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Predictive value of SS-B precipitating antibodies in Sjögren's syndrome

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Abstract

As part of a screening programme several patients were identified with antibodies to the nuclear antigen SS-B. Fifteen were examined and 11 found to have Sjögren's syndrome, though this had not been suspected by most of the referring physicians. In contrast, among a group of 17 patients with overt Sjögren's syndrome, most of whom also had rheumatoid arthritis, only one had antibodies to SS-B. Patients presenting with polyarthralgia found to be SS-B positive may be likely to develop Sjögren's syndrome but unlikely to develop rheumatoid arthritis. The detection of SS-B antibodies may antedate clinical evidence of Sjögren's syndrome by months or even years.

These results emphasise the clinical heterogeneity of Sjögren's syndrome.

Introduction

Sjögren's syndrome is an autoimmune disease characterised by a pronounced infiltration of lymphocytes and plasma cells into the salivary and lacrimal glands.^{1,2} Features of its autoimmunity include the frequent presence of hypergammaglobulinaemia (50% of cases), rheumatoid factor (75-90% of cases), and anti-nuclear antibody (up to 80% of cases).² Less common findings include the presence of antibodies to DNA, salivary duct tissue, smooth muscle, mitochondria, thyroglobulin, thyroid microsomes, and gastric parietal cells.¹⁻⁴

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In the past five years it has also been shown that some patients with Sjögren's syndrome develop antibodies to the recently described nuclear antigens SS-A and SS-B.⁵ The nomenclature has become confused as other groups have identified similar antibodies but given them different names. SS-A seems to correspond to the Ro antigen described by Reichlin² and SS-B to both the so-called La and Ha antigens.^{6,7} Of the two, SS-B has been more closely associated with Sjögren's syndrome.²

Most of the reports linking SS-B antibodies with Sjögren's syndrome have concentrated on patients in whom the diagnosis was well established. We have studied a population who were known to be SS-B positive but whose accompanying diseases were not initially fully known. We were particularly interested to see whether finding antibodies to SS-B might be of predictive value so far as an underlying diagnosis of Sjögren's syndrome was concerned. Sera from patients with rheumatoid arthritis, systemic lupus erythematosus, and polyendocrine syndromes who had no evidence of Sjögren's syndrome were used as controls. In addition to assessing their sera for antibodies to SS-B we also tested for a panel of other autoantibodies.

Patients and methods

In the past two years 55 patients whose sera showed speckled anti-nuclear antibody (ANA) by indirect immunofluorescence were identified as having SS-B antibodies by counterimmunodiffusion. Fifteen of these patients agreed to undergo examination to see if the antibodies correlated with the presence of Sjögren's syndrome. This was diagnosed on the basis of their having (a) four or more of the symptoms of Sjögren's syndrome described by Anderson *et al*⁸; (b) a positive Schirmer's test result (the filter paper strips (Clement Clarke, London) being damped to less than 10 mm after challenge with smelling salts for five minutes); (c) a positive labial biopsy appearance (graded 3 or 4 according to the criteria of Chisolm and Mason⁹).

A detailed medical history of these 15 patients was obtained from their referring physicians, who also gave written permission for their patients to be studied.

Sera from a further 17 patients with Sjögren's syndrome, who had already been diagnosed in the rheumatology department at University College Hospital, were also studied. All of these patients also met the above criteria. All were women and their mean age was 64 ± (SD) 9.7 years. Twelve also had rheumatoid arthritis, one with renal tubular acidosis, one with fibrosing alveolitis, and one with hypothyroidism; one had systemic sclerosis; two had polyarthralgia of uncertain cause;

one had arthralgia and bullous pemphigoid; and one had primary Sjögren's syndrome alone.

As further controls 25 patients with systemic lupus erythematosus were studied: they each had four or more of the criteria for the classification of the disease.¹⁰ Twenty-four patients with definite or classical rheumatoid arthritis¹¹ were also reviewed. The patients in these two control groups were all examined and had no symptoms or signs (including Schirmer's test) of Sjögren's syndrome. The provisional diagnosis on the 40 patients we could not examine ourselves but who were SS-B positive are shown in table I. The information was supplied by the referring physicians. The sera from 11 patients

TABLE I—Clinical diagnoses of the 40 patients not examined during the study

Diagnosis	No of cases
Systemic lupus erythematosus (or suspected)	13
Mixed connective tissue disease (or suspected)	3
Rheumatoid arthritis (or suspected)	5
Rheumatoid arthritis and Sjögren's syndrome	1
Sjögren's syndrome	4
Other collagenoses	4
Drug eruptions	2
No diagnosis supplied	8

