The Prevalence and Clinical Significance of Anti-U1 RNA Antibodies in Patients with Systemic Sclerosis

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We studied the prevalence and clinical significance of anti-U1 RNA antibodies in patients with systemic sclerosis. The presence of anti-U1 RNA antibodies was determined using immunoprecipitation in systemic sclerosis patients with anti-U1 RNP antibodies (n = 36), antitopoisomerase 1 antibodies (n = 20), or anticentromere antibodies (n = 20), mixed connective tissue disease patients (n = 23), systemic lupus erythematosus patients with anti-U1 RNP antibodies (n = 26), and normal controls (n = 20). Moreover, antigen specificities for anti-U1 RNP antibodies were examined in patients with systemic sclerosis by immunoblotting and enzyme-linked immunosorbent assay. Anti-U1 RNA antibodies was detected in 22 of 36 systemic sclerosis patients (61%) with anti-U1 RNP antibodies, 14 of 23 patients (61%) with mixed connective tissue disease, and eight of 26 systemic lupus erythematosus patients (31%) with anti-U1 RNP antibodies. Anti-U1 RNA antibodies were not detected in other groups. As for systemic sclerosis patients, the frequencies of pulmonary fibrosis and reduced percentage diffusion capacity for carbon monoxide were significantly greater in patients with anti-U1 RNA antibodies than in those without (76% vs 18%, p < 0.005; 82% vs 27%, p < 0.005, respectively). Moreover, patients with anti-U1 RNA antibodies had significantly lower percentage diffusion capacity for carbon monoxide and percentage vital capacity values than those without (51.9 ± 16.8 vs 79.4 ± 16.4, p < 0.001; 83.8 ± 21.4 vs 101.4 ± 12.9, p < 0.05, respectively). Regarding the antigen specificities of anti-U1 RNP antibodies in systemic sclerosis patients, the frequency of anti-70 kDa antibodies determined by immunoblotting was significantly higher in patients with anti-U1 RNA antibodies than in those without (77% vs 43%, p < 0.05). This finding was also confirmed by enzyme-linked immunosorbent assay for anti-70 kDa antibodies (86% vs 43%, p < 0.05). These results indicate that anti-U1 RNA antibodies may be a serologic indicator for pulmonary fibrosis in systemic sclerosis patients with anti-U1 RNP antibodies. Key words: anti-U1 RNP antibody/pulmonary fibrosis/scleroderma. J Invest Dermatol 120:204–210, 2003
MATERIALS AND METHODS

Patients Serum samples were obtained from 125 systemic rheumatic disease patients first evaluated by rheumatologists in our division during 1990–99. 36 patients with SSC or SSc overlap syndrome positive for anti-U1 RNP by immunodiffusion [30 patients with SSC, two patients with SSc and SLE, and four patients with SSc, SLE, and polymyositis (PM)], 20 SSc patients with antitopoisomerase-I antibodies negative for anti-U1 RNP, 20 SSc patients with antitopoisomerase-I antibodies positive for anti-U1 RNP, 23 patients with MCTD, and 26 SLE patients positive for anti-U1 RNP by immunodiffusion. SSc patients with antitopoisomerase-I antibodies and antitrycerebellum antibodies, MCTD patients, and SLE patients with anti-U1 RNP were randomly selected. Three patients who had been treated with corticosteroids or immunosuppressives were excluded. The diagnosis was previously associated with previous reports indicating that these treatments might improve the skin and internal involvements. As normal controls, 20 serum samples were also obtained from healthy controls. Informed consent was obtained from all subjects. Of 36 patients with SSC or SSc overlap syndrome positive for anti-U1 RNP, seven were positive for antitopoisomerase-I antibodies and one patient was positive for antitrycerebellum antibodies. The patients with SSc were grouped according to the classification system proposed by Okano and Medsger (1990); 49 patients with limited cutaneous SSC (lCSSc), and 27 patients with diffuse cutaneous SSc (dcSSc). All patients with dcSSc and 44 with lCSSc fulfilled the criteria proposed by the American College of Rheumatology (formerly, the American Rheumatism Association) (Subcommittee for Scleroderma Criteria of the American Rheumatism Association Diagnostic Criteria Committee, 1980). All patients with lCSSc who did not meet these criteria had sclerodactyly and at least two other features of theCREST syndrome (calcinosis, Raynaud’s phenomenon, esophageal dysmotility, telangiectasia). For a diagnosis of MCTD, the criteria proposed by Alarcon-Segovia and Cardiel (1989) were used. In this study, patients with MCTD had anti-U1 RNP and clinical features of SLE, SSc, and PM, but did not satisfy the criteria for other connective tissue diseases, such as SLE, SSc, or PM/dermatomyositis (Bohan and Peter, 1975). Patients with SLE met the American Rheumatism Association criteria for definite SLE (Tan et al, 1982). Patients who met two or more diagnostic criteria for these connective tissue diseases were distinguished from MCTD and diagnosed with overlap syndrome. Aliquots of sera were frozen at −80°C until assayed.

