

Prevalence and clinical relevance of 52-kDa and 60-kDa Ro/SS-A autoantibodies in Japanese patients with systemic sclerosis

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Abstract

Objective—To determine the prevalence of 52-kDa and 60-kDa Ro/SS-A autoantibodies in serum samples from Japanese patients with systemic sclerosis (SSc).

Methods—Serum samples from 263 Japanese patients with SSc were examined by double immunodiffusion and enzyme linked immunosorbent assay (ELISA).

Results—By double immunodiffusion, 29 serum samples from patients with SSc were found to possess anti-Ro/SS-A antibodies. By ELISA, 31 serum samples were positive for anti-Ro52 and/or anti-Ro60. Of 27 serum samples that were positive by both methods, 15 reacted with both Ro52 and Ro60, five with Ro52 alone, and seven with Ro60 alone. Eleven of 12 patients with both anti-Ro52 and anti-Ro60 and all five patients with anti-Ro52 alone had Sjögren's syndrome, while only one of six patients with anti-Ro60 alone had this disorder.

Conclusions—Anti-Ro52 may be a serological marker for the presence of Sjögren's syndrome in anti-Ro/SS-A-positive patients with SSc.

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Sjögren's syndrome is a chronic exocrinopathy of possible autoimmune origin, characterised by keratoconjunctivitis sicca, xerostomia, and parotid gland enlargement.¹ This disease may occur as a distinct entity (primary Sjögren's syndrome) or in association with other rheumatic diseases including systemic sclerosis (SSc). In patients with SSc, the coexistence of Sjögren's syndrome has been found at high frequencies.²

Although detected most frequently in Sjögren's syndrome and systemic lupus erythematosus (SLE), anti-Ro/SS-A antibodies (anti-Ro/SS-A) are the most prevalent type of autoantibody detected during routine immunological studies of patients with various connective tissue diseases.³ Recent investigations have shown that the main components of the Ro/SS-A system are two major distinct proteins with molecular weights of 60 kDa (Ro60) and 52 kDa (Ro52).⁴ The Ro/SS-A antigen consists of a family of ribonucleoprotein (RNP) complexes, each containing one of four small RNA of the hY family, non-covalently bound to Ro60, whereas Ro52 is

now not considered to be part of the RoRNP.⁵ Patients with anti-Ro/SS-A have been found to have several types of Ro/SS-A reactivity: some have antibodies against both Ro52 and Ro60, while others have antibodies against only one of them. Furthermore, the reactivity to the Ro/SS-A proteins has been clarified to correlate with the clinical features of Sjögren's syndrome and SLE.⁶

Antibodies to Ro/SS-A antigens have been demonstrated in 15-20% of patients with SSc,⁴ and have been reported to be useful serological markers of the presence of Sjögren's syndrome.⁷ However, there have been no reports concerning the reactivity to individual Ro/SS-A antigens and its clinical significance in SSc. Thus, in this study, we analysed the prevalence of reactivity types to Ro/SS-A proteins in SSc, and investigated the possible correlations with clinical manifestations.

Methods

PATIENTS

Serum samples were obtained from 263 Japanese patients with SSc who were seen at University of Tokyo Hospital between 1984 and 1996. These patients were grouped according to the classification by LeRoy *et al*.⁸; 146 had limited cutaneous SSc (lcSSc) and 117 had diffuse cutaneous SSc (dcSSc). All of the patients with dcSSc and 107 of the patients with lcSSc fulfilled the criteria proposed by the American College of Rheumatology (ACR).⁹ The remaining 39 patients with lcSSc who did not meet the criteria had sclerodactyly and at least two other features of the CREST syndrome (calcinosis, Raynaud's phenomenon, oesophageal dysmotility, telangiectasia). Serum samples from 10 patients with non-SSc primary Sjögren's syndrome were used as controls.

Clinical manifestations and laboratory findings of each patient were obtained from the medical records. Organ system involvements were defined as described by Steen *et al*¹⁰ with some modifications¹¹: lung = bibasilar fibrosis on chest radiograph; oesophagus = hypomotility shown by barium radiography; heart = pericarditis, congestive heart failure, or arrhythmias requiring treatment; joint = inflammatory polyarthralgias or arthritis; kidney = malignant hypertension and/or progressive renal failure. Sjögren's syndrome was diagnosed according to the criteria proposed by the Research Committee on Sjögren's Syndrome of the Ministry

of Health and Welfare of the Japanese Government¹² and by the European Community.¹³ SLE was diagnosed according to the criteria proposed by ACR.¹⁴

DOUBLE IMMUNODIFFUSION

Antibodies against Ro/SS-A antigen were detected using double immunodiffusion in 0.5% agarose as described previously.^{15,16} Porcine spleen extract was used as an antigen source.

ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA) FOR ANTIBODIES TO RO52 AND RO60 PROTEINS

The concentrations of antibody to Ro52 (anti-Ro52) and antibody to Ro60 (anti-Ro60) were determined by a commercially available IgG isotype specific ELISA method (MBL, Aichi, Japan), according to the manufacturer's instructions. Briefly, recombinant proteins of Ro52 or Ro60 were precoated onto microtitre wells. Serum samples (diluted 1:200) were added to each well. All serum samples were tested in duplicate. The bound antibodies were detected with peroxidase conjugated monoclonal antihuman γ chain antibodies, using tetramethylbenzidine and hydrogen peroxide as the substrate. Anti-Ro52 and -Ro60 activities were shown with an index by reference standard samples. Absorbance values greater than 20 index (anti-Ro52) and 4 index (anti-Ro60) were considered to be positive.

STATISTICS

Fisher's exact probability test was performed to analyse frequencies. *p* Values less than 0.05 were considered significant.

Results

DIAGNOSIS OF SJÖGREN'S SYNDROME

Of 263 patients with SSc, 102 had been examined for the presence of Sjögren's syndrome. According to the Japanese criteria,¹² 66 (65%) were diagnosed as having definite Sjögren's syndrome. On the other hand, by the European criteria,¹³ 37 (36%) were classified into definite secondary Sjögren's syndrome, and 29 (28%) into probable secondary Sjögren's syndrome.

Table 1 Clinical association of antibodies to 52-Kda (Ro52) and 60-Kda(Ro60) Ro/SS-A proteins in patients with SSc

	Reactivity to Ro/SS-A antigens			Ro/SS-A antigen negative
	Ro52 and Ro60	Ro52 alone	Ro60 alone	
Number	15	5	7	236
Clinical features				
Diffuse:limited	5:10	3:2	3:4	135:101
Raynaud's phenomenon	14/15	4/5	7/7	213/236
Pitting scars	5/15	3/5	4/7	141/236
Telangiectasia	5/11	2/4	3/5	152/222
Internal involvements				
Oesophagus	9/12	2/4	5/5	99/172
Lungs	7/14	2/5	5/7	89/212
Heart	1/14	0/7	0/7	14/210
Joints	6/13	1/5	3/7	96/198
Kidneys	1/15	0/5	0/7	9/236
Coexistence of other connective tissue diseases				
Sjogren's syndrome	11/12	5/5	1/6**	49/79
Systemic lupus erythematosus	1/15	0/5	2/7	9/236

** *p* < 0.01, compared with patients with Ro52 and Ro60, and those with Ro52 alone, respectively.

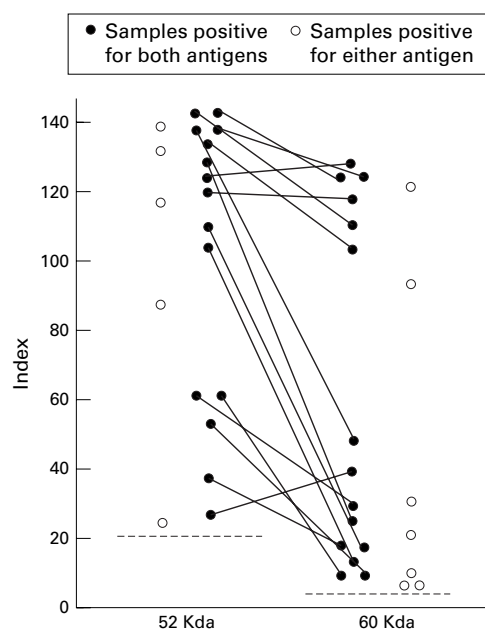


Figure 1 Levels of antibodies to 52-kDa and 60-kDa Ro/SS-A proteins in patients with systemic sclerosis determined by ELISA. Horizontal dotted lines show cut off values.

The Japanese criteria were used in the following analyses.

