Clinical manifestation and prognostic factor in anti-melanoma differentiation-associated gene 5 antibody-associated interstitial lung disease as a complication of dermatomyositis

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Abstract

Objective. The aim of this study is to evaluate the clinical manifestation and prognostic factors of anti-melanoma differentiation-associated gene 5 (MDA5) antibody-associated interstitial lung disease (ILD) with DM.

Methods. Fourteen patients who presented with anti-MDA5 antibody and 10 patients with anti-aminoacyl-tRNA synthetase (ARS) antibody were enrolled. All patients were diagnosed as having DM with ILD. Clinical manifestations in the patients with anti-MDA5 antibody were compared with those in the patients with anti-ARS antibody.

Results. The frequencies of acute/subacute interstitial pneumonia (A/SIP) and fatal outcome were significantly higher in the subset with anti-MDA5 antibody. The creatine kinase (CK) value was significantly lower and the γ-glutamyl transpeptidase and ferritin values were significantly higher in the subset with anti-MDA5 antibody. Significant correlations were found between PaO₂/FiO₂ and ferritin (rₛ = −0.59, P = 0.035), alveolar–arterial oxygen difference (A–aDO₂) and KL-6 (rₛ = 0.73, P = 0.016) and A–aDO₂ and ferritin (rₛ = 0.66, P = 0.013) in the subset with anti-MDA5 antibody. The most significant prognostic factor was ferritin. The cumulative survival rate was significantly lower (P < 0.0001) in the subset with ferritin ≥1600 ng/ml than that in the subset with ferritin <1600 ng/ml in anti-MDA5 antibody-associated ILD.

Conclusion. Both serum ferritin and anti-MDA5 antibody are powerful indicators for the early diagnosis of A/SIP with DM. Ferritin also predicts disease severity and prognosis for patients with anti-MDA5 antibody. Intensive treatment should be administered to cases that have anti-MDA5 antibody-associated ILD with DM showing hyperferritinaemia, especially if the ferritin level is ≥1600 ng/ml.

Key words: Dermatomyositis, Interstitial lung disease, Melanoma differentiation-associated gene 5, Ferritin, Macrophage activation.

Introduction

DM is characterized by inflammation of the skin and muscle [1]. DM is occasionally complicated with interstitial lung disease (ILD), which is classified into two subsets, acute/subacute interstitial pneumonia (A/SIP) and chronic interstitial pneumonia (CIP) [2–6]. A/SIP is of prime importance in the clinical management of patients with DM because it is an intractable and life-threatening complication [4, 6–8]. Clinically amyopathic DM (C-ADM) includes typical skin lesions with amyopathy or hypomyopathy [9] and was recently reported to be complicated by A/SIP, especially in those patients with anti-C-ADM-140 antibody [10]. C-ADM with A/SIP showed a rapid progressive pattern with a 6-month survival rate of 40.8–45% [11, 12]. Recently, the anti-C-ADM-140 antibodies in
patients’ sera specifically reacted with melanoma differentiation-associated gene 5 (MDA5) protein, which plays a role in the innate immune system, thus confirming MDA5 as the C-ADM-140 autoantigen [13]. We reported that serum ferritin predicts the development and severity of ILD with DM [14]. Additionally, we have observed several patients in whom the serum ferritin level was high and correlated with disease activity in A/SIP with DM.

Taken together, the characteristic manifestation and prognostic factors should be analysed and understood for the appropriate management in anti-MDA5-associated ILD with DM because the fatal outcome is of a relatively high frequency. In the present study, we compared ILD with anti-MDA5 antibody with ILD with anti-aminocyl-tRNA synthetase (ARS) antibody and evaluated the clinical manifestation and prognostic factors in anti-MDA5 antibody-associated ILD with DM.