Clinical assessment The clinical and laboratory data reported in this study were obtained at the time the blood samples were drawn. Patients with SSc were evaluated for the presence of gastrointestinal, pulmonary, cardiac, renal or joint involvement, as follows. Esophagus hypomotility was defined as distal esophageal hypomotility on barium-contrast radiographs and/or clinical symptoms consistent with esophageal motility disorders. Cardiac involvement was defined as symptomatic pericarditis, clinical evidence of left ventricular congestive heart failure, or arrhythmias requiring treatment. Renal involvement was defined as malignant hypertension and/or rapidly progressive renal failure. Skeletal muscle involvement was defined as proximal muscle weakness and elevated serum creatine kinase levels, plus abnormal electromyographic findings consistent with myopathy and/or histopathologic changes in inflammatory myopathy. Joint involvement was defined as inflammatory polyarthritis or arthritis. Disease onset was defined as the date of the first appearance of Raynaud’s phenomenon. Duration of disease was defined as the interval between the onset and the first physician diagnosis. All SSc patients were followed through June 2000.

Analysis of antinuclear antibodies Antinuclear antibodies were detected via immunofluorescence using HEp-2 cells as a substrate, as described previously (Takehara et al, 1983; Ihn et al, 1996).

Double immunodiffusion Antibodies against U1 RNP and topoisomerase-I antigens were detected using double immunodiffusion in 0.5% agarose, as described previously (Fujimoto et al, 1997).

Immunoprecipitation Immunoprecipitation using HeLa cell extracts was performed. A total of 10 μl of each patient’s sera was mixed with 2 μg of protein A–Agarose CL-4B (Pharmacia Biotech, Uppsala, Sweden) in 500 μl of an IPP buffer (20 mM Tris–HCl, pH 8.0, 500 mM NaCl, 0.1% Nonidet P-40) and incubated with rotation overnight at 4°C. The IgG-coated agarose was washed three times in 750 μl of an IPP buffer using 15 s spins in a microfuge tube, and then resuspended in 500 μl of NET-2 buffer (50 mM Tris–HCl, pH 8.0, 150 mM NaCl, 0.05% Nonidet P-40).

For analysis of antibodies against nucleosomes, this suspension was incubated with 200 μl of an extract of protein-free naked RNA, which was prepared from 3 × 106 cells by deproteinization with phenol/ chloroform/isoamyl alcohol (50 : 50 : 1; containing 0.1% 8-hydroxyquinolone), on a rotator for 2 h at 4°C. The Ag-bound nucleosome was then collected via a 15 s centrifugation in a microfuge, washed three times with NET-2 buffer, and resuspended in 300 μl of NET-2 buffer. To extract bound RNAs, 30 μl of 50 M sodium acetate, 30 μl of 10% sodium dodecyl sulfate (SDS), 1 μl of glycogen, and 300 μl of phenol/chloroform/ isoamyl alcohol were added to the agarose beads. After agitation in a Vortex mixer and spinning in a microfuge for 3 min, RNAs were recovered in the aqueous phase, precipitated with ethanol, and dissolved in 20 μl of an electrophoresis sample buffer composed of 10 M urea, 0.025 M Tris–HCl, pH 8.0, 6% sodium dodecyl sulfate (SDS), 0.1% Nonidet P-40, and 0.1 M ethylenediaminetetraacetic acid (EDTA).

