Clinical Significance of Serum Chitotriosidase Level in Anti-MDA5 Antibody–positive Dermatomyositis-associated Interstitial Lung Disease

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ABSTRACT. ***Objective.*** To assess prognostic factors of antimelanoma differentiation-associated gene 5 antibody (anti-MDA5)–positive dermatomyositis/clinically amyopathic DM–associated interstitial lung disease (DM/CADM-ILD) and evaluate the use of serum chitotriosidase, a marker for macrophage activation, as a potential biomarker in anti-MDA5-positive DM/CADM-ILD.

Methods. This retrospective study included 30 patients with anti-MDA5–positive DM/CADM-ILD. The clinical characteristics and laboratory findings at the time of diagnosis were analyzed. Serum chitotriosidase levels were measured in the 30 patients, in 21 healthy controls, and in 25 patients with anti-aminoacyl- tRNA synthetase antibody–positive (anti-ARS)-polymyositis (PM)/DM/CADM-ILD, and the potential of serum chitotriosidase as a prognostic biomarker in anti-MDA5–positive DM/CADM-ILD was assessed.

Results. The median serum chitotriosidase level in patients with anti-MDA5–positive DM/CADM-ILD was 17.3 ng/ml, which was higher than that in healthy controls and anti-ARS–PM/DM/CADM-ILD (2.0 and 8.9 ng/ml, respectively). Of the 30 patients, 10 died of respiratory failure associated with DM/CADM-ILD deterioration. Cox hazard analysis demonstrated that higher serum chitotriosidase level and lower PaO_2 value were significant predictors of a poor outcome. Using optimal cutoff levels according to receiver-operating characteristic curve analyses, chitotriosidase ≥ 23.5 ng/ml, ferritin ≥ 800 ng/ml, and Krebs von den Lungen–6 ≥ 720 U/ml were significantly associated with a poor prognosis. Serum chitotriosidase levels were negatively correlated with PaO_2 and percentage predicted forced vital capacity. The survival rate was significantly poorer in patients with high chitotriosidase levels (≥ 23.5 ng/ml) than in those with low chitotriosidase levels (< 23.5 ng/ml).

Conclusion. Serum chitotriosidase may be a potential biomarker for predicting a poor prognosis in patients with anti-MDA5–positive DM/CADM-ILD. (First Release May 15 2019; J Rheumatol 2019;46:935–42; doi:10.3899/jrheum.180825)

Key Indexing Terms:
DERMATOMYOSITIS INTERSTITIAL LUNG DISEASE PROGNOSTIC FACTORS
CHITOTRIOSIDASE ANTI-MDA5 ANTIBODY FERRITIN

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Accepted for publication November 15, 2018.

Polymyositis (PM) and dermatomyositis (DM) are systemic autoimmune diseases affecting the skeletal muscles, skin, and other organs, such as the lungs and joints[1]. Clinically amyopathic dermatomyositis (CADM) is a distinct subgroup of DM that involves a rash typical of classic DM, but with little or no evidence of muscular manifestations[2,3]. Patients with PM, DM, or CADM frequently show interstitial lung disease (ILD) as a pulmonary complication. The clinical course of ILD varies among patients[4,5,6]; however, this condition has been reported to be a major cause of morbidity and mortality[3,7,8,9,10].

Recent studies have demonstrated the importance of assessing serum myositis–specific antibodies (MSA) for predicting the clinical course and prognosis in patients with PM, DM, or CADM[11,12]. Antimelanoma differentiation–associated gene 5 antibody (anti-MDA5) is an MSA found in 20%–35% of patients with DM or CADM[13,14,15], and the detection of this antibody has been shown to be related to a

high ILD incidence and a poor prognosis[14,16,17,18,19]. We previously evaluated the MSA status in 60 patients with PM/DM/CADM-associated ILD and reported that 25% of the patients were positive for anti-MDA5 and that the seropositive status of anti-MDA5 was significantly associated with a poor prognosis[17]. Additionally, the major cause of death in anti-MDA5–positive patients was respiratory failure associated with acute/subacute deterioration of ILD within 6 months (90-day survival rate of 66.7% among the anti-MDA5–positive patients). Considering the poor prognosis of patients with anti-MDA5–positive DM/CADM–associated ILD (DM/CADM-ILD), the prognostic factors and serum biomarkers of disease severity should be evaluated for appropriate management and treatment in clinical practice. However, the factors and biomarkers for predicting a poor outcome in patients with anti-MDA5–positive DM/CADM-ILD remain to be determined.