**Materials and methods**

**Patients and controls**

This retrospective study included patients admitted to our hospital from August 1992 to August 2009. All of the enrolled patients suffered from skin rash, myopathy or respiratory symptoms (or a combination thereof) on admission. These patients were diagnosed as having DM or C-ADM based on the criteria of Bohan and Peter [15] or Sontheimer [16], respectively. In general, C-ADM presents with typical skin lesions and amyopathy or hypomyopathy for >6 months. A subset of the C-ADM group included patients who developed fatal ILD within the first 6 months of the present study. Clinical data were obtained from medical records on admission. The study was approved by the ethical committee in our institution (Institute of Rheumatology, Tokyo Women’s Medical University), and informed consent was obtained from all patients. Blood tests included evaluation of liver enzymes [alanine aminotransferase (ALT), aspartate aminotransferase (AST) and γ-glutamyl transpeptidase (γ-GTP)], creatine kinase (CK), lactate dehydrogenase (LD), CRP ferritin, KL-6 and ANA. Anti-SS-A, anti-SS-B and anti-U1 RNP antibodies were detected in five, one and one patients, respectively. As shown in Table 1, 14 patients with anti-MDA5 antibody and 10 patients with anti-ARS antibody were enrolled into this study. Clinical manifestations in the patients with anti-MDA5 antibody were compared with those in the patients with anti-ARS antibody. Table 1 shows the results of the first examinations on admission. The following describes the results in the patients with anti-MDA5 antibody compared with those in patients with anti-ARS antibody. The age at onset was 43.6 (14.6) years [mean (S.D.)], with no significant difference between each subset. The frequency of C-ADM was 57%, which is significantly higher (P = 0.033). The median value of CK was significantly lower (P = 0.01). The frequency of A/SIP was 71%, which is significantly higher (P = 0.036). The value of KL-6 and pulmonary function showed no significant differences. γ-GTP concentrations were significantly higher (P = 0.0089). Although the value of CRP was not significantly different, as shown in Fig. 1, serum ferritin was significantly higher (P = 0.016). The frequency of the fatal outcome was 36%, which is significantly higher (P = 0.047).

Clinical manifestations in patients with anti-MDA5 antibody compared with those in patients without anti-MDA5 antibody in C-ADM

Anti-MDA5 antibody was positive in 8 (53.3%) of 15 patients with C-ADM. In seven C-ADM patients without anti-MDA5 antibody, three patients had anti-Jo-1 antibody, anti-SSA antibody or ACA, respectively. The complication of A/SIP was revealed in six (75%) of eight C-ADM patients with anti-MDA5 antibody. On the other hand, the
complication of CIP was revealed in five (71%) of seven C-ADM patients without anti-MDA5 antibody. Taken together, A/SIP was complicated more frequently in C-ADM patients with anti-MDA5 antibody than in those without anti-MDA5 antibody.

Correlation coefficients between parameters in patients with anti-MDA5 antibody

As shown in Table 2, the correlation coefficients between parameters were established in patients with anti-MDA5 antibody. The parameters included pulmonary function markers (%VC, P/F ratio and A-aDO2) and laboratory markers (KL-6, CRP and ferritin). Although the correlations between pulmonary function markers and CRP were not definitive, significant correlations were found between P/F ratio and ferritin \( r_s = -0.59, P = 0.035 \), A-aDO2 and KL-6 \( r_s = 0.73, P = 0.016 \), and A-aDO2 and ferritin \( r_s = 0.66, P = 0.013 \) in patients with anti-MDA5 antibody.

Comparison of clinical manifestations between surviving and non-surviving patients who had ILD with anti-MDA5 antibody

As shown in Table 3, clinical manifestations were compared between the surviving and non-surviving patients.
who had ILD with anti-MDA5 antibody. The following indicate the significant results for the non-surviving patients with anti-MDA5 antibody: age at onset \((P = 0.045)\), A-aDO2 \((P = 0.045)\), AST \((P = 0.027)\), \(\gamma\)-GTP \((P = 0.028)\), and ferritin \((P = 0.016)\). Combination therapy including prednisolone (PSL) and immunosuppressive agents (IAs), such as cyclophosphamide and calcineurine inhibitor, were administered to eight surviving patients and four non-surviving patients, respectively. The content of therapy did not significantly differ between each subset.