Preparation of nuclear extracts for immunoblotting Nuclear extracts were prepared as described previously (Habets et al, 1983; Ihn et al, 1997). Briefly, confluent HeLa cells from two 150-mm dishes were washed with ice-cold phosphate-buffered saline (10 mM HEPES–KOH pH 7.9, 1.5 mM MgCl2, 10 mM KCl, 1 mM dithiothreitol, 0.2 mM phenylmethylsulfonfluoride (PMSF)) and tri-chloroacetic acid (100 μg/ml). The cells were harvested and washed for 10 min and then resuspended in ice-cold nuclear extraction buffer (90 mM Tris–HCl, pH 8.6, 90 mM borate, 1 mM ethylenediaminetetraacetic acid (EDTA)). The RNA samples were denatured at 65°C for 5 min, resolved in a 7 M urea–10% polyacrylamide gel, and stained with silver (Bio-Rad Laboratories, Hercules, CA). In certain experiments, for analysis of antibodies against protein–RNA complex, the same procedures were performed using an extract of RNA–protein complex, which was prepared from 3 × 106 cells without deproteinization.

Immunoblotting Immunoblotting was performed as described previously (Sato et al, 1994; Ihn and Trojanowska, 1997). Briefly, nuclear antigens from HeLa cells were subjected to electrophoresis on 4%–20% gradient SDS–polyacrylamide slab gels, and then electrotransferred from the gels onto nitrocellulose sheets. The nitrocellulose sheets were then immersed in 1% skim milk in TBS (10 mM Tris–HCl, pH 7.5, 0.15 M NaCl) for 1 h at 25°C. Bound antibodies were detected with an alkaline-phosphatase-conjugated goat antihuman IgG antibody, and color was developed with nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate.

Enzyme-linked immunosorbent assay (ELISA) for anti-70 kDa antibodies A specific ELISA kit for anti-70 kDa antibodies (MBL, Aichi, Japan) was used to determine the prevalence of anti-70 kDa antibodies in SSC or SSc overlap syndrome patients with anti-U1 RNP. This ELISA kit was prepared as described previously with minor modification (Habets et al, 1989; ter Borg et al, 1991). In brief, the insoluble β-galactosidase–70 kDa fusion protein produced by MBL was solubilized in 6 M urea and diluted (1:100) in coating buffer (15 mM Na2CO3, 35 mM NaHCO3, pH 9.6). One hundred microliters of fusion protein diluted in coating buffer were brought into each well of microtiter plates and allowed to coat overnight at 4°C. Plates were washed five times with PBST (PBS + 0.05% Tween 20) and remaining binding sites were blocked with 1% bovine serum albumin in PBST for 2 h at room temperature. Plates were washed again five times with PBST.

According to the manufacturer’s instructions, 36 sera from SSC or SSc overlap syndrome patients with anti-U1 RNP were measured. As normal controls, 20 sera from the healthy population used in immunoprecipitation were also measured. In brief, a microtiter plate prepared as described above was incubated with 100 μl of 201-fold diluted serum at room temperature for 1 h. Then, the plate was washed and incubated at room temperature for 1 h with 100 μl of 80-fold diluted horseradish peroxidase-conjugated antihuman IgG. Next, the plate was washed again, 100 μl of tetramethylbenzene was added, and incubation was performed at room temperature for 30 min. Finally, 2 mmol per 1 NaOH was added to terminate the peroxidase reaction and the absorbance at 450 nm was measured. Sera with optical density (OD) 450 nm values more than 2SD greater than the mean value in the normal controls were regarded as positive for anti-70 kDa antibodies.
Prevalence of anti-U1 RNA Figure 1 shows representative results of immunoprecipitation. RNP immunoprecipitation was performed using an extract of RNA–protein complex, whereas RNA immunoprecipitation was performed using an extract of protein-free naked RNA. Sera with U1 RNA in both RNP and RNA immunoprecipitation were regarded as positive for anti-U1 RNP and anti-U1 RNA (Fig 1, lanes 4, 5). Sera with U1 RNA only in RNP immunoprecipitation were regarded as sera with anti-U1 RNP negative for anti-U1 RNA (Fig 1, lanes 6, 7). As shown in Fig 1(b), many sera from SSc or SSc overlap syndrome patients with anti-U1 RNP revealed U1 RNA in RNA immunoprecipitation. Results are summarized in Table I. Anti-U1 RNA were present in 22 of 36 patients (61%) with SSc or SSc overlap syndrome positive for anti-U1 RNP, 17 of 30 patients (57%) with SSc positive for anti-U1 RNP, 14 of 23 patients (61%) with MCTD, and eight of 26 patients (31%) with SLE positive for anti-U1 RNP. There were significant differences between the SLE patient group and the other three groups described above (p < 0.05, respectively). There were no cases with anti-U1 RNA in 20 SSc patients positive for antitopoisomerase I antibodies, 20 SSc patients positive for anticientromere antibodies, and 20 normal controls.

