SARS-CoV with a detection limit of 1 copy RNA per reaction. This strategy is expected to be applicable to many areas requiring ultrasensitivity and/or early detection of target sequences.

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References


The antigens to which anti-RNP antibodies react reside in many proteins (70 kD, protein A, and protein C) complexed with small nuclear U1-RNA. This complex is called small nuclear U1 ribonucleoprotein (snU1-RNP). Similarly, the antigen to which anti-Sm antibodies bind is composed of a complex of proteins (B, B′/H11032, D, E, F, and G) and snRNAs (U1, U2, U4–U6, and U5). Anti-Sm antibodies are highly specific for SLE (2).

The snRNPs (U1-RNP/Sm) are involved in the splicing of precursor messenger RNA. Traditionally, anti-RNP antibodies have been detected by techniques such as passive hemagglutination, immunodiffusion, counterimmunoelectrophoresis, and ELISA using purified antigen. More recently, recombinant antigens have been used increasingly to identify anti-RNP antibodies, thus allowing identification of antibodies to the individual proteins of the snU1-RNP complex (70 kD, protein A, and protein C) (3).

In the present study, we investigated whether the presence of antibodies to individual proteins of the snU1-RNP complex is associated with clinical symptoms and/or organ involvement in SLE patients and patients with MCTD. We collected 37 consecutive serum samples obtained from different individuals in which anti-RNP antibodies were identified by dot blot with a purified antigen (BMD) and measured antibodies to recombinant RNP-A, RNP-C, and RNP-70 kD by use of Varelisa Split ANA Profile (Pharmacia Diagnostics). The source population was university hospital-based, and the patients had symptoms at the time anti-RNP testing was ordered. Each sample corresponded to one individual.

Of the 37 anti-RNP-positive samples, 14 were from patients with SLE and 20 from individuals with MCTD. SLE was diagnosed according to the American College of Rheumatology classification criteria revised in 1997 (3), and MCTD was diagnosed according to the criteria proposed by Alercón-Segovia et al. (4). One patient had chronic idiopathic urticaria, 1 patient had rheumatoid arthritis, and 1 had bacterial parotitis. None of these 3 individuals had signs of systemic disease. The clinical symptoms and organ involvement of the patients are summarized in Table 1. Raynaud phenomenon was present in 18 (90%) of the 20 MCTD patients and in 10 (70%) of the 14 SLE patients, consistent with the previously reported association of anti-RNP antibodies and Raynaud phenomenon (5). In both diseases, arthritis was present in 50%–55% of the patients. Lymphopenia was present in 11 (78%) of the 14 SLE patients. Glomerulonephritis and lymphopenia were found mainly in SLE.