### Table 3: Comparison of clinical manifestations between patients who are alive and dead patients in ILD with anti-MDA5 antibody

<table>
<thead>
<tr>
<th>Variables</th>
<th>Alive</th>
<th>Dead</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>9</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>37.9 (14.5)</td>
<td>53.8 (8.3)</td>
<td>0.045</td>
</tr>
<tr>
<td>C-ADM, n (%)</td>
<td>5 (56)</td>
<td>3 (60)</td>
<td>1</td>
</tr>
<tr>
<td>CK, IU/l</td>
<td>165 (65–422)</td>
<td>231 (74–599)</td>
<td>0.84</td>
</tr>
<tr>
<td>A/SIP, n (%)</td>
<td>5 (56)</td>
<td>5 (100)</td>
<td>0.22</td>
</tr>
<tr>
<td>KL-6, U/ml</td>
<td>732 (285–1084)</td>
<td>717 (602–1361)</td>
<td>0.67</td>
</tr>
<tr>
<td>P/F ratio, torr</td>
<td>344.3 (335.5–486.9)</td>
<td>291.7 (173.7–358.2)</td>
<td>0.09</td>
</tr>
<tr>
<td>A-aDO2, mmHg</td>
<td>25.6 (4.2–30.3)</td>
<td>89.6 (28.8–253.6)</td>
<td>0.045</td>
</tr>
<tr>
<td>LD, IU/l</td>
<td>415 (342–618)</td>
<td>512 (384–637)</td>
<td>0.55</td>
</tr>
<tr>
<td>AST, IU/l</td>
<td>70 (35–142)</td>
<td>175 (110–421)</td>
<td>0.027</td>
</tr>
<tr>
<td>ALT, IU/l</td>
<td>77 (41–130)</td>
<td>193 (106–455)</td>
<td>0.053</td>
</tr>
<tr>
<td>(\gamma)-GTP, IU/l</td>
<td>40 (13–86)</td>
<td>90 (82–363)</td>
<td>0.028</td>
</tr>
<tr>
<td>CRP, mg/dl</td>
<td>0.8 (0.15–1.15)</td>
<td>0.2 (0.08–2.53)</td>
<td>0.89</td>
</tr>
<tr>
<td>Ferritin, ng/ml</td>
<td>301 (180–680)</td>
<td>1730 (1610–2120)</td>
<td>0.016</td>
</tr>
<tr>
<td>MDA5 ELISA, U/ml</td>
<td>217.1 (42.7–439.2)</td>
<td>221.6 (48.4–555.4)</td>
<td>0.76</td>
</tr>
<tr>
<td>Treatment</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>PSL alone</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>PSL + IA</td>
<td>8</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

The values of age indicate mean (S.D.), and those of laboratory markers and pulmonary function tests indicate median (interquartile range). P-value is established by using the Fisher’s exact test, t-test or Mann–Whitney U-test. Bold indicates significant values.

### Table 4: Prognosis factors in ILD with anti-MDA5 antibody

<table>
<thead>
<tr>
<th>Variables</th>
<th>Hazard ratio (per unit) (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>P/F ratio, torr</td>
<td>1 (0.97, 1.02)</td>
<td>0.86</td>
</tr>
<tr>
<td>A-aDO2, mmHg</td>
<td>1 (0.99, 1.03)</td>
<td>0.67</td>
</tr>
<tr>
<td>AST, IU/l</td>
<td>1.005 (1, 1.01)</td>
<td>0.084</td>
</tr>
<tr>
<td>(\gamma)-GTP, IU/l</td>
<td>1 (0.99, 1.02)</td>
<td>0.64</td>
</tr>
<tr>
<td>Ferritin, ng/ml</td>
<td>1.005 (1.004, 1.014)</td>
<td>0.027</td>
</tr>
</tbody>
</table>

Hazard ratio and P-value established by age- and sex-adjusted Cox regression analysis. Bold indicates significant values.

### Prognostic factor in ILD with anti-MDA5 antibody

As shown in Table 4, age- and sex-adjusted Cox regression analysis was performed to identify a prognostic factor for ILD with anti-MDA5 antibody. The results indicate that the most significant prognostic factor was serum ferritin (hazard ratio per unit 1.005; 95% CI 1.004, 1.014; P = 0.027).

