Clinical features of seven Japanese patients with anti-PL-12 antibody: frequent positivity for anti-cyclic citrullinated peptide antibody

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Abstract

We report the clinical features of 7 Japanese patients with anti-PL-12 antibody. Rheumatoid arthritis (RA) was the most common diagnosis (4/7 patients). Interstitial lung disease (ILD) was detected in all 7 patients, polyarthritis was present in 5 patients, and elevation of creatine kinase was noted in 2 patients. The 4 patients with RA were all positive for anti-cyclic citrullinated peptide (CCP) antibody, and the titer was high in 3 of them. Bone erosions were detected in 3 patients. One patient developed rapidly progressive ILD. In conclusion, a high frequency of anti-CCP-positive RA was detected among Japanese patients with anti-PL-12 antibody.

(Key words: antisynthetase syndrome, dermatomyositis, polymyositis, rheumatoid arthritis, systemic sclerosis)

Introduction

Polymyositis (PM) and dermatomyositis (DM) are autoimmune diseases characterized by inflammation of the muscles. Myositis-specific autoantibodies are positive in about half of all patients with PM/DM¹. While the most common autoantibody in PM/DM patients is anti-aminooacyl-tRNA synthetase (ARS) antibody, a total of 8 autoantibodies have been described¹. Anti-Jo-1 (anti-histidyl-tRNA synthetase) antibody is the best known of them, and is positive in 15 – 25% of PM/DM patients². Patients with antisynthetase antibody have distinctive clinical characteristics, which are fever, arthritis, myositis, interstitial lung disease (ILD), Raynaud’s phenomenon, and mechanic’s hands. However, the frequency of each symptom and the timing of onset for myositis, ILD, and skin manifestations differ slightly between each antibody³.

Anti-PL-12 antibody is one of the antisynthetase antibodies and it targets alanyl-tRNA synthetase. Compared with patients who are positive for anti-Jo-1 antibody, arthritis and muscle weakness are less frequent in patients with anti-PL-12 antibody³. Although joint manifestations are common in patients with anti-PL-12 antibody, such manifestations have not been investigated for patients showing positivity for anti-cyclic citrullinated peptide (CCP) antibody, which is highly specific for rheumatoid arthritis (RA). Therefore, we studied the clinical characteristics of 7 consecutive Japanese patients with anti-PL-12, including anti-CCP antibody.

Methods

We routinely measure anti-ARS antibodies in patients with suspected inflammatory myopathy. From January 2007 to March 2014, anti-ARS antibodies were examined in patients admitted to our department with the following diagnoses: inflammatory myopathy, unclassified arthritis, ILD, and unclassified connective tissue diseases (among others). Detection of anti-ARS antibodies was performed with a line-blotting kit from Orgentec Diagnostika (Mainz, Germany) or a kit from Euroimmun AG (Luebeck, Germany). Diagnosis of PM and DM was based on the criteria of Bohan and Peter⁴. Clinically amyopathic DM (CADM) was diagnosed according to the criteria proposed by Sontheimer et al⁵. The diagnosis of systemic sclerosis (SSc) and Sjögren’s syndrome (SS) was based on the former American College of Rheumatology criteria and the Japanese criteria, respectively⁶. Rheumatoid arthritis (RA) was diagnosed by either the former or new criteria.
for the classification of RA\(^2\). Fever was defined as an axillary temperature \(\geq 38^\circ C\), either during admission or measured by the patient before admission. Bone erosion was detected on plain radiographs. ILD was diagnosed by computed tomography (CT). Rapidly progressive ILD was defined as progressive dyspnea and hypoxia with worsening of interstitial changes on chest CT scans within 1 month of the onset of respiratory manifestations.

### Results

During the study period, anti-ARS antibodies were examined in 170 patients. The final diagnoses of these 170 patients were as follows: PM or DM in 115, RA in 33, SSc in 10, SS in 2, systemic lupus erythematosus in 2, isolated ILD in 2, adult onset Still’s disease in 1, mixed connective tissue disease in 1, viral myositis in 1, and undetermined diagnosis in 3. Patients with overlapping diagnoses were included in either category, although all patients who had RA overlapping with other connective tissue diseases were included in the RA category. Among the 170 patients, 7 patients (4.1%) were positive for anti-PL-12 antibody. Only 2 patients with PM/DM (1.7%) were positive for anti-PL-12 antibody, while 4 out of 33 patients with RA (12.1%) were positive for this antibody. Two representative cases are presented briefly.

