pm SD. After testing for normality, data were compared with Student's *t* test (paired and unpaired) or Mann-Whitney U-test and correlated with Spearman's rank correlation. Categorical variables were compared using chi-squared test. Individual relative risk and 95% confidence intervals were calculated using separate logistic regression for variables found to be significant or approaching significance in the previous analysis. For all tests, $p < 0.05$ was considered statistically significant.

RESULTS

Frequency of SNHL in MCTD patients. Thirty-three (46.47%) of 71 patients with MCTD were proved to have SNHL by audiogram, versus only 11 (21.5%) controls ($p < 0.007$) (Table 2). Hearing loss was bilateral in all patients and control individuals.

There was no significant difference between MCTD patients with or without SNHL with regards to the patient age, duration of disease, previous treatment with cytotoxic drugs, or corticosteroids (CS). Ten (30.3%) of 33 MCTD patients with SNHL complained of diminished hearing acuity, while the remaining 23 patients were asymptomatic, and hearing loss was detectable only on audiograms. The audiometric curve was flat in 11 patients and disclosed high frequency loss in 22 patients. In the control group one (9.0%) woman complained of hearing loss. Tinnitus was present in 3 (9.0%) MCTD patients, while vertigo was found in 2 (6.0%) patients with SNHL.

We evaluated the clinical symptoms and immunoserolog-

ical abnormalities of MCTD patients in order to determine their predictive value for inner ear involvement (Table 3). An association was found between Raynaud's phenomenon, secondary APS, and SNHL [Raynaud's phenomenon: $p < 0.03$; RR: 3.125 (1.128–8.659); secondary APS: $p < 0.05$; RR: 3.3 (1.007–10.816)]. We found a close association between the serum levels of anti-U1RNP and SNHL [anti-RNP: $p < 0.05$; RR: 2.683 (1.025–7.026)]. We identified a correlation between the presence of AECA and IgG type aCL antibodies and SNHL [AECA: $p < 0.0001$; RR: 8.750 (2.986–25.639); aCL IgG: $p < 0.0001$; RR: 36.96 (9.694–140.982)].

Serum levels of autoantibodies in MCTD patients with and without sensorineural hearing loss. In MCTD patients with SNHL, significantly higher levels of anti-U1RNP autoantibodies were found compared with the normal hearing MCTD group (MCTD+SNHL: 19.3 ± 10.2 U/ml; MCTD–SNHL: 13.8 ± 11.3 U/ml; $p < 0.05$). Further, AECA levels were significantly elevated in MCTD patients with SNHL (MCTD+SNHL: 41.2 ± 31.8 U/ml; MCTD–SNHL: 23.9 ± 19.0 U/ml; $p < 0.001$).

In our series, aCL was found in 19/33 (57.5%) MCTD patients with SNHL versus in 5 MCTD patients (13.1%) without SNHL ($p < 0.001$). Three out of 11 SNHL controls had aCL, while the 40 non-SNHL control sera samples contained no aCL autoantibodies ($p < 0.0079$).

These data underline that presence of aCL autoantibody is related to SNHL rather than to MCTD (Table 4). Eight of 19 (42.1%) MCTD patients with SNHL had transitory aCL positivity, and aCL elevation persisted after 12 weeks in 11 patients positive for anti- β_2 -GPI antibodies. All 11 MCTD patients with SNHL had had thrombotic events and fulfilled diagnostic criteria for APS (aCL: MCTD patients with SNHL and thrombosis: 62.3 ± 16.1 U/ml, after 12 weeks: 53.3 ± 13.0 U/ml, $p = 0.143$).

In control subjects with SNHL aCL elevation was tran-

Table 2. Sensorineural hearing loss (SNHL) patients with mixed connective tissue disease (MCTD) compared to controls. Values are number (%) unless otherwise indicated.