Antigen specificity for anti-U1 RNP Figure 2 shows representative results of immunoblotting and these results are summarized in Table II. In immunoblotting, anti-70 kDa antibodies, anti-A protein antibodies, and anti-C protein antibodies were detected in 23 of 36 patients (64%), 30 of 36 patients (83%), and four of 36 patients (11%) respectively with SSc or SSc overlap syndrome positive for anti-U1 RNP.

As described above, there is a significant difference in the presence of anti-70 kDa antibodies between sera from SSc or SSc overlap syndrome patients with anti-U1 RNA and those without. To confirm this finding, we also performed ELISA for anti-70 kDa antibodies using sera from SSc or SSc overlap syndrome patients with anti-U1 RNA and those without. The cut-off value (mean + 2SD) was set at 0.50, based on data of the 20 healthy control sera. As shown in Fig 3, there was a significant difference in the frequencies of anti-70 kDa antibodies between patients with anti-U1 RNA and those without (86% vs 43%, p < 0.005). Excluding six patients with overlap syndrome (shown as open circles and crosses in Fig 3), the frequency of anti-70 kDa antibodies was again significantly higher in patients with anti-U1 RNA than in those without (82% vs 38%, p < 0.005). All of 23 sera that reacted with the 70 kDa protein in immunoblotting were also positive for anti-70 kDa antibodies in ELISA. Of 13 sera without anti-70 kDa in immunoblotting, two sera were positive and the remaining 11 sera were negative for anti-70 kDa antibodies in ELISA. These results indicate that ELISA is a more sensitive technique for the detection of anti-70 kDa antibodies than immunoblotting.

Clinical and serologic correlations The clinical and serologic features of SSc or SSc overlap syndrome patients with or without anti-U1 RNA are shown in Table III. To focus on the significance of anti-U1 RNA, SSc or SSc overlap syndrome patients with antitopoisomerase I antibodies or anticientromere antibodies were excluded. There was no significant difference between these groups in terms of sex. Age of onset in patients...
Table I. Frequencies of anti-U1 RNA antibodies (anti-U1 RNA) in patients with systemic sclerosis (SSc) or SSc overlap syndrome, systemic lupus erythematosus (SLE), and mixed connective tissue disease (MCTD), detected by immunoprecipitation

<table>
<thead>
<tr>
<th>Antibody Type</th>
<th>Frequency (%)</th>
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<tbody>
<tr>
<td>SSc or SSc overlap syndrome patients with anti-U1 RNP (n = 36)</td>
<td>22 (61)</td>
</tr>
<tr>
<td>SSc patients with anti-U1 RNP (n = 30)</td>
<td>17 (57)</td>
</tr>
<tr>
<td>SSc patients with anti-topo I negative for anti-U1 RNP (n = 20)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>SSc patients with ACA negative for anti-U1 RNP (n = 20)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>SLE patients with anti-U1 RNP (n = 26)</td>
<td>8 (31)</td>
</tr>
<tr>
<td>MCTD patients (n = 23)</td>
<td>14 (64)</td>
</tr>
<tr>
<td>Normal controls (n = 20)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Unless indicated otherwise, values are numbers.

anti-U1 RNP, anti-U1 RNP anti-body; anti-topo I, anti-topoisomerase I antibody; ACA, anti-centromere antibody.