### Table 1. Association between autoantibodies and clinical symptoms.

<table>
<thead>
<tr>
<th>Symptom</th>
<th>SLE</th>
<th>MCTD</th>
<th>RNP-A + RNP-C + RNP-70 kD</th>
<th>RNP-A + RNP-C</th>
<th>RNP-A + RNP-70 kD</th>
<th>RNP-C + RNP-70 kD</th>
<th>RNP-70 kD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raynaud phenomenon</td>
<td>10</td>
<td>18</td>
<td>18 (7/11)</td>
<td>5 (3/2)</td>
<td>2 (0/2)</td>
<td>1 (0/1)</td>
<td>2 (0/2)</td>
</tr>
<tr>
<td>Fever</td>
<td>5</td>
<td>3</td>
<td>5 (4/1)</td>
<td>1 (1/0)</td>
<td>1 (0/1)</td>
<td></td>
<td>1 (0/1)</td>
</tr>
<tr>
<td>Xerostomia</td>
<td>1</td>
<td>3</td>
<td>1 (0/1)</td>
<td>2 (1/1)</td>
<td></td>
<td></td>
<td>1 (0/1)</td>
</tr>
<tr>
<td>Xerostomia and xerophthalmia</td>
<td>2</td>
<td>1</td>
<td>3 (2/1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rash</td>
<td>8</td>
<td>8</td>
<td>12 (6/6)</td>
<td>3 (2/1)</td>
<td></td>
<td></td>
<td>1 (0/1)</td>
</tr>
<tr>
<td>Photosensitivity</td>
<td>4</td>
<td>4</td>
<td>6 (3/3)</td>
<td>2 (1/1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hair loss</td>
<td>4</td>
<td>2</td>
<td>5 (4/1)</td>
<td>1 (0/1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aphthosis</td>
<td>2</td>
<td>3</td>
<td>3 (2/1)</td>
<td>1 (0/1)</td>
<td></td>
<td></td>
<td>1 (0/1)</td>
</tr>
<tr>
<td>Arthritis</td>
<td>7</td>
<td>11</td>
<td>12 (5/7)</td>
<td>4 (2/1)</td>
<td>1 (0/1)</td>
<td>1 (0/1)</td>
<td></td>
</tr>
<tr>
<td>Pleuritis/pericarditis</td>
<td>5</td>
<td>3</td>
<td>4 (3/1)</td>
<td>2 (0/2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scleroderma/sclerodactyly</td>
<td>1</td>
<td>9</td>
<td>6 (1/5)</td>
<td>1 (0/1)</td>
<td>1 (0/1)</td>
<td>1 (0/1)</td>
<td></td>
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<tr>
<td>Esophageal dysmotility</td>
<td>0</td>
<td>2</td>
<td>1 (0/1)</td>
<td>1 (0/1)</td>
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<td></td>
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<tr>
<td>Polymyositis</td>
<td>0</td>
<td>4</td>
<td>3 (0/3)</td>
<td>1 (0/1)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Decreased lung diffusion</td>
<td>4</td>
<td>3</td>
<td>2 (1/1)</td>
<td>4 (3/1)</td>
<td></td>
<td>1 (0/1)</td>
<td></td>
</tr>
<tr>
<td>Proteinuria (&gt;300 mg/day)</td>
<td>6</td>
<td>2</td>
<td>6 (5/1)</td>
<td>1 (0/1)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Cellular casts</td>
<td>0</td>
<td>0</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Glomerulonephritis</td>
<td>5</td>
<td>0</td>
<td>4 (4/0)</td>
<td>1 (1/0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Involvement central nervous system</td>
<td>1</td>
<td>1</td>
<td>2 (1/1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphopenia</td>
<td>11</td>
<td>3</td>
<td>7 (7/0)</td>
<td>3 (3/0)</td>
<td>1 (0/1)</td>
<td>1 (1/0)</td>
<td>2 (0/2)</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>7</td>
<td>5</td>
<td>8 (6/2)</td>
<td>2 (1/1)</td>
<td>1 (0/2)</td>
<td>1 (0/1)</td>
<td></td>
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<tr>
<td>Thrombocytopenia</td>
<td>1</td>
<td>1</td>
<td>1 (1/0)</td>
<td>1 (0/1)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Anemia</td>
<td>7</td>
<td>7</td>
<td>11 (6/5)</td>
<td>2 (1/1)</td>
<td></td>
<td></td>
<td>1 (0/1)</td>
</tr>
<tr>
<td>Repeated miscarriage</td>
<td>3</td>
<td>1</td>
<td>3 (2/1)</td>
<td>1 (1/0)</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

a Values in parentheses indicate the number of patients with SLE (first value) vs the number of patients with MCTD (second value).
b This group contains a patient with rheumatoid arthritis.
patients, whereas polymyositis, esophageal dysmotility, and scleroderma were found mainly in MCTD patients. In one sample (obtained from a SLE patient), no antibodies to recombinant protein A, protein C, and the 70 kD protein could be identified. In this patient, anti-RNP antibodies were revealed only when a purified antigen was used.