Chitotriosidase, a member of the glycosyl hydrolase family, is a chitinase in humans that catalyzes the hydrolysis of both chitin and chitin-like substrates[20,21]. An increase in chitotriosidase activity was originally noted in Gaucher disease, a genetic disorder in which lipid accumulation in macrophages occurs in certain organs (e.g., spleen, liver, lungs, kidneys, and brain)[20,22]. Chitotriosidase is mainly produced by activated macrophages and neutrophils under inflammatory conditions[20,21,22], and it plays an important role in infectious diseases (e.g., fungal infections) as an innate immune response component against chitin present in the cell structure of pathogens. Chitotriosidase is considered a marker for macrophage activation and differentiation. Studies have demonstrated that serum chitotriosidase is a potential biomarker for monitoring several lung diseases, such as tuberculosis, sarcoidosis, chronic obstructive pulmonary disease, and asthma[23,24,25,26,27].

Anti-MDA5–positive DM/CADM can occasionally cause macrophage activation syndrome (MAS). In fatal cases of anti-MDA5–positive CADM-related rapidly progressive ILD, systemic activation of macrophages is observed in many organs, and it might be related to the pathogenesis[28]. Serum chitotriosidase has been highlighted as an indicator of macrophage activation and a potential biomarker; however, the clinical significance of the serum chitotriosidase level in patients with anti-MDA5–positive DM/CADM-ILD remains unclear. In our present study, we assessed the clinical features and prognostic factors of anti-MDA5–positive DM/CADM-ILD and evaluated the use of serum chitotriosidase as a potential biomarker in patients with anti-MDA5–positive DM/CADM-ILD.

MATERIALS AND METHODS

Subjects. This retrospective study included patients diagnosed with anti-MDA5-positive DM/CADM-ILD at Hamamatsu University Hospital and its affiliated hospitals in Japan between 2000 and 2017. DM was diagnosed according to the Bohan and Peter criteria[29], as described previously[4,6]. Patients with definite or probable DM were included. CADM was diagnosed according to the presence of a rash characteristic of DM and absence of clinical evidence of muscular disorders along with little or no increase in the serum creatine kinase level, as described previously[5,6,30]. To evaluate serum chitotriosidase levels, the study enrolled 21 age- and sex-matched healthy controls and 25 patients with anti-aminoacyl-tRNA synthetase antibodies (anti-ARS)–positive PM/DM/CADM-ILD (3 PM, 9 DM, 13 CADM). This study was approved by the Institutional Review Board of Hamamatsu University School of Medicine (approval number: E15-062).

Evaluation of ILD. ILD was diagnosed according to respiratory symptom presence, physical examination findings, chest high-resolution computed tomography (HRCT) findings, and pulmonary function test results. ILD was classified into the following 3 types: acute, subacute, and chronic. Acute ILD was defined as rapidly progressive ILD showing acute worsening or development of dyspnea with a new widespread alveolar abnormality on chest HRCT within 1 month from the onset of respiratory symptoms or the initial visit[17,31]. Subacute ILD was defined as progressive ILD with deterioration in 1–3 months[5,6,17,32]. Chronic ILD was defined as stable or slowly progressive ILD presenting with gradual deterioration over a period longer than 3 months[5,6,17,32]. ILD deterioration was assessed according to the modified version of the International Consensus Statement of idiopathic pulmonary fibrosis of the American Thoracic Society[33,34]. It was defined as the presence of 2 or more of the following findings during the followup period: (1) symptomatic exacerbation (e.g., dyspnea upon exertion); (2) an increase in opacity on chest HRCT; and (3) > 10% decrease in the percentage predicted forced vital capacity (%FVC) or > 10 mmHg decrease in arterial oxygen tension (PaO_2).

Data collection, measurement of anti-MDA5 titers, and measurement of serum chitotriosidase levels. Clinical data, including symptoms, physical findings, laboratory findings, and pulmonary function test results, were obtained from the medical records collected at the first encounter that led to the diagnoses of ILD and DM/CADM. Stored serum samples collected at the initial diagnosis of DM/CADM-ILD were used to measure anti-MDA5 titers and serum chitotriosidase levels. Anti-MDA5 titers were measured using ELISA[15], and serum chitotriosidase levels were determined using chitotriosidase ELISA (CircuLex Human Chitotriosidase ELISA Kit; MBL), according to the manufacturer's instructions.