Figure 2. Immunoblot analysis of sera from SSc patients with anti-U1 RNP antibodies positive for anti-U1 RNA antibodies (a) and patients with anti-U1 RNP antibodies negative for anti-U1 RNA antibodies (b). Nitrocellulose blot strips contained HeLa nuclear extracts transferred after separation on 4%–20% gradient SDS–polyacrylamide gels. Each strip was incubated with the patient’s serum at a 1 : 100 dilution and bound antibodies were detected with an alkaline-phosphatase-conjugated goat anti-human IgG antibody; color was developed with nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate.

with anti-U1 RNA was significantly higher than in those without (43.3 ± 11.3 y vs 33.3 ± 7.9 y, p < 0.005) and disease duration prior to diagnosis in patients with anti-U1 RNA was significantly shorter than in those without (4.6 ± 3.9 y vs 12.6 ± 7.1 y, p < 0.005). There was no significant difference in the frequency of dcSSc between patients with anti-U1 RNA and those without. Though there were no clinically significant differences, neither calcinosis nor renal disease was detected in SSc patients with anti-U1 RNA. The frequencies of pulmonary fibrosis (PF) and reduced percentage diffusion capacity for carbon monoxide were significantly greater in patients with anti-U1 RNA than in those without (76% vs 18%, p < 0.005; 82% vs 27%, p < 0.005, respectively). The frequency of reduced percentage vital capacity was also greater in patients with anti-U1 RNA than in those without (41% vs 18%), but there was no significant difference. Moreover, patients with anti-U1 RNA had significantly lower percentage diffusion capacity for carbon monoxide values and percentage vital capacity values than those without (51.9 ± 16.8 vs 79.4 ± 16.4, p < 0.01; 83.8 ± 21.4 vs 101.4 ± 12.9, p < 0.05, respectively).

To further confirm the significance of anti-U1 RNA, we investigated the prevalence of PF in SSc patients with antitopoisomerase-I antibodies, anticentromere antibodies, or anti-U1 RNA. The prevalence of PF in SSc patients with antitopoisomerase-I antibodies is significantly elevated compared with those without (78% vs 38%, p < 0.005). The prevalence of PF in SSc patients with anticentromere antibodies is significantly decreased compared with those without (9% vs 67%, p < 0.0005). These results are consistent with previous reports (Steen et al., 1988). The prevalence of PF in patients with U1 RNA was also elevated compared with those without (80% vs 39%, p < 0.05).

We also investigated the association of anti-U1 RNA with PF in patients with MCTD. The prevalence of PF in MCTD patients with anti-U1 RNA was elevated compared with those without (50% vs 30%), but there was no significant difference. Regarding SLE, no patients had interstitial pulmonary involvement.

**DISCUSSION**

This study was a comprehensive analysis of the distribution of anti-U1 RNA in patients with connective tissue disease. Anti-U1 RNA was detected in 44 of 85 patients (52%) with anti-U1 RNP, including 22 of 36 patients (61%) with SSc or SSc overlap syndrome, 14 of 23 patients (61%) with MCTD, and eight of 26 patients (31%) with SLE. No cases with anti-U1 RNA were detected in SSc patients with antitopoisomerase-I antibodies or anticentromere antibodies negative for anti-U1 RNP, and in normal controls. These results are consistent with previous reports that anti-U1 RNA was always accompanied by anti-U1 RNP and was detected in about 45%–60% of connective tissue disease patients with anti-U1 RNP, about 30% of SLE patients with anti-U1 RNP, and about 60% of MCTD patients (van Venrooij et al., 1995; Hoffman et al., 1995). Previous reports suggested that anti-U1 RNA was confined to sera from patients with SLE or SLE overlap syndrome, but was rarely found in sera from patients with other connective tissue diseases (Wilusz and Keene, 1986; van Venrooij et al., 1990; Hoffman et al., 1995). In this study, excluding six patients with overlap syndrome, however, 17 of 30 sera samples (57%) from SSc patients with anti-U1 RNP were positive for anti-U1 RNA. This result suggests that the presence of anti-U1 RNA in sera from patients with SSc is not rare. To our knowledge, this is the first report indicating the prevalence of anti-U1 RNA in SSc patients and the prevalence of this antibody in other ethnic groups remains to be confirmed. The frequencies of different autoantibodies in patients with SSc show considerable variation among different ethnic groups (McNeilage et al., 1989; Reveille et al., 1992; Kuwana et al., 1994). Multiple factors linked to ethnicity, including genetic and environmental factors, appear to influence autoantibody status in SSc (Kuwana et al., 1999). More information from different ethnic groups would give insight into the exact prevalence of anti-U1 RNA in patients with SSc.
This study and previous studies indicated that anti-U1 RNA was always accompanied by anti-U1 RNP. These results strengthen the hypothesis that the induction of an immune response to one component of an autoantigenic (U1) snRNP complex, such as 70K, can induce the diversification of autoantibodies to other components of the (U1) snRNP complex, including U1 RNA. Recent reports have suggested that predictable, orderly patterns of emergence of anti-U1 RNP responses occur in humans as well as animal models of autoimmune diseases (James et al., 1995).

