

Sicca Symptoms and Anti-SSA/Ro Antibodies Are Common in Mixed Connective Tissue Disease

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ABSTRACT. Objective. To examine a large well characterized cohort of patients with mixed connective tissue disease (MCTD) and determine longitudinally the prevalence of clinical and serologic features of Sjögren's syndrome in these patients.

Methods. Patients were followed longitudinally up to 30 years with systematic clinical and serologic analysis. Sera were analyzed for reactivity with SSA/Ro, SSB/La, and snRNP polypeptide U1-70kD and for anticardiolipin antibodies.

Results. Among a well characterized patient population with MCTD, 18/55 (32.7%) had antibodies to SSA/Ro, while 2/55 (3.6%) had antibodies to SSB/La, either initially or during the course of followup. All patients had antibodies to U1-70kD small nuclear ribonucleoprotein antigen. Sicca symptoms were common, occurring in 23/55 (41.8%) patients. Patients with MCTD who were anti-SSA/Ro positive had increased incidence of malar rash ($p < 0.03$) and photosensitivity ($p < 0.001$) compared to anti-SSA/Ro negative patients.

Conclusion. Sicca symptoms are frequent in patients with MCTD, occurring in up to one-third of the patients studied. The presence of anti-SSA/Ro antibodies identifies a group of MCTD patients with a very high incidence of malar rash and photosensitivity. (J Rheumatol 2002;29:487-9)

Key Indexing Terms:

SJÖGREN'S SYNDROME
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Sjögren's syndrome (SS) occurs as a primary disease entity and commonly as a secondary condition associated with other rheumatic diseases, such as systemic lupus erythematosus (SLE), systemic sclerosis (SSc), polymyositis, and rheumatoid arthritis (RA)¹. Although precise epidemiologic data on the incidence and prevalence of secondary SS in these 4 rheumatic diseases is limited, secondary SS has been reported to occur in roughly 25% of patients with SLE, 30% of patients with SSc, 15% of patients with polymyositis, and 40% of patients with RA.

Mixed connective tissue disease (MCTD) is character-

ized by overlapping features of SLE, SSc, polymyositis, and RA². Substantial evidence now supports the concept that MCTD represents a distinct subset of patients, and this topic has been recently reviewed³. The core clinical features of Raynaud's phenomenon, arthralgia/arthritis, swollen hands, esophageal motility abnormalities, myositis and pulmonary dysfunctions, unique serologic features, and distinctive immunogenetics support the concept of MCTD²⁻⁴. Although SS is known to be present in SLE, SSc, polymyositis, and RA, there are limited data on the occurrence of SS in MCTD. Early studies reported a wide range of findings, with 7-50% of patients with MCTD stated to have secondary SS^{5,6}. Our study of a well characterized population of patients with MCTD was designed to test the hypothesis that secondary SS is common in MCTD, and that both clinical and serologic features of SS are present in MCTD. This study includes comprehensive clinical and serologic characterization of patients followed for up to 30 years.

MATERIALS AND METHODS

Patients and controls. The University of Missouri Institutional Review Board approved the studies. Fifty-five patients followed longitudinally in the Rheumatology Clinics at the University of Missouri Hospital and Harry S. Truman Memorial Veterans Hospital between 1969 and 2000 were selected for study based on the criteria of Kasukawa, *et al* for MCTD⁷. Systematic and longitudinal clinical and laboratory observations and numerous serologic analyses were performed on many of these patients, initially according to a clinical research center protocol. Sicca was defined as daily oral dryness including frequent use of liquids because of oral dryness or daily dry eyes.

Clinical database. Patients were systematically evaluated, all medical

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records were reviewed, and data were entered into a computerized database using the definitions and algorithm as described⁸. The patients' sex, age at disease onset, age at diagnosis, and duration of followup were also recorded.

Sera samples. Serum specimens were obtained on multiple occasions, from the early phase of the disease to the latest followup, on a regular basis, in most cases every 6 months, and were stored frozen or lyophilized at -20°C or -70°C . In some cases, more than 50 specimens from the same subject were studied.