TABLE II—Concurrent clinical diagnoses in the 11 SS-B positive patients found to have Sjögren's syndrome

Case No	Sex	Age	Diagnosis
1	F	43	Systemic lupus erythematosus
2	F	41	Raynaud's phenomenon, arthralgia
3	M	70	Systemic lupus erythematosus
4	F	53	Intermittent arthralgia, mouth ulcers
5	F	52	Atypical collagen disease
6	F	48	Mixed connective tissue disease
7	F	51	Rheumatoid arthritis
8	M	51	Mixed connective tissue disease
9	F	65	Polymyositis, Addison's disease
10	M	59	Rheumatoid arthritis
11	M	31	Systemic lupus arthritis
Mean age 51.2 (SD) 10.4 years			

TABLE III—Results of antibody screening

	Antinuclear antibody	Extractable nuclear antibody rabbit thymus	Raised DNA binding (normal < 80 U/ml)	RAHA titre (normal < 20)	Others*
SS-B positive patients (n = 11)	11	11 SS-B	2	4	1 T
Known Sjögren's syndrome (n = 17)	9	1 SS-B, 1 unidentified	2	2	3 T 3 G 1 M/R
Rheumatoid arthritis (n = 24)	11	0	2	12	5 T 1 G 7 T
Systemic lupus erythematosus (n = 25)	25	2 SS-B, 5 RNP, 2 SM, 1 SM and RNP	19	4	2 G 1 M 2 R 8 T 5 G 2 M 2 T
Polyendocrine syndromes (n = 11)	0	0	0	0	
Normal controls	2	0	0	0	

*T = Thyroglobulin and/or thyroid microsomal antibodies. G = Gastric parietal cell antibodies. M = Mitochondrial antibodies. R = Ribosomal antibodies.

with polyendocrine syndromes and 11 normal controls were included to show that SS-B precipitins occurred only when sera showed speckled antinuclear antibodies by immunofluorescence.

DETECTION OF PRECIPITATING ANTIBODIES TO EXTRACTABLE NUCLEAR ANTIGENS

Antigen was prepared from a lyophilised acetone extract of rabbit thymus (Pel Freeze Biologicals, Rogers, Arkansas) and counter-immunoelectrophoresis was carried out as described by Kurata and Tan.¹² Precipitin reactions were seen to occur when sera contained antibodies to the antigens present in the extract. Specificities of these antibodies were indicated by the sensitivity of the precipitin reactions to enzyme or heat treatment of the antigen and confirmed by immuno-

diffusion against control sera containing antibodies to Sm, RNP, and SS-B antigens.

An extract of the Wil₂ human lymphocyte cell line was used as an antigen source for detecting anti-SS-B antibodies.¹³ Cells were recovered by low-speed centrifugation, sonicated in phosphate buffered saline and centrifuged at 50 000 g for 20 minutes. Double diffusion in agarose was carried out, sera being tested at dilutions ranging from neat to 1/64 against a reference serum containing SS-B antibodies.

Representative samples of sera from the original 55 patients were sent to Dr E M Tan at Colorado University, who confirmed the presence of antibodies to SS-B antigen in all of them.

DETECTION OF OTHER AUTOANTIBODIES

Sera were screened by the standard immunofluorescence sandwich technique using 5-μm tissue sections. Sera were tested at a dilution of 1/10 on rat liver and kidney sections and undiluted on human stomach and submaxillary tissue. Positive reactions were titred out to end-point. Commercial haemagglutination kits were used for detecting thyroglobulin and thyroid microsomal antibodies (Wellcome Reagents Ltd) and DNA antibodies (Fujizoki). Rheumatoid factor was detected by RA latex agglutination and RAHA haemagglutination (Fujizoki).

The opportunity was taken to reassess the prevalence of submaxillary duct antibody¹⁴ in Sjögren's syndrome and a variety of other autoimmune conditions. Altogether 3442 sera were evaluated. Clinical diagnoses were provided in most cases by the referring physicians.

Results

Of the 15 patients known to be SS-B positive, four had no symptoms of Sjögren's syndrome and had a normal Schirmer's test result (one had thyroiditis, one viral pneumonia and transient arthralgia, and two intermittent vague arthralgia). The concurrent diagnoses in the 11 with Sjögren's syndrome are shown in table II. Ten patients meet all three of the criteria set out in the introduction. One patient, with systemic lupus erythematosus, had six of the symptoms described by Anderson and damped the filter paper strip to just 1 mm. He refused

a labial biopsy but we thought it reasonable to diagnose him as having Sjögren's syndrome.