	MCTD Patients, N = 71		Controls, N = 51	
	With SNHL ^a , N = 33 (46.47)*	Without SNHL ^b , N = 38 (53.53)	With SNHL ^c , N = 11 (21.57)*	Without SNHL ^d , N = 40 (78.43)
Male/female	2/31	0/38	0/11	2/35
Mean age \pm SD**, yrs	57.84 \pm 8.2	56.4 \pm 7.65	54.7 \pm 5.7	57.2 \pm 8.1
Mean followup [†] , yrs	15.36 \pm 8.3	13.81 \pm 7.81	—	—
Treatment with cytotoxic drugs ⁺	18 (54.5)	21 (55.2)	—	—
Treatment with corticosteroids ⁺⁺	25 (75.7)	29 (76.3)	—	—
Auditory symptoms				
Subjective hearing loss	10 (30.3)	0	1 (9.0)	0
Subjective tinnitus	3 (9.0)	0	0	0
Vertigo	2 (6.0)	0	1 (9.0)	0

* a vs c = $p < 0.007$; ** a vs b = NS; a vs c = NS; b vs c = NS [‡] a vs b = NS; [†] a vs b = NS; ^{††} a vs b = NS.

Table 3. Association of sensorineural hearing loss with clinical and laboratory characteristics of mixed connective tissue disease.

	RR (95% CI)	p
Polyarthritis	2.083 (0.457–9.483)	0.4587
Raynaud's phenomenon	3.125 (1.128–8.659)	0.03
Myositis	1.086 (0.384–3.070)	1.0
Esophageal dysmotility	0.847 (0.332–2.155)	0.813
Serositis	0.92 (0.325–2.601)	1.0
Interstitial lung disease	0.937 (0.3374–2.602)	1.0
Pulmonary arterial hypertension	0.681 (0.187–2.481)	0.741
Secondary Sjögren's syndrome	1.031 (0.345–3.075)	1.0
Secondary antiphospholipid syndrome	3.3 (1.007–10.816)	0.05
Autoantibodies		
Anti-RNP	2.683 (1.025–7.026)	0.05
IgG-aCL	36.96 (9.694–140.982)	0.0001
Anti-β ₂ -GPI	1.167 (0.218–6.218)	1.0
Antiendothelial cell antibody	8.750 (2.986–25.639)	0.0001
ANTI-SSA	2.667 (0.858–8.284)	0.10
Anti-SSB	0.790 (0.225–2.778)	0.761
ANCA	1.167 (0.218–6.218)	1.0

aCL: anticardiolipin; anti-β₂-GPI: anti-β₂ glycoprotein I; ANCA: anti-neutrophil cytoplasmic antibodies.

sient, and there was no thrombosis in the control group. In 5 MCTD patients without SNHL aCL levels also persisted after 12 weeks (aCL: 57.5 ± 15.8 U/ml, after 12 weeks: 37.3 ± 11.5 U/ml, p = 0.560). Sera from 11 MCTD patients with SNHL and from 5 MCTD patients without SNHL all contained anti-β₂-GPI antibodies, and they had APS. Transitory aCL positivity was found in 8/33 MCTD patients with SNHL (24.2%). The aCL level decreased after 12 weeks, and the patients' sera did not contain anti-β₂-GPI (IgG-aCL: 56.4 ± 10.7 U/ml, after 12 weeks: 13.5 ± 4.4 U/ml; p < 0.001). Transitory aCL elevation was found in 3 control subjects with SNHL, without thrombosis.

Serum cytokine levels. Serum concentrations of IFN-γ, TNF-α, and IL-10 were significantly higher in patients with MCTD compared to controls (Figure 1). However, serum levels of IFN-γ and TNF-α further increased in MCTD patients with SNHL compared to MCTD patients without SNHL (MCTD+SNHL: IFN-γ: 51.8 ± 22.1 pg/ml, without SNHL: 39.5 ± 32.0 pg/ml, p < 0.05; TNF-α with SNHL:

34.7 ± 23.2 pg/ml, without SNHL: 22.1 ± 14.7 pg/ml, p < 0.05). MCTD patients with SNHL showed an increase in serum IL-10 levels compared to patients with intact hearing (MCTD+SNHL: 26.5 ± 10.4 pg/ml, MCTD patients without SNHL: 22.8 ± 17.8 pg/ml, p < 0.05), while serum IL-4 levels were similar in MCTD patients with SNHL than in controls.