FREQUENCY OF ANTI-RO/SS-A ANTIBODIES IN SSC

Of 263 serum samples from patients with SSc, 29 were shown to be positive for anti-Ro/SS-A by double immunodiffusion, while, by ELISA, 31 reacted with Ro52 or Ro60, or both. Among them, 27 patients were positive by both methods and were considered to have anti-Ro/SS-A antibodies in the following analyses. The remaining patients who were positive by only one of the methods all showed low titres, and were excluded. All 10 patients with primary Sjögren's syndrome were positive by both methods. The incidence of anti-Ro/SS-A was almost identical in patients with dcSSc (11 of 117, 9%) and those with lcSSc (16 of 146, 11%). The patients who also had anti-U1RNP antibody (anti-U1RNP) showed a significantly higher frequency of positive anti-Ro/SS-A (8 of 31, 21%), compared with those who also had anti-topoisomerase I antibody (anti-topo I) (9/103, 9%) or anticentromere antibody (ACA) (5 of 70, 7%). Sjögren's syndrome was found in 15 of 23 (65%) of patients positive for anti-Ro/SS-A, which was equal to the incidence in those who were negative (51 of 79, 65%).

REACTIVITY TO RO52 AND RO60 RO/SS-A PROTEINS DETERMINED BY ELISA

In the 27 patients who were considered to have anti-Ro/SS-A antibodies, 15 had antibodies against both Ro52 and Ro60, five had anti-Ro52 alone, and seven had anti-Ro60 alone by ELISA (table 1). Figure 1 shows the levels of anti-Ro52 and anti-Ro60. In both antibodies, the values exhibited rather bipolar distribution, and could be divided into a high titre and a low titre group (> 90 index: high titre; < 65 index, low titre). Among the 10 patients with primary

Table 2 Correlation of the positivity and levels of antibodies to 52-Kda (Ro52) and 60-Kda (Ro60) Ro/SS-A proteins with other autoantibodies in patients with SSc

Positivity	Ro52 and Ro60		Ro52 alone	Ro60 alone
Anti-Ro/SS-A alone (n=6)	3		3	0
+ Anti-topoisomerase I (n=9)	4		1	4
+ Anti-centromere (n=5)	3		0	2
+ Anti-U1RNP (n=8)	5		1	2
+ Anti-La/SS-B (n=8)	4		1	3
Antibody levels				
High	Ro52 and Ro60	Ro52	Ro60	None
Low or negative	None	Ro60	Ro52	Ro52 and Ro60
Anti-Ro/SS-A alone	0	4	0	2
+ Anti-topoisomerase I	3	2	1	3
+ Anti-centromere	0	0	1	4
+ Anti-U1RNP	4	1	1	2
+ Anti-La/SS-B	3	2	0	3

Values are numbers of patients.

Sjögren's syndrome, six had anti-Ro52 and anti-Ro60, and the other four had anti-Ro52 alone.

CLINICAL ASSOCIATION

Table 1 shows the association of antibodies to Ro52 and Ro60 with the clinical features of SSc. There were no significant differences in the incidence of features between patients with or without Ro/SS-A antigens. However, the patients with antibodies to both proteins and those with anti-Ro52 alone had Sjögren's syndrome at a high incidence (11 of 12 and 5 of 5, respectively), while those with anti-Ro60 alone had this disorder at a significantly lower incidence (one of six, $p < 0.01$). No significant association was found between the presence of Sjögren's syndrome and other autoantibodies coexisting with anti-Ro/SS-A. Nor did the presence of Sjögren's syndrome show any correlation with high or low titres of anti-Ro52 or anti-Ro60 (data not shown).

CORRELATION OF ANTI-RO52 AND ANTI-RO60 WITH OTHER AUTOANTIBODIES

Table 2 shows the correlations of the positivity and levels of anti-Ro52 and anti-Ro60 with the presence of other autoantibodies. All patients with high titres of both anti-Ro52 and anti-Ro60 had anti-topo I and/or anti-U1RNP. In contrast, all patients positive for ACA had low titres of anti-Ro52 and anti-Ro60, except for only one patient with a high titre of anti-Ro60. Additionally, no patients with anti-Ro/SS-A alone were found to be positive for anti-Ro60 alone; most of these patients (four of six) had a high titre of anti-Ro52 with a low titre of anti-Ro60 or with no anti-Ro60.

Discussion

In earlier studies of SSc,^{17,18} the coexistence of Sjögren's syndrome was reported as 1–2% of all cases. After Ararcón-Segovia *et al*² described a high frequency of this coexistence, the relation of these disorders has become widely appreciated. However, because of the lack of well defined criteria for the diagnosis of Sjögren's syndrome, reports of its prevalence in SSc have ranged from 17% to 90%.^{2,7,19–21} In our series of Japanese patients with SSc, Sjögren's syndrome was found in 66 of 102 (65%).