### Case 1

A 49-year-old woman was diagnosed with SS and ILD because of anti-SSA antibody positivity and the results of lip biopsy. She started treatment with prednisolone (PSL) for ILD. Six years later, she developed polyarthritis. Because both anti-CCP antibody and rheumatoid factor were negative, the arthritis was considered to be related to SS, and she was treated with disease-modifying anti-rheumatic drugs. Elevation of creatine kinase (CK) was found at the age of 61. DM was diagnosed from Gottron’s sign and the results of muscle biopsy. Anti-PL-12 antibody was positive at this time. She was treated with high-dose PSL and cyclosporine.

### Case 2

A 49-year-old woman presented to her local hospital with Raynaud’s phenomenon, polyarthralgia, and edema of the hands. SSc was diagnosed and she was treated with low-dose PSL. Polyarthritis recurred after several months and she was referred to our hospital for evaluation. She had sclerosis of the skin on her face and fingers, but antinuclear antibody was negative. ILD was detected by chest CT and esophageal dysmotility was revealed by barium esophagography. CK was mildly elevated, which was considered to be due to myositis associated with SSc. RA was also diagnosed because of positivity for anti-CCP antibody and rheumatoid factor. The dose of PSL was increased to 20 mg/day and azathioprine was added. Eight months later, she was readmitted with exacerbation of ILD, which did not respond to treatment with PSL at 1 mg/kg/day. Home oxygen therapy was introduced. She died of respiratory failure despite treatment with methylprednisolone pulse therapy and intravenous cyclophosphamide. Anti-PL-12 antibody was positive at this admission.

The characteristics of the 7 patients with anti-PL-12 antibody are listed in Table 1. Their mean age (SD) was 59.3 (6.8) years and 5 patients (71%) were women. The diagnosis was RA in 2 patients, RA with SS in 1,

### Table 1. Clinical and laboratory characteristics of 7 patients with anti-PL-12 antibody.

<table>
<thead>
<tr>
<th>No.</th>
<th>Patient Age</th>
<th>Dx</th>
<th>Duration (months)</th>
<th>Sex</th>
<th>Arthritis</th>
<th>Seroody</th>
<th>Muscle Weakness</th>
<th>CK</th>
<th>RF</th>
<th>CCP</th>
<th>SSA</th>
<th>CT</th>
<th>RP-12</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>61/F</td>
<td>DM, SS</td>
<td>144</td>
<td>Yes</td>
<td>Yes/No</td>
<td>NA</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>1063</td>
<td>Neg</td>
<td>Neg</td>
<td>Yes NSIP No</td>
</tr>
<tr>
<td>2</td>
<td>49/F</td>
<td>RA, SSc</td>
<td>6</td>
<td>Yes</td>
<td>Yes/Yes</td>
<td>2.53</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>544</td>
<td>2560²</td>
<td>278</td>
<td>Neg NSIP No</td>
</tr>
<tr>
<td>3</td>
<td>62/F</td>
<td>RA</td>
<td>126</td>
<td>No</td>
<td>Yes/Yes</td>
<td>5.42</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>58</td>
<td>111</td>
<td>6.7</td>
<td>Neg OP No</td>
</tr>
<tr>
<td>4</td>
<td>56/F</td>
<td>RA</td>
<td>8</td>
<td>Yes</td>
<td>Yes/Yes</td>
<td>NA</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>40</td>
<td>63</td>
<td>1443</td>
<td>Yes NSIP Yes</td>
</tr>
<tr>
<td>5</td>
<td>56/M</td>
<td>CADM</td>
<td>11</td>
<td>No</td>
<td>No/NA</td>
<td>NA</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>70</td>
<td>40²</td>
<td>Neg</td>
<td>Neg NSIP No</td>
</tr>
<tr>
<td>6</td>
<td>59/F</td>
<td>RA, SS</td>
<td>2</td>
<td>No</td>
<td>Yes</td>
<td>3.77</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>89</td>
<td>145</td>
<td>117</td>
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</tr>
<tr>
<td>7</td>
<td>72/M</td>
<td>SS</td>
<td>2</td>
<td>Yes</td>
<td>No/NA</td>
<td>NA</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>110</td>
<td>325</td>
<td>Neg</td>
<td>Yes NSIP No</td>
</tr>
</tbody>
</table>