Regulatory T cells in MCTD patients with and without SNHL. The percentage and absolute number of CD4+CD25^{high}FoxP3⁺ natural regulatory T cells (nTreg) were significantly lower in peripheral blood of patients with MCTD compared to controls (Table 5, all data are absolute values). Interestingly, the percentage and the absolute number of CD4+CD25^{high}nTregs showed a decrease in MCTD patients with SNHL compared to patients without impaired hearing (MCTD+SNHL: 2.01 ± 1.33% vs controls: 4.36 ± 0.99%, p < 0.001; MCTD–SNHL: 3.14 ± 1.74% vs controls: p < 0.05; MCTD with and without SNHL: 2.01 ± 1.33% vs 3.14 ± 1.74% p < 0.001; absolute number: MCTD+SNHL: 0.028 ± 0.005 G/l, MCTD–SNHL: 0.011 ± 0.012 G/l, p < 0.05, controls: 0.04 ± 0.016 G/l, MCTD–SNHL vs controls: p < 0.001, MCTD–SNHL vs controls: p < 0.001).

In contrast, in MCTD patients with and without SNHL the percentage and absolute number of CD4+/IL-10+ Tregs significantly increased compared to controls. Although the percentage and absolute number of CD4+/IL-10+ cells were higher in MCTD patients with SNHL, the difference was not statistically significant.

We found a negative correlation between the absolute number of Treg cells and the serum levels of aCL (r = –0.742, p < 0.001), and Treg cells and anti-U1RNP autoantibodies (r = –0.598, p < 0.01).

DISCUSSION

SNHL in patients with MCTD has not previously been described. We found SNHL in 33 (46.47%) of 71 patients with MCTD, which was significantly higher compared to healthy individuals. The presence of SNHL did not correlate with patient age, duration of disease, or previous therapy with cytostatic drugs and/or CS.

Clinically, SNHL generally occurred slowly over several

Table 4. Anticardiolipin antibody (aCL) positivity and thrombotic events in mixed connective tissue disease (MCTD) patients with and without sensorineural hearing loss (SNHL) and in control individuals. Values are number (%).

	MCTD with SNHL, N = 33	MCTD without SNHL, N = 38	p	Controls with SNHL, N = 11	Controls without SNHL, N = 40	p
aCL positivity	19 (57.5)	5 (13.1)	0.0001	3 (27.2)	0	0.0079
Thrombotic events (aCL, anti-β ₂ -GPI)	11 (33.3)	5 (13.1)	0.05	0	0	
Transitory aCL positivity	8 (24.2)	0	0.0013	3 (27.2)	0	0.0079

aCL: anticardiolipin; anti-β₂-GPI: anti-β₂ glycoprotein I antibody.