Autoantibody assays. Serum samples from study patients were tested for fluorescent antinuclear antibody reactivity by indirect immunofluorescence, for antibodies to RNP, Sm, SSA/Ro, SSB/La, PM-1/PM-Scl, and Scl-70 antigens by double immunodiffusion; for antibodies to extractable nuclear antigen (ENA) by passive hemagglutination (PHA) before and after RNase digestion of ENA; for antibodies to double-stranded DNA by the *Crithidia lucilliae* test; and for reactivity with individual snRNP polypeptides by immunoblotting⁸. An ELISA was used to determine the presence and level of autoantibodies reactive with the U1-70kD polypeptide, employing a recombinant U1-70kD fusion protein as antigen⁸. An ELISA was used to quantitate IgG and IgM antiphospholipid (anticardiolipin) autoantibodies⁹. Although detailed serologic results were recorded from previous testing, comprehensive autoantibody analyses were performed during the past 6 months on sera from all patients that had been obtained and stored frozen in their early disease course, during the mid-course, and at the time of the most recent clinical followup. These repeated analyses confirmed the accuracy and reproducibility of our methods.

Statistical analyses. Summary statistics were calculated as appropriate. Exact 95% confidence intervals were calculated to estimate the proportion of the population with each of the clinical findings. Between-group comparisons were performed using Fisher's exact tests for categorical variables. When multiple comparisons were made, $p < 0.01$ was considered significant. Statistical analyses were performed using Prism software (Graph Pad Software, San Diego, CA, USA).

RESULTS

Demographic and immunogenetic data. Fifty-five patients with a diagnosis of MCTD were followed in the Division of Immunology and Rheumatology for a period of 3–30 years (mean 9 years). Forty-seven (86%) were female. The mean age at diagnosis of MCTD was 33 years (range 5–70). All patients met the classification criteria of Kasukawa, *et al* for MCTD⁷. Patients classified as MCTD did not evolve to other diagnoses, such as SLE or SSc.

Clinical characteristics. The clinical characteristics of the patients are summarized in Table 1. The most prevalent clinical manifestations of disease were those typical of MCTD: Raynaud's phenomenon (89%), swollen hands (73%), gastroesophageal reflux (71%), arthritis/arthralgia (63%), myalgia (63%), esophageal hypomotility (56%), pleuritis/pericarditis (55%), lymphopenia (55%), and sclerodactyly (51%). Sicca symptoms were present in 23/55 (42%) patients. Photosensitivity was present in 25/55 (45%) patients and malar rash in 22/55 (40%) patients overall. Ocular or oral symptoms could not be associated with concomitant drug use.

Clinical features of anti-SSA/Ro-positive versus anti-SSA/Ro-negative patients. Patients were further classified into those who were anti-SSA/Ro-positive versus those who were anti-SSA/Ro-negative and clinical features were

Table 1. Clinical characteristics of the 55 patients with mixed connective tissue disease.

Clinical Characteristics	Percentage Positive
Raynaud's phenomenon	89 (49/55)
Swollen hands	73 (40/55)
Gastroesophageal reflux	71 (39/55)
Arthritis/arthralgia	62 (34/55)
Myalgias	62 (34/55)
Esophageal hypomotility	56 (31/55)
Pleuritis/pericarditis	55 (30/55)
Lymphopenia	53 (29/55)
Sclerodactyly	51 (28/55)
Photosensitivity	45 (25/55)
Sicca symptoms*	42 (23/55)
DLCO < 70% of predicted	47 (23/49)
Malar rash	40 (22/55)
Telangiectasias	38 (21/55)
Pulmonary hypertension	52 (13/25)
Renal disease	24 (13/55)
Oral or nasal ulcers	24 (13/55)
Leukopenia	24 (13/55)
Anemia	22 (12/55)
Nervous system disease	2 (1/55)

*Defined as daily oral dryness, frequent use of liquid because of oral dryness, or daily ocular dryness. DLCO: Pulmonary diffusion capacity for carbon monoxide.

compared between the 2 groups (Table 2). There were statistically significant differences between the groups for photosensitivity ($p < 0.0013$) and malar rash ($p < 0.0025$). There was also a difference between groups for anemia ($p < 0.013$), but this difference was not statistically significant at the threshold selected to compensate for multiple comparisons. Sicca symptoms were equally distributed between the anti-SSA/Ro-positive (8/18, 44%) and negative groups (15/37, 41%).