*CDAM, clinically amyopathic dermatomyositis; CK, creatine kinase (normal, <150 U/L); CCP, anti-cyclic citrullinated peptide antibody (normal, <4.5 U/mL); CT, computed tomography findings; DAS, disease activity score; DM, dermatomyositis; Dx, diagnosis; NA, not available; NSIP, nonspecific interstitial pneumonia; OP, organizing pneumonia; RA, rheumatoid arthritis; RF, rheumatoid factor (normal, <15 U/mL or <1:20); RP, Raynaud’s phenomenon; RP-ILD, rapidly progressive interstitial lung disease; SSA, anti-SSA antibody; SSc, systemic sclerosis; SS, Sjögren’s syndrome.

₁Duration from the onset of first symptoms to admission. ²Rheumatoid factor was measured by the rheumatoid arthritis particle agglutination (RAPA) method.
RA with limited SSc in 1, CADM in 1, SS in 1, and DM with SS in 1. Patient No. 1 developed DM 12 years after the diagnosis of SS and ILD. Patient No. 5 with CADM underwent skin biopsy (the forearm) because overlap with early SSc was suspected due to mild sclerodactyly and diffuse pigmentation of the trunk. Although he did not have skin sclerosis clinically, histologic examination showed an increase of collagen fibers around the cutaneous appendages. One patient with SS (No. 7) had concurrent lung cancer and had undergone endoscopic resection of gastric cancer one month earlier.

Polyarthritis were noted in 5 out of 7 patients. Both fever and Raynaud’s phenomenon were present in 4 patients, while sclerodactyly was seen in 3 patients. Whether mechanic’s hands existed was not known due to the lack of data in the clinical records. Muscle weakness and elevation of creatine kinase (CK) were present in 2 patients each. All 7 patients had ILD. In 6 patients, CT revealed nonspecific interstitial pneumonia, while the remaining 1 patient (No. 3) developed biopsy-proven organizing pneumonia after treatment with etanercept plus methotrexate for 4 years. Rapidly progressive ILD was only observed in 1 patient (No. 4), and occurred 8 months after the diagnosis of RA while the patient was taking prednisolone and methotrexate. None of the patients had pulmonary hypertension or cardiac involvement. Pericardial effusion was detected in 1 patient (No. 5).

Serological tests showed that rheumatoid factor was positive in 6 out of 7 patients, while anti-CCP antibody was positive in 4 patients and anti-SSA antibody in 3 patients. None of the patients had antinuclear antibody, but anticytoplasmic antibody was positive in all 7 patients. Bone erosions were detected in 3 out of 5 patients who underwent radiological examination. The following differences were noted between patients with or without anti-CCP antibody: the diagnosis (4/4 had RA vs. none), sex (4/4 were women vs. 1/3), the presence of arthritis (4/4 vs. 1/3), rheumatoid factor positivity (4/4 vs. 1/3), and anti-SSA antibody positivity (1/4 vs. 2/3).

All of the patients were initially treated with glucocorticoids at medium to high doses and all of them were mainly treated for ILD, except patient No. 2. Calcineurin inhibitors were administered to 4 out of 7 patients. The patient with rapidly progressive ILD (No. 4) showed a good response to high-dose prednisolone alone. Exacerbation of ILD occurred in 3 patients (Nos. 1, 2, and 7) after tapering of prednisolone. Only 1 patient (No. 2) died, despite treatment with methylprednisolone pulse therapy and intravenous cyclophosphamide. Polyarthritis recurred in one patient (No. 3) after tapering of prednisolone. She was sequentially treated with tocilizumab, and there was no recurrence of organizing pneumonia thereafter.