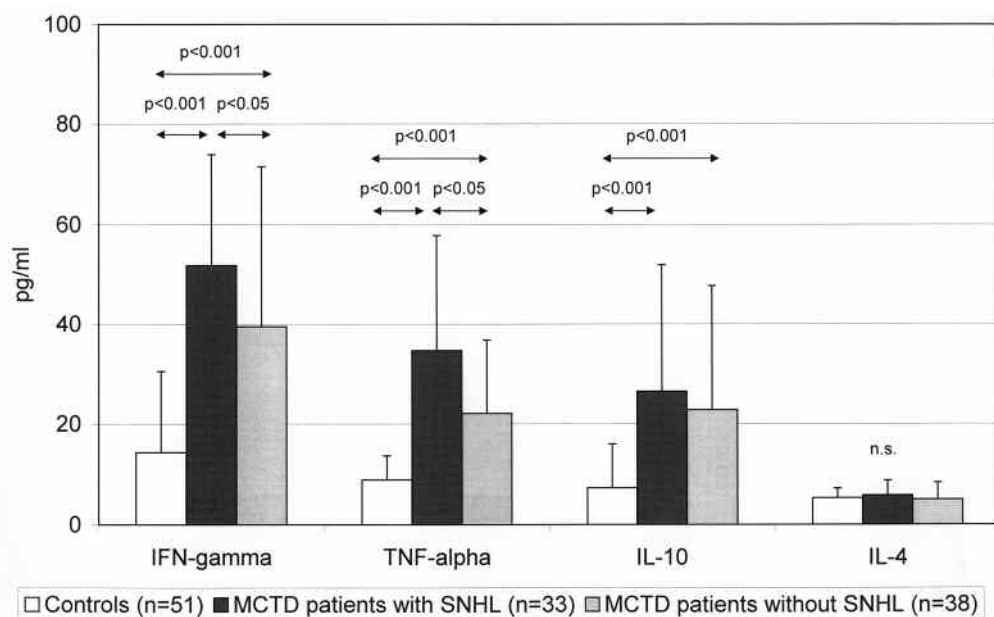


Figure 1. Serum interferon- γ (IFN-gamma), tumor necrosis factor- α (TNF-alpha), interleukin (IL-10), and IL-4 levels in mixed connective tissue disease (MCTD) patients with and without sensorineural hearing loss.

Table 5. The absolute number of CD4+CD25^{high}FoxP3+ and CD4+/IL-10+ T regulatory cells in mixed connective tissue disease (MCTD) patients with and without sensorineural hearing loss (SNHL).

Regulatory T Cells	Controls, n = 51	MCTD with SNHL ^a , n = 33	MCTD without SNHL ^b , n = 38	a vs b p	c vs a p	c vs b p
CD4+ CD25 ^{high} FoxP3+, g/l	0.4 ± 0.016	0.019 ± 0.005	0.028 ± 0.012	0.05	0.001	0.001
CD4+/IL-10+, g/l	0.062 ± 0.02	0.18 ± 0.078	0.16 ± 0.09	NS	0.001	0.001

C: controls.

months in MCTD patients and was bilateral in all patients. Our finding was in accordance with previous observations, namely that inner-ear diseases are usually to some degree bilateral in systemic autoimmune diseases^{6,22}. The presence of Raynaud's phenomenon and secondary APS associated with MCTD had predictive value for the subsequent development of SNHL in MCTD patients.

The high levels of anti-U1RNP, IgG type aCL, and AECA were found in association with SNHL in MCTD patients.

In systemic autoimmune diseases prevalence of auditory disorders is relatively frequent, especially with involvement of the middle ear, which is high in patients with Wegener granulomatosis, with an incidence of 35% to 47%²³. In patients with SLE Andonopoulos, *et al*²³ observed hearing loss in 57.5% of 40 patients investigated, while Kastanioudakis and colleagues²⁴ showed auditory manifestations in 22.5% of 38 patients with SLE. Auditory symptoms were found in 55.5% of 45 patients with SLE; however, SNHL appeared only in 7 cases (15.6%)²⁵. Tumiat, *et al* found a high prevalence of aCL antibodies in patients

with Sjögren's syndrome who had SNHL⁷. Twelve of 31 patients were positive for aCL antibodies, and 9 of these patients had SNHL. Casoli, *et al* described a 55-year-old woman with a 6-year history of Sjögren's syndrome with positive IgG and IgM antiphospholipid antibody titers, who developed a sudden onset of sensorineural hearing loss associated with vertigo²⁶.

The pathogenesis of immune-mediated SNHL is unclear, but it may be related to immune complex-mediated vasculitis in the inner ear; or pathogen antibodies may directly damage the inner ear structures²⁷⁻²⁹. Toubi, *et al*³⁰ reported that antiphospholipid antibodies are involved in the pathogenesis of inner ear dysfunction.

In our series aCL was found in 19/33 (57.5%) MCTD patients with SNHL, while aCL was found in 5 MCTD patients (13.1%) without SNHL ($p < 0.0001$). Three of 11 SNHL controls had aCL antibody, while 40 non-SNHL control sera contained no aCL ($p < 0.0079$). These data emphasize that the presence of aCL autoantibody is more closely related to SNHL than to MCTD.

Eight out of 19 (42.1%) MCTD patients with SNHL had

transitory aCL antibody positivity, and the aCL elevation persisted in 11 patients with anti- β_2 -GPI. All of the 11 MCTD patients with SNHL had APS.

In the 11 control persons with SNHL, elevation of aCL was transient, and there was no thrombosis in the control group.