Antibodies to (a) RNP-A, RNP-C, and RNP-70 kD; (b) RNP-A and RNP-70 kD; (c) RNP-A and RNP-C; (d) RNP-C and RNP-70 kD; (e) RNP-A only; (f) RNP-C only; and (g) RNP-70 kD only were found in, respectively, 9, 0, 3, 1, 0, 0, and 0 of the 14 SLE patients and in 13, 2, 2, 1, 0, 0, and 2 of the 20 MCTD patients. These distributions were comparable in SLE and MCTD. The patients with chronic idiopathic urticaria, rheumatoid arthritis, or parotitis had antibodies to, respectively, RNP-70 kD only, RNP-A and RNP-C, and RNP-70 kD only. Table 1 contains a survey of the symptoms and the antibodies to the individual snU1-RNP proteins in patients with anti-RNP antibodies. The statistical significance of associations between autoantibodies and symptoms was explored by Fisher 2-tailed exact test. Within the patient group with anti-RNP antibodies, patients with a specific combination of antibodies were compared with all other patients. This was done for each symptom and for each specific combination of antibodies. No correction for multiple testing was made, but only a P value <0.01 was taken to indicate significance.

We found no associations between antibodies to the individual snU1-RNP proteins and symptoms or organ involvement except for an association between decreased lung diffusion and the presence of antibodies to RNP-A as well as to RNP-C (in the absence of antibodies to RNP-70 kD; \( P = 0.0068 \)). Antibodies to proteins A and C were present in 4 of the 7 patients with anti-RNP antibodies and decreased lung diffusion but were present in only 2 of the 30 individuals with anti-RNP antibodies but without decreased lung diffusion. Such an association needs to be confirmed in future studies.

In addition to antibodies to RNP-A, RNP-C, and RNP-70 kD, we also measured antibodies to recombinant Sm-B/B’ (Varelisa Split ANA Profile) and to purified Sm-D (Elia-Sm; Pharmacia Diagnostics) in the 37 samples with anti-RNP antibodies. Anti-Sm-D antibodies were found in 5 of the 14 SLE patients and in none of the MCTD patients \(( P = 0.0046 \)). Anti-Sm-B/B’ antibodies were found in 9 of the 14 SLE patients and in 11 of the 20 MCTD patients \(( P = 0.728 \)). Four of the 5 patients with anti-Sm-D antibodies also had anti-Sm-B/B’ antibodies. These data indicate that anti-Sm-D antibodies are specific for SLE, whereas anti-Sm-B/B’ are not, confirming a previous report \cite{6}. The detection of antibodies to Sm-B/B’ in the absence of anti-Sm-D antibodies in patients with MCTD is most probably attributable to the reported cross-reactivity of antibodies to RNP-A and RNP-C with Sm-B/B’ \cite{7,8}. Both antigens share proline-rich octapeptide epitopes.

In conclusion, (a) we confirmed that a high percentage of patients with anti-RNP antibodies have Raynaud phenomenon; (b) assay systems composed of recombinant antigens may miss rare samples that contain anti-RNP antibodies; (c) anti-Sm-D antibodies are specific for SLE, whereas anti-Sm-B/B’ are not; (d) within a group of patients with antibodies to RNP, no associations between antibodies to individual snU1-RNP proteins and symptoms and/or organ involvement were found, except for a possible association between decreased lung diffusion and the presence of antibodies to proteins A and C. The latter finding requires confirmation.

The Varelisa Split ANA Profile assays were kindly provided by Sweden Diagnostics (Freiburg, Germany).

References


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Evaluation of 3 Internal Standards for the Measurement of Cyclosporin by HPLC–Mass Spectrometry, Paul J. Taylor, Scott R. Brown, Donald P. Cooper, Paul Salm, Michael R. Morris, Peter I. Pillans, and Stephen V. Lynch (Departments of Clinical Pharmacology and Surgery, Princess Alexandra Hospital, Brisbane, Australia; Australian Bioanalytical Services Pty Ltd, Princess Alexandra Hospital, Brisbane, Australia; Clinical Applications Group, Waters Corporation, Manchester, United Kingdom; * address correspondence to this author at: Department of Clinical Pharmacology, Level 3, R-Wing, Bldg. 1, Princess Alexandra Hospital, Ipswich Road, Woolloongabba, Brisbane, Queensland 4102, Australia; fax 61-7-3240-5031, e-mail ptaylor@soms.uq.edu.au)

The calcineurin inhibitor cyclosporin A (CsA) is used as a primary immunosuppressant in solid organ transplantation and for the treatment of many autoimmune diseases. Individualization of therapy is required because CsA has