Statistical analysis. We used either the chi-square test or Fisher's exact test (as appropriate for the sample size) for 2-group comparisons involving binary data. Comparisons involving continuous data were performed using the Mann–Whitney U test. Receiver-operating characteristic (ROC) curve analyses were performed to identify the optimal cutoff values of serum markers [ferritin, chitotriosidase, and Krebs von den Lungen–6 (KL-6)] for predicting a poor outcome. The cutoff value of each serum marker was decided as the point that has the highest value of sensitivity + specificity – 1 (Youden's index). Correlations between serum chitotriosidase levels and clinical variables were evaluated using the Spearman correlation coefficients test. Cox proportional hazard models were used to identify the variables associated with survival. Akaike information criterion (AIC) was used to compare relative abilities to predict poor outcome. The cumulative survival rate was calculated by using the Kaplan–Meier method. The log-rank test was used to compare the survival rate between patient groups. All statistical analyses were performed using commercially available software programs (JMP version 9.0: SAS Institute Inc., and R version 3.4.3: The R Foundation for Statistical Computing). A p value < 0.05 was considered statistically significant.

RESULTS

Baseline characteristics. The study included 30 patients with anti-MDA5-positive DM/CADM-ILD. Of these 30 patients, 13 (43%) had DM-ILD and 17 (57%) had CADM-ILD. The clinical characteristics of the enrolled patients are presented in Table 1. The median patient age was 54 years, and 23

Table 1. Clinical characteristics of study patients with anti-MDA5–positive DM/CADM-ILD.

Characteristics	n = 30
Median age, yrs	54.0 (31–80)
Female	23 (77)
Median observation period, yrs	1.01 (0.02–18.8)
Smoking status	
Never/former/current	18 (60)/8 (27)/4 (13)
Myositis diagnosis	
DM/CADM	13 (43)/17 (57)
ILD form	
Acute	10 (33)
Subacute	18 (60)
Chronic	2 (7)
Laboratory data	
Anti-MDA5 antibody, U/ml	185 (56–285)
Ferritin, ng/ml	664 (107–12,701)
Chitotriosidase, ng/ml	17.3 (0–53.2)
CPK, IU/l	110 (20–1790)
Aldolase, U/l	6.7 (3.0–27.2)
KL-6, U/ml	739 (249–2450)
SP-D, ng/ml	41.7 (17.1–228)
Pulmonary function	
PaO_2, Torr	69.0 (42.6–109)
%FVC, %	72.3 (36.6–125.5)*
Mortality	10 (33)

*Four patients in fatal cases could not undergo pulmonary function tests. Data are expressed as number (percentage) or median (range). DM: dermatomyositis; CADM: clinically amyopathic dermatomyositis; ILD: interstitial lung disease; anti-MDA5 antibody: antimelanoma differentiation-associated gene 5 antibody; CPK: creatine phosphokinase; KL-6: Krebs von den Lungen-6; SP-D: surfactant protein D; FVC: forced vital capacity.

(77%) of the 30 patients were women. Of the 30 patients, 10 (33%) had acute ILD, 18 (60%) had subacute ILD, and 2 (7%) had chronic ILD. The median serum ferritin and KL-6 levels were elevated [ferritin: 664 ng/ml (reference range: male, 13–277 ng/ml; female, 5–153 ng/ml); KL-6: 739 U/ml (reference range: ≤ 500 U/ml)]. The median (range) serum chitotriosidase levels in the patients with anti-MDA5-positive DM/CADM-ILD were significantly higher than those in healthy controls [17.3 (0–53.2 ng/ml) vs 2.0 (1.6–27.6 ng/ml), p = 0.0013] and relatively higher than those in patients with anti-ARS–positive PM/DM/CADM-ILD [8.9 (1.9–31.2 ng/ml), p = 0.36; Supplementary Figure 1, available with the online version of this article]. The median PaO_2 and %FVC values were relatively low. Of the 30 patients, 10 died of respiratory failure associated with DM/CADM-ILD deterioration within 4 months after diagnosis.

Comparisons of the clinical findings and treatments between survivors and nonsurvivors. Comparisons of the clinical findings between patients who survived (survivor group, n = 20) and those who died of respiratory failure (nonsurvivor group, n = 10) are presented in Table 2. The median patient age and patient sex were not different between the groups. In the survivor group, 1 patient (5%) had acute ILD, 17 (85%) had subacute ILD, and 2 (10%) had chronic ILD. In the nonsurvivor group, 9 patients (90%) had acute ILD and 1 (10%) had subacute ILD. No patient from the nonsurvivor group had chronic ILD. Regarding laboratory data, serum ferritin, chitotriosidase, and KL-6 levels were significantly

Table 2. Comparisons of the clinical findings between survivors and nonsurvivors.