DISCUSSION

This study summarizes the clinical and serologic features of 55 patients followed during the past 30 years who fulfilled criteria for MCTD⁷. This longitudinal analysis of a large number of patients at a single center over a period of up to 30 years provides important insights into clinical outcomes

Table 2. Comparison of clinical features in anti-SSA/Ro autoantibody-positive versus anti-SSA/Ro antibody-negative patients reveals association with photosensitivity and malar rash, but not sicca symptoms.

Clinical Characteristics	Anti-SSA/Ro Autoantibody		p*
	Percentage Positive	Percentage Negative	
Photosensitivity	78 (14/18)	30 (11/37)	0.0013
Malar rash	72 (13/18)	24 (9/37)	0.0025
Sicca symptoms	44 (8/18)	41 (15/37)	NS

* Fisher's 2 tailed exact test. NS: not significant.

of such patients. The patients' characteristics (Table 1) are similar to those previously reported from this institution and others²⁻⁴. Sicca symptoms were common among these patients, occurring in 23/55 (42%) patients (Table 1).

Autoantibodies against the SS associated antigens SSA/Ro and SSB/La were also examined. Anti-U1-RNP and anti-U1-70kD polypeptide antibodies were detected among all the patients. Anti-SSA/Ro antibodies were detected in 18/55 (32.7%) and anti-SSB/La antibodies were detected in 2/55 (3.6%) patients. The clinical features among the SSA/Ro positive versus negative groups were then compared (Table 2). The presence of anti-SSA/Ro antibodies was associated with photosensitive rashes and malar rashes. Previously, the association of anti-SSA/Ro with photosensitive rashes (including facial involvement in some studies) was well recognized to occur in SLE¹⁰⁻¹². Interestingly, the presence of anti-SSA/Ro antibodies was not associated with sicca symptoms (Table 2). Recently, Greidinger, *et al* reported that patients with lupus skin disease had higher recognition of apoptotic U1-70kD than patients without skin disease¹³. We are now examining this possibility in our patient groups.

We would consider the patients described here as having "secondary" SS, in that the primary presentation and clinically dominant spectrum of their illness was most consistent with MCTD. Further, we have extensive immunogenetics studies (including in some cases complete HLA-DR, DQ, and DP genotyping) for many of these patients. From these studies we know that the patients do not have HLA phenotypes or genotypes typical of cases classified as primary SS by other investigators (i.e., the HLA-DR3, DQ1/DQ2, and molecular subtypes of these).

Limitations of our study include the fact that data were collected retrospectively for some patients and that the original study design was not intended to characterize the full range of manifestations of SS using highly sensitive and invasive methods, such as serial labial biopsy, sialography, or salivary scintigraphy¹⁴. These limitations do not diminish the fact that this study includes a large number of MCTD patients followed up to 30 years with serial observations. Clearly, this work establishes that sicca symptoms are common in MCTD and occur at a frequency similar to that described in SLE and SSc¹. It is clear from other studies that sicca symptoms can occur in the absence of SSA/Ro and/or SSB/La autoantibodies. Further, this work shows that antibodies against SSA/Ro are common in MCTD, and their presence is associated with photosensitivity rash and malar

rash, supporting a potentially pathogenic role for these antibodies regardless of the underlying disease¹⁵.

In summary, we find that sicca symptoms are frequent in patients with MCTD, occurring in up to one third of the patients studied. Furthermore, the presence of anti-SSA/Ro antibodies identifies a group of MCTD patients with a very high incidence of malar rash and photosensitivity.

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