### Survival rates of patients with anti-MDA5 antibody and those with anti-ARS antibody

The cumulative 60-month survival rates were 62.9 and 100% for ILD with anti-MDA5 antibody and ILD with anti-ARS antibody, respectively (Fig. 2A). The cumulative 60-month survival rates were significantly different between each subset (log-rank test, P = 0.042). The present study found that the serum ferritin level was the most significant prognostic factor. The cut-off value for a high serum ferritin concentration according to the 2004 diagnostic guidelines for haemophagocytic lymphohistiocytosis (HLH) is \(\geq 500\) ng/ml [15]. The two subsets of ILD with anti-MDA5 antibody were classified according to whether the cases had a serum ferritin concentration of 500 ng/ml. The cumulative 60-month survival rates were 37.5 and 100% in the subsets with ferritin levels of \(\geq 500\) and \(<500\) ng/ml, respectively (Fig. 2B). The cumulative 60-month survival rate was significantly lower (P = 0.027) in the subset with \(\geq 500\) ng/ml ferritin than in the subset with \(<500\) ng/ml ferritin.

To define the optimal cut-off point with the highest diagnostic accuracy, we performed receiver operating characteristic analyses for a mathematical expression of different serum ferritin concentrations. The highest area under the curve was calculated for a baseline serum ferritin concentration of 1600 ng/ml. The two subsets were classified according to whether the cases had a serum ferritin concentration of 1600 ng/ml. The cumulative 60-month survival rates were 28.8 and 100% in the subsets with ferritin levels of \(\geq 1600\) and \(<1600\) ng/ml, respectively (Fig. 2D). The cumulative 60-month survival rate was significantly lower (P = 0.027) in the subset with \(\geq 1600\) ng/ml ferritin than in the subset with \(<1600\) ng/ml ferritin.
The survival rate was significantly ($P < 0.0001$) lower in the subset with $\geq 1600$ ng/ml ferritin than that with $< 1600$ ng/ml ferritin (Fig. 2C). Our study has limitations. A part of the patients has not been prospectively observed for 60 months in the present study. The actual survival rate could be different from that estimated using Kaplan–Meier method, although most patients were observed for several years.

**Discussion**

We have demonstrated the clinical manifestations and a prognostic factor for anti-MDA5 antibody-associated ILD with DM. The clinical manifestations of anti-MDA5 antibody-associated ILD with DM were basically similar to those of C-ADM with A/SIP described previously [6, 10, 11]. These manifestations were characterized by a low value of CK, high frequency of A/SIP and a high rate of mortality. In the present study, the following findings were newly revealed. First, although anti-MDA5 antibody was a specific disease marker for C-ADM with A/SIP, A/SIP with anti-MDA5 antibody could also be complicated with classical DM. Secondly, the levels of liver enzyme $\gamma$-GTP and ferritin were high in anti-MDA5 antibody-associated ILD with DM. Although elevation of liver enzymes can be attributed to muscle enzymes, it can also be attributed to liver injury, because $\gamma$-GTP is specific to the liver, and CK was lower in the present study. These aspects indicate that the liver, as well as the lung and skin, is involved in anti-MDA5 antibody-associated ILD with DM. Previous reports have described that the increase in ESR, CRP, fibrinogen and ferritin could be due to acute-phase reactants in patients with PM/DM [19, 20]. In the present study, however, CRP levels were relatively low in anti-MDA5 antibody-associated ILD with DM. Additionally, although ferritin levels were increased, CRP levels were lower in the non-surviving patients than those in the surviving patients. These results are different from those of previous reports. Serum ferritin levels also correlated with pulmonary function in the present study. Taken together, serum ferritin levels can be associated with the pathophysiology rather than just the acute-phase reactant in anti-MDA5 antibody-associated ILD with DM.

The present study sought to identify prognostic factors in ILD with anti-MDA5 antibody. The most significant prognostic factor was serum ferritin. The cumulative 60-month survival rate differed significantly between patients with high baseline serum ferritin concentrations ($\geq 500$ ng/ml) and patients with low baseline ferritin concentrations, which indicates that the baseline serum ferritin concentrations could predict survival. Additionally, using a statistical method, we established a serum ferritin...
concentration cut-off value of 1600 ng/ml as the best indicator of survival in our cohort of patients with anti-MDA5 antibody-associated ILD. These results indicate that the serum ferritin concentration predicts the severity and prognosis of anti-MDA5 antibody-associated ILD with DM.