### Discussion

In the present case series, the clinical diagnosis of these 7 patients with anti-PL-12 antibody was heterogeneous. RA was the most common diagnosis, being detected in 4 out of 7 patients. This result is inconsistent with previous reports that less than 10% of patients with anti-PL-12 antibody have RA. The difference is probably due to referral bias. The frequency of arthritis in patients with anti-PL-12 antibody shows wide variation (12% to 80%) according to previous reports. This may be because patients with joint symptoms are referred to rheumatologists, while patients with pulmonary involvement alone or rapidly progressive ILD tend to be referred to pulmonologists. In another study, 65% of patients with ILD initially presented to a pulmonologist.

Anti-CCP antibody can also be positive in patients with anti-PL-12 antibody. In fact, 4 of our 7 patients also had anti-CCP antibody and the titer was very high in 3 of them. Three patients had bone erosions. So far, there have only been 2 case reports about patients who were positive for anti-CCP antibody as well as anti-PL-12. Coexistence of antisynthetase antibodies and anti-CCP antibody has rarely been investigated. Among patients with anti-Jo-1 antibody, 2 out of 12 patients were reported to be positive for anti-CCP antibody. Kaneko et al. reported a high frequency of both RF (45%) and anti-CCP antibody (29%) in patients positive for antisynthetase antibodies, especially patients with bone erosion or ankylosis on hand radiographs. This suggests that not only anti-PL-12 antibody, but also other antisynthetase antibodies, may frequently coexist with anti-CCP antibody. However, whether coexistence of anti-CCP and anti-PL-12 antibody is common in other ethnic groups needs to be elucidated, because this has only been reported in Japanese patients to date.

Rapidly progressive ILD rarely occurs in Japanese patients with anti-PL-12 antibody. Hamaguchi et al. reported that none of their 18 patients with anti-PL-12 developed rapidly progressive ILD during follow-up. In the present series, only 1 out of 7 patients had rapidly progressive ILD, and drug-induced pneumonia cannot be excluded because ILD developed during treatment with methotrexate. This contrasts sharply with reports from Western countries that more than half of the patients who are positive for anti-PL-12 antibody present with progressive dyspnea. This difference may also be due to referral bias or there may be an influence of ethnicity. The reported Japanese study was mainly conducted by dermatologists, and patients with progressive ILD may not often be referred to dermatologists.

The limitation of this study is that not all the patients with RA are prospectively examined for anti-PL-12 antibody. Only a small portion of the inpatients with RA along with ILD who are suspected of having antisynthetase syndrome by the attending physicians were examined for this antibody. The
overall frequency of anti-PL-12 antibody among patients with RA was not known from this study. Anti-PL-12 antibody was measured by commercial line-blotting kit alone, and was not confirmed by immunoprecipitation, but this assay method is only available at specialized facilities.

In conclusion, over half of our patients with anti-PL-12 had RA and showed anti-CCP positivity. Because all the patients were positive for anticytoplasmic antibody, RA patients with ILD as well as anticytoplasmic antibody positivity may also have undetected antisynthetase syndrome.

**Declaration of interest**: The authors have no conflict of interest to declare.

**References**


抗PL-12抗体陽性患者7例の臨床的特徴

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要　約

抗PL-12抗体陽性患者7例の臨床的特徴について報告する。基礎疾患として関節リウマチが最も高頻度にみられた(7例中4例)。間質性肺疾患は7例全例に認められ、多発性関節炎は5例、クレアチンキナーゼ高値は2例に認められた。抗環状シトルリン化ペプチド抗体（抗CCP抗体）は、関節リウマチ患者4例全例が陽性であり、4例中3例の抗体価は高価であった。骨びらんは5例中3例に認められた。1例は急速進行性間質性肺炎を合併した。日本人の抗PL-12抗体陽性患者では抗CCP抗体陽性が半数以上に認められた。
（キーワード：関節リウマチ、抗シンセターゼ抗体症候群、全身性強皮症、多発性筋炎、皮膚筋炎）