Anti-Ro/SS-A was demonstrated in 27 of 263 (10%) of our patients with SSc, but there

was no significant difference in the prevalence of Sjögren's syndrome between those with and without anti-Ro/SS-A. Osial *et al*⁶ reported that Sjögren's syndrome was found in 29% of patients with SSc and that anti-Ro/SS-A and anti-La/SS-B are useful serological markers for the presence of Sjögren's syndrome in patients with SSc. Thus, our results are contradictory to theirs. This may be because of one or more of the following reasons. Firstly, the difference in diagnostic criteria because the incidence of Sjögren's syndrome is considerably different between their study and ours (29% *v* 65%). While Osial *et al* evaluated the presence of Sjögren's syndrome by minor salivary gland biopsy, our criteria depended largely on the clinical findings of dry eyes and dry mouth. Secondly, biases may exist in our examination for the presence of Sjögren's syndrome. We were able to retrospectively confirm whether Sjögren's syndrome was present in 102 of 263 patients based on medical charts. Although we routinely try to screen for the presence of Sjögren's syndrome, the patients who had clinical symptoms of Sjögren's syndrome might have been likely to have further examinations. Finally, racial differences might be an important factor that would explain the differences, as anti-Ro/SS-A has been known to be related with specific human leucocyte antigens.²² However, there have been no other reports describing the prevalence of Sjögren's syndrome in Asian patients with SSc. Our results show that the coexistence of Sjögren's syndrome may not be simply associated with anti-Ro/SS-A in SSc.

Ben-Chetrit *et al*⁶ reported that anti-Ro52 alone (without concomitant anti-Ro60) was detected only in serum samples from primary Sjögren's syndrome patients, whereas anti-Ro60 alone was apparently confined to patients with SLE. In contrast with primary Sjögren's syndrome and SLE, reactivity to Ro52 and Ro60 in SSc was heterogeneous in this study; anti-Ro/SS-A positive serum samples were found to have a variety of types of Ro/SS-A reactivity. It is of interest that the coexistence of Sjögren's syndrome was observed at a high incidence in patients with both anti-Ro52 and anti-Ro60 and in those with anti-Ro52 alone, while only one of six patients positive for anti-Ro60 alone also had Sjögren's syndrome. Therefore, not Ro60 but Ro52 is a major autoantigen in anti-Ro/SS-A positive SSc patients who have Sjögren's syndrome. As mentioned above, the concurrence of Sjögren's syndrome with SSc is not simply associated with anti-Ro/SS-A in SSc; however, among anti-Ro/SS-A positive patients, anti-Ro52 may be a serological marker for the presence of Sjögren's syndrome. In addition, our findings in SSc may be compatible with those of Ben-Chetrit *et al* in primary Sjögren's syndrome and SLE,⁶ because anti-Ro52 is related to the presence of Sjögren's syndrome in both conditions.

Rader *et al*²³ reported that there are qualitative and quantitative differences associated with the presence of other autoantibodies in the specificity of anti-Ro/SS-A serum samples. In our study, a tendency of different reactivities

was observed according to the coexisting autoantibodies, although there were no significant differences, probably because of the small number of patients. Anti-Ro/SS-A was more common in serum samples with anti-U1RNP compared with those with anti-topo I or ACA. Additionally, the patients with anti-U1RNP or anti-topo I showed high titres of both anti-Ro52 and anti-Ro60, whereas those with anti-Ro/SS-A alone showed a high titre of anti-Ro52 alone. Drosos *et al*²⁰ reported that virtually no patients with CREST with Sjögren's syndrome had antibodies to Ro/SS-A. In our study, anti-Ro/SS-A was positive in only 7% of patients with ACA, who moreover showed low antibody levels. Therefore, it may be concluded that ACA and anti-Ro/SS-A are largely dissociated in patients with SSc.

To conclude, the most striking finding in this study is the association of anti-Ro52 with the presence of Sjögren's syndrome in anti-Ro/SS-A-positive patients with SSc. Although no evidence has been presented that anti-Ro/SS-A participates in the pathogenesis of SSc, it may be useful to determine the exact antigen specificity of anti-Ro/SS-A, to clarify how anti-Ro/SS-A are related to the disease manifestations of SSc and Sjögren's syndrome.

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