In MCTD there is evidence of T cell dependent B cell responses driven by antigenic stimulation against self-antigen, possibly due to subtle antigenic structural modifications¹². Antinuclear antibodies and anti-UIRNP are characteristic of the disease, and other antibodies such as aCL, AECA may determinate the clinical symptoms and the disease course^{31,32}. The presence of aCL antibodies has been well described in various autoimmune diseases, including malignancies and chronic infectious diseases.

Recently it has been shown that environmental factors, bacterial or viral cell products via pathogen associated pattern recognition receptors (TLR), can activate autoreactive B cells, and finally B cells can mediate tissue injury through a variety of antibody-directed mechanisms¹².

In MCTD patients, aCL antibodies were found in 15–20%, while aCL antibodies occurred more frequently (40–50%) among patients with SLE. In contrast, a large followup study reported 33% of aCL antibodies in MCTD³³. In a series of 28 patients who had MCTD (18 of whom had aCL antibodies), it was suggested that aCL antibodies correlated with thrombocytopenia but not with recurrent thrombosis or recurrent miscarriages³⁴. However, classic APS can occur in MCTD and can be fatal³⁵. Recently the presence of aCL antibodies was identified as a risk factor for development of pulmonary arterial hypertension; moreover, pulmonary arterial hypertension is the primary disease-associated cause of death in MCTD³⁶.

At the site of inflammation Treg cells operate in association with T effector cells, leading to the modulation of immune reactions³⁷. T regulatory cells repress the catalytic capacity of T effector cells after antigen-specific sensitization by decreasing cytokine production. This regulatory mechanism may involve various cytokines, such as IFN- γ . In MCTD patients with SNHL, increased production of IFN- γ was observed. INF- γ and other proinflammatory cytokines such as TNF- α provoke inflammation of the endothelial cells in the blood vessels and provoke tissue damage. Moreover, if this inflammatory process takes place in the ear, hearing loss can occur.

The elevated levels of IL-10 cytokine, producing inducible T regulatory cells, may be a compensatory mechanism that directly inhibits activity of T effector cells. However, B cells in MCTD patients have intrinsic B cell overactivity, and different viral infections such as parvovirus, cytomegalovirus, and herpes simplex also may induce persistent or transient aCL overproduction.

aCL and other autoantibodies such as AECA reacting to endothelial cell targets may cause endothelial cell activation

or damage in the inner ear microcirculation³⁸. Toubi, *et al*³⁹ did not find an association between presence of Epstein-Barr virus, cytomegalovirus, or hepatitis C virus and elevated levels of aCL and anti- β_2 -GPI in patients with immune mediated hearing loss, but the potential role of viral infection could not be excluded. Possibly the disturbance of a regulatory mechanism and external agents, such as viral infections, provoke increased autoantibody formation.

Identification of the pathogenetic mechanism that determines inner ear disturbance would be essential for development of therapy. SNHL was treated in other autoimmune diseases especially in patients with SLE, but it is not known which treatment is most appropriate and most effective. CS and cyclophosphamide in the acute stage may be effective as therapy. Nineteen MCTD patients with SNHL received 2 mg/kg bodyweight/day CS (methylprednisolone) for 2 weeks, followed by a reduced dosage CS (1 mg/kg bodyweight/day) for 1 month, then tapered to a daily dose of 8–10 mg. In 5 patients with sudden hearing loss CS treatment was supplemented with intravenous cyclophosphamide. Recently it was reported that anti-interleukin-1 (anti-IL-1) agent anakinra improved SNHL in patients in a dominantly inherited autoinflammatory syndrome in Muckle-Wells syndrome⁴⁰. In future, cytokine-related SNHL such as Kawasaki disease or hemophagocytic lymphohistiocytosis may be improved by anticytokine therapy. The elevated levels of Th1 cytokines, IFN- γ and TNF- α , raise the possibility that anti-TNF- α monoclonal antibodies or TNF- α receptor blocker may be useful in the therapy of SNHL associated with autoimmune diseases, or in immune mediated SNHL.

The inner ear target antigen epitopes have not been investigated in patients with MCTD. We suggest that SNHL is associated to MCTD, but the future studies need to investigate the exact pathomechanism and the effective therapy.

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