Greidinger and Hoffman (2001) reported that 70K is predominant in early RNP antigens and immunity to A and C develops most often as a consequence of spreading. Our finding that the prevalence of anti-70 kDa antibodies is significantly increased in SSc patients with anti-U1 RNA is consistent with this theory.

We previously reported that anti-U1 RNP was closely correlated with PF and joint involvement in patients with SSc (Ihn et al., 1999). In this study, we showed that the presence of anti-U1 RNA was closely correlated with PF in patients with SSc or SSc overlap syndrome positive for anti-U1 RNP. Regarding joint involvement, there was no significant correlation with the presence of anti-U1 RNA. These results indicate that anti-U1 RNA may serve as a useful serologic indicator of PF in SSc patients with anti-U1 RNP. We also investigated the association of anti-U1 RNA with PF in all SSc patients. The prevalence of PF in patients with anti-U1 RNA was also elevated compared with those without (80% vs 39%, p < 0.05). These results suggest that anti-U1 RNA also serve as a marker of PF in all SSc patients.

Table II. Antigen specificity of anti-U1 RNP antibodies (anti-U1 RNP) determined by immunoblotting in patients with systemic sclerosis (SSc) or SSc overlap syndrome positive or negative for anti-U1 RNA antibodies (anti-U1 RNA)

<table>
<thead>
<tr>
<th>Antigens</th>
<th>(n=36)</th>
<th>(n=22)</th>
<th>(n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>70 kDa (%)</td>
<td>23 (64)</td>
<td>17 (77)*</td>
<td>6 (43)*</td>
</tr>
<tr>
<td>A (%)</td>
<td>30 (83)</td>
<td>19 (86)</td>
<td>11 (79)</td>
</tr>
<tr>
<td>C (%)</td>
<td>4 (11)</td>
<td>2 (9)</td>
<td>2 (14)</td>
</tr>
</tbody>
</table>

Unless indicated otherwise, values are numbers.
A, anti-A protein antibody; C, anti-C protein antibody.
* p < 0.05.

Table III. Correlation of anti-U1 RNA with clinical features in 28 patients with SSc

<table>
<thead>
<tr>
<th>Clinical features</th>
<th>Patients with anti-U1 RNA</th>
<th>Patients without anti-U1 RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>1 : 16</td>
<td>0 : 11</td>
</tr>
<tr>
<td>Age of onset (y)</td>
<td>43.3±11.3*</td>
<td>33.3±7.9*</td>
</tr>
<tr>
<td>Duration of disease (y)</td>
<td>4.6±3.9*</td>
<td>12.6±7.1*</td>
</tr>
<tr>
<td>Skin sclerosis (lcSSc : dcSSc)</td>
<td>13 : 4</td>
<td>11 : 1</td>
</tr>
<tr>
<td>Pitting scars/ulcers</td>
<td>41 (7/17)</td>
<td>45 (5/11)</td>
</tr>
<tr>
<td>Short sublingual frenulum</td>
<td>53 (9/17)</td>
<td>45 (5/11)</td>
</tr>
<tr>
<td>Contracture of phalanges</td>
<td>41 (7/17)</td>
<td>36 (4/11)</td>
</tr>
<tr>
<td>Hyperpigmentation</td>
<td>18 (3/17)</td>
<td>9 (1/11)</td>
</tr>
<tr>
<td>Calcification</td>
<td>0 (0/17)</td>
<td>36 (4/11)</td>
</tr>
<tr>
<td>Telangiectasia</td>
<td>71 (12/17)</td>
<td>55 (6/11)</td>
</tr>
<tr>
<td>Nailfold bleeding</td>
<td>65 (11/17)</td>
<td>64 (7/11)</td>
</tr>
<tr>
<td>Raynaud's phenomenon</td>
<td>100 (17/17)</td>
<td>100 (11/11)</td>
</tr>
</tbody>
</table>