Characteristics	Survivor, n = 20	Nonsurvivor, n = 10	p
Median age, yrs	54.5 (31–69)	54 (32–80)	0.48
Female	16 (80)	7 (70)	0.55
Median observation period, yrs	2.67 (0.33–18.8)	0.14 (0.02–0.28)	< 0.001
Myositis diagnosis			0.29
DM/CADM	10 (50)/10 (50)	3 (30)/7 (70)	< 0.001
ILD form, n (%)			
Acute	1 (5)	9 (90)	
Subacute	17 (85)	1 (10)	
Chronic	2 (10)	0	
Laboratory data			
Anti-MDA5 antibody, U/ml	183 (113–285)	213 (56–264)	0.16
Ferritin, ng/ml	511 (107–1480)	1078 (197–12,701)	0.022
Chitotriosidase, ng/ml	12.4 (0–39.8)	27.8 (3.8–53.2)	0.017
CPK, IU/l	95 (20–1498)	133 (24–1790)	0.63
Aldolase, U/l	6.15 (3.1–12.7)	8.95 (3.0–27.2)	0.26
KL-6, U/ml	660 (249–2450)	1026 (297–2230)	0.039
SP-D, ng/ml	56.6 (17.1–137)	30.6 (17.2–338)	0.17
Pulmonary function			
PaO_2, Torr	75.0 (57.9–109)	63.3 (42.6–69.4)	0.004
%FVC	79.3 (36.6–125.5)	51.3 (38.1–82.8)*	0.021

*Four patients in the nonsurvivor group could not undergo pulmonary function tests. Data are expressed as number (percentage) or median (range). DM: dermatomyositis; CADM: clinically amyopathic dermatomyositis; ILD: interstitial lung disease; anti-MDA5 antibody: antimelanoma differentiation-associated gene 5 antibody; CPK: creatine phosphokinase; KL-6: Krebs von den Lungen-6; SP-D: surfactant protein D; FVC: forced vital capacity.

higher in the nonsurvivor group than in the survivor group (p = 0.022, p = 0.017, and p = 0.039, respectively). However, the creatine phosphokinase and aldolase levels were not different between the groups. Additionally, the surfactant protein D levels were almost identical between the groups. The PaO_2 and %FVC values were significantly lower in the nonsurvivor group than in the survivor group (p = 0.004 and p = 0.021, respectively). However, 4 patients in the nonsurvivor group could not undergo pulmonary function tests.

Comparisons of treatments between the survivor and nonsurvivor groups are presented in Supplementary Table 1, available with the online version of this article. All patients in both groups received corticosteroids and immunosuppressive agents. The initial dose of prednisolone was comparable between the groups; however, the frequency of intravenous methylprednisolone pulse therapy (500–1000 mg/day for 3 days) was significantly higher in the nonsurvivor group than in the survivor group (p = 0.020). Combination therapy, including corticosteroids, calcineurin inhibitors (cyclosporine or tacrolimus), and intravenous cyclophosphamide (IV CYC), was administered to 8 patients (80%) from the nonsurvivor group and 1 patient (5%) from the survivor group (p < 0.0001). IV immunoglobulins were administered to 7 patients (70%) from the nonsurvivor group and 3 patients (16%) from the survivor group (p = 0.0035).

Cutoff levels of serum markers and correlations between serum chitotriosidase levels and clinical variables. ROC curve analyses were performed for ferritin, chitotriosidase, and KL-6 (Supplementary Figure 2, available with the online version of this article). The area under the curve (AUC) of serum ferritin for predicting a poor outcome was 0.760. Using 800 ng/ml as the cutoff level of serum ferritin, the sensitivity and specificity were 80.0% and 70.0%, respectively (Supplementary Figure 2A). The AUC of serum chitotriosidase for predicting a poor outcome was 0.778. Using 23.5 ng/ml as the cutoff level of serum chitotriosidase, the sensitivity and specificity were 70.0% and 80.0%, respectively (Supplementary Figure 2B). The AUC of serum KL-6 for predicting a poor outcome was 0.733. Using 720 U/ml as the cutoff level of serum KL-6, the sensitivity and specificity were 90.0% and 55.0%, respectively (Supplementary Figure 2C). The AUC of serum chitotriosidase was larger than the AUC of serum ferritin and KL-6.