Serum ferritin is an important laboratory finding of HLH [21]. Macrophage activation syndrome (MAS) is now an accepted term used to refer to a form of secondary HLH seen in the context of rheumatic disorders [21, 22]. The pathophysiology of MAS involves the lack of regulation of T lymphocytes and excessive production of cytokines, such as TNF-α, IL-1β, IL-6 and IL-18, resulting in the activation of macrophages [22, 23]. In the present study, patients with anti-MDA5 antibody-associated ILD were not complicated by HLH. Most patients with CIP observed in DM appeared to be well controlled by corticosteroids and IAs [24–26]. In contrast, patients with A/SIP observed in DM were resistant to a variety of treatments, including corticosteroids, cyclophosphamide and calcineurin inhibitor [6–8, 11]. This distinction in treatment responses might be responsible for the cellular phenotypes affecting the pathogenesis of ILD. Alveolar macrophages, which are activated by some antigens, microbes and autoimmune stimuli, are induced to produce leucotriene B4 and IL-8. These factors stimulate neutrophils to induce the fibrosis process in the lung [27]. In our study, we found an elevation of γ-GTP in anti-MDA5 antibody-associated ILD with DM. The elevation of a liver enzyme and hyperferritinemia suggest that T lymphocytes and macrophages, such as Kupffer cells and alveolar macrophages, are intensively activated and cause injury to the skin, lung and liver in DM with anti-MDA5 antibody, although we did not obtain direct evidence of macrophage activation. Whether alveolar macrophages are activated should be investigated using bronchoalveolar lavage or lung biopsy in anti-MDA5 antibody-associated ILD with DM.

On the other hand, the elevation of serum ferritin levels correlates with the general disease activity of SLE [28–30]. Although it was not revealed whether the elevated ferritin levels correlated with the activity of other clinical features such as dermatitis and myositis in the present study, serum ferritin may be a marker of general disease activity rather than a specific marker for ILD with anti-MDA5 antibody. Additionally, patients who have A/SIP with DM experienced a shorter disease duration than the other patients at the time at which ferritin was investigated. The intensity of inflammation may correlate with the serum ferritin concentration.

C-ADM with A/SIP showed a rapidly progressive pattern with a 6-month survival rate of 40.8–45% [11, 12]. Although the clinical manifestations of C-ADM may be a useful indicator for the occurrence of A/SIP retrospectively, ILD with C-ADM does not always develop as a feature of A/SIP. Additionally, the two different subsets of ILD with C-ADM may be A/SIP and CIP [31]. Fathi et al. [32] reported that patients with myositis with ILD require careful evaluation of clinical features as well as pulmonary function tests and radiological features during follow-up, because the course of ILD could not be predicted at the first examination. Recently, anti-MDA5 antibody was detected [13], as well as a specific disease marker for C-ADM with A/SIP. Investigation of the anti-MDA5 antibody is useful for predicting the onset of A/SIP and enables earlier and more appropriate treatment before the disease progresses. However, the present study revealed that the frequency of CIP was 29% in anti-MDA5 antibody-associated ILD. We consider that investigations of both serum ferritin and anti-MDA5 antibody are the most useful predictors of the onset of A/SIP. Additionally, we have experienced several patients in whom the serum ferritin level was high and correlated with the disease activity in A/SIP with DM (manuscript under preparation). Thus, the intensity of immunosuppressive treatment can be decided according to serum ferritin levels as serum ferritin may also be a useful biomarker of disease activity.

In conclusion, both the serum ferritin concentration and the anti-MDA5 antibody are powerful indicators for the early diagnosis of A/SIP with DM. Serum ferritin also predicts the disease severity and prognosis for A/SIP with anti-MDA5 antibody. Intensive treatment with a combination of various IAs should be implemented as soon as possible for DM patients with ILD showing hyperferritinemia, especially for ferritin levels >1600 ng/ml.

Rheumatology key messages

- Ferritin and anti-MDA5 antibody are powerful indicators for early diagnosis of A/SIP with DM.
- Ferritin also predicts disease severity and prognosis for patients with anti-MDA5 antibody.

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References