Unless noted otherwise, values are percentages.
SSc, diffuse cutaneous SSc; lcSSc, limited cutaneous SSc; VC, vital capacity; DLCO, diffusion capacity for carbon monoxide; LDH, lactate dehydrogenase; ESR, erythrocyte sedimentation rate (normal <15 mm per h); CRP, c-reactive protein (normal <0.3 mg per dl). * p < 0.05.
In this study, we demonstrated that disease duration prior to significant discovery was shorter in SSc patients with anti-U1 RNA than in those without. Most patients without anti-U1 RNA had a prolonged clinical phase of Raynaud’s phenomenon prior to definitive diagnosis of SSc. This may reflect that SSc patients with anti-U1 RNA usually reveal typical symptoms whereas anti-U1 RNP positive, anti-U1 RNA negative SSc patients had mild symptoms. Hoffman et al. (1995) reported that an anti-RNP positive, anti-U1 RNA negative group consisted of patients with various symptoms of mild MCTD and SLE, whereas an anti-U1 RNA positive group comprised patients with more typical MCTD features. These results suggest that anti-U1 RNA may be useful in the classification of patients with connective tissue diseases, including SSc, SLE, and MCTD, who are positive for anti-U1 RNP and may serve as a serologic indicator for the typical type of these diseases.

We also determined the antigen specificity of anti-U1 RNP in SSc patients with anti-U1 RNP. In immunoblotting, the frequencies of anti-70 kDa, anti-A, and anti-C protein antibodies were 64%, 83%, and 11%, respectively. These results were consistent with our previous reports (Ihn et al., 1998). The frequency of anti-70 kDa antibodies was significantly higher in patients with SSc or SSc overlap syndrome positive for anti-U1 RNA than in those negative for anti-U1 RNA (77% vs 43%, p < 0.05). This difference in the frequency of anti-70 kDa antibodies is also confirmed using ELISA (86% vs 43%, p < 0.05). Regarding the titer of anti-70 kDa antibodies in patients with overlap syndrome, previous papers reported as follows. (i) In the group of overlap syndrome patients with SLE and SSc, anti-U1 RNP were directed in lower titers against the 70 kDa protein. (ii) In the group of overlap syndrome patients with SLE, SSc, and PM, a strong anti-70 kDa antibody level was predominated (Habets et al., 1983; van Venrooij, 1987). In this study, all sera from six patients with overlap syndrome had anti-70 kDa antibodies. Four sera from patients with SLE, SSc, and PM (shown as crosses in Fig. 3) revealed OD 450 nm values ranging from 1.68 to 2.67, whereas two sera from patients with SLE and SSc (shown as open circles in Fig. 3) revealed OD 450 nm values of 0.93 and 1.17. These results seem to be consistent with previous reports. Excluding these six patients, the frequencies of anti-70 kDa, anti-A, and anti-C protein antibodies in patients with SSc were also consistent with our previous reports (57%, 83%, and 13%, respectively) and the frequencies of anti-70 kDa antibodies determined by immunoblotting and ELISA were also significantly higher in SSc patients with anti-U1 RNA than in those without (71% vs 38%, p < 0.05; 82% vs 38%, p < 0.05, respectively). As there was a significant correlation between the presence of anti-U1 RNA and anti-70 kDa antibodies in patients with SSc or SSc overlap syndrome, we also assessed whether or not anti-70 kDa antibodies were correlated with PF. There was no significant difference in the frequency of PF between patients with anti-70 kDa antibodies and those without (64% vs 50%).

In conclusion, we demonstrated that anti-U1 RNA may be useful in the classification of patients with anti-U1 RNP in SSc, as well as SLE and MCTD, and serve as a useful indicator of PF in SSc patients with anti-U1 RNP.

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