In only three of these 11 cases had the diagnosis of Sjögren's syndrome been suspected by the referring physician. Six of the other eight patients had developed the symptoms of dry eyes in the period (up to two years) between having the original blood test and being examined specifically for Sjögren's syndrome. The remaining two patients were uncertain about how long the symptom of dry eyes had been present.

The results of the antibody screening in these 11 patients, in the 17 with clinically overt Sjögren's syndrome, and in the other control groups is shown in table III. As expected, the prevalence of antinuclear antibody was high in the patients with collagen disorders. The "speckled" immunofluorescence pattern correlated closely with the extractable nuclear antigen precipitins.

The prevalence of other antibodies was in agreement with previous reports.

The prevalence of the submaxillary duct antibodies is shown in table IV. Among patients with Sjögren's syndrome and rheumatoid arthritis 20% and 22% respectively showed positive reactions specific to salivary ducts. These antibodies were found only in 4-7% of all other groups tested.

Only five of the 40 patients from the original SS-B positive group were suspected of having Sjögren's syndrome (table I). Six were thought to have rheumatoid arthritis, though in three cases this was suspected rather than confirmed.

TABLE IV—Prevalence of submaxillary duct-fluorescent antibodies in Sjögren's syndrome and other autoimmune conditions

	No tested	No (%) positive
Sjögren's syndrome	121	24 (20)
Rheumatoid arthritis	929	204 (22)
Systemic lupus erythematosus	227	15 (7)
Other collagen diseases	210	10 (5)
Organ specific autoimmune diseases	931	51 (5)
Mixed hospital patients, excluding those with autoimmune diseases	1024	39 (4)
Total	3442	343

Discussion

Antibodies to SS-B have been reported in up to 73% of cases of primary Sjögren's syndrome—that is, sicca complex alone—and up to 85% patients with Sjögren's syndrome and systemic lupus erythematosus.² In contrast, patients with rheumatoid arthritis and Sjögren's syndrome have generally been reported to have a much lower incidence of SS-B antibodies, perhaps 5-8%.^{15 16}

Most research groups studying these antigens have examined patients in whom the diagnosis of Sjögren's syndrome had already been established. We have considered the problem of disease associations from a different perspective. By studying patients known to have antibodies to SS-B, we have shown that the presence of this antibody can act as a predictive marker for sicca syndrome, especially when a patient presents with a polyarthralgia. We have thus extended the findings of Martinez-Lavin *et al.*¹⁷ who first showed that recognition of SS-A and SS-B antibodies may aid the diagnosis of Sjögren's syndrome in certain patients in whom the symptoms of dry eyes or mouth, or both, are either not sought by the clinician or overshadowed by disease of other organs.

Our data suggest further conclusions. When a patient presents with polyarthralgia and is found to be SS-B positive it is unlikely that he will develop rheumatoid arthritis but this test may well be a prognostic indicator for both the subsequent development of Sjögren's syndrome and a more unusual arthropathy such as systemic lupus erythematosus or mixed connective tissue disease. A positive test result for SS-B auto-antibodies may antedate the development of clinical symptoms of sicca syndrome by months or even years. These findings parallel the appearance of serological markers in diabetes¹⁸ or polyendocrine disorders,¹⁹ where organ specific antibodies to the affected organ can be detected years before the clinical expression of the disease.

Had we been allowed to examine the other 40 patients in the group originally found to have SS-B antibodies our results may have been different. This seems unlikely, however, since the information available to us about these patients (table I) appears to confirm the relative infrequency in diagnosing either Sjögren's syndrome or rheumatoid arthritis in this group.

The existence of autoantibodies, both tissue specific and non-tissue specific, in Sjögren's syndrome has been recognised for some time.^{20 21} In theory these might help to demarcate the clinical heterogeneity long known to exist among patients with Sjögren's syndrome. As our data in tables III and IV show,

however, the more commonly sought antibodies, such as those to thyroid microsomes, gastric parietal cells, and submaxillary duct tissue, are of little value. The presence of SS-B antibodies does, however, appear to provide a useful clue to the clinical subtypes occurring among patients with Sjögren's syndrome. We suggest that SS-B antibodies should be searched for in any patient presenting with polyarthralgia or thought to have a connective-tissue disorder in whom a speckled antinuclear antibody can be shown in the immunofluorescence screening test.

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