Correlations between serum chitotriosidase levels and conventional clinical variables are presented in Table 3. Serum chitotriosidase levels showed significant negative correlations with PaO_2 (r = –0.52, p = 0.005) and %FVC (r = –0.43, p = 0.028).

Survival analysis. The results of Cox proportional hazard regression analysis are shown in Table 4. In univariate analysis, acute ILD, high serum chitotriosidase levels, and low PaO_2 values were significantly associated with a poor outcome in patients with anti-MDA5-positive DM/CADM-ILD. Using the optimal cutoff levels, serum ferritin (≥ 800 ng/ml), chitotriosidase (≥ 23.5 ng/ml), and KL-6 (≥ 720 U/ml) were significantly associated with a poor prognosis. Because serum markers can be evaluated noninvasively, even when patients have severe symptoms (e.g., respiratory failure), we focused on serum markers, ferritin, chitotriosidase, and KL-6. We performed separate multivariate Cox proportional hazard regression analyses of each serum marker, adjusted by age and sex. Multivariate Cox analyses demonstrated that higher serum chitotriosidase levels and higher serum ferritin levels, adjusted by age and sex, were significantly associated with a poor prognosis, and serum KL-6 levels were not (Table 4). Serum ferritin (≥ 800 ng/ml), chitotriosidase (≥ 23.5 ng/ml), and KL-6 (≥ 720 U/ml), adjusted by age and sex, were also associated with a poor prognosis (Table 4). To compare the predictive abilities of each model we evaluated the respective AIC indices. The AIC index of the chitotriosidase model (chitotriosidase, age, and sex) was 60.57, lower than the ferritin model (ferritin, age, and sex) and the KL-6 model (KL-6, age, and sex; 62.88 and 65.42, respectively). Thus, a higher serum chitotriosidase level is a better prognostic indicator in patients with anti-MDA5–positive DM/CADM-ILD. We performed survival analysis using the optimal cutoff level of serum chitotriosidase. The 30 study patients were divided into the following 2 groups according to the cutoff level of serum chitotriosidase: chitotriosidase^{high} (≥ 23.5 ng/ml, n = 11) and chitotriosidase^{low} (< 23.5 ng/ml, n = 19). Kaplan–Meier survival curves are presented in Figure 1. The mortality rate was significantly higher in the chitotriosidase^{high} group than in the chitotriosidase^{low} group (60% vs 20%, p = 0.003).

Table 3. Correlations between serum chitotriosidase levels and clinical variables.

Variables	r	p
Ferritin, ng/ml	0.14	0.46
CPK, IU/l	–0.07	0.71
Aldolase, U/l	0.10	0.58
KL-6, U/ml	0.30	0.11
SP-D, ng/ml	–0.21	0.27
PaO_2, Torr	–0.52	0.005
%FVC, %	–0.43	0.028

CPK: creatine phosphokinase; KL-6: Krebs von den Lungen-6, SP-D: surfactant protein D; FVC: forced vital capacity.

DISCUSSION

In our study, we evaluated the clinical significance of serum chitotriosidase as a potential biomarker in patients with anti-MDA5-positive DM/CADM-ILD. We found that the serum chitotriosidase level was significantly higher in patients with DM/CADM-ILD than in healthy controls. In particular, the serum chitotriosidase level was higher in patients with DM/CADM-ILD who had a fatal outcome than in surviving patients. A high serum chitotriosidase level and

Table 4. Cox proportional hazard regression analysis of mortality.

Analytical Data	HR	95% CI	p
Univariate analysis			
Age, yrs	1.04	0.979–1.11	0.21
Female	0.66	0.181–3.05	0.55
DM vs CADM	0.48	0.103–1.73	0.27
Acute ILD	34.7	6.34–647	< 0.0001
Ferritin, ng/ml	1.00	0.999–1.000	0.20
Chitotriosidase, ng/ml	1.06	1.01–1.10	0.01
CPK, IU/l	1.00	0.999–1.001	0.79
Aldolase, U/l	1.07	0.968–1.16	0.17
KL-6, U/ml	1.00	0.999–1.001	0.12
SP-D, ng/ml	1.00	0.988–1.02	0.61
PaO_2, Torr	0.87	0.783–0.946	0.0004
Ferritin ≥ 800 ng/ml	5.42	1.35–35.9	0.015
Chitotriosidase ≥ 23.5 ng/ml	6.09	1.68–28.5	0.006
KL-6 ≥ 720 U/ml	7.62	1.42–140.9	0.014
Multivariate analysis, adjusted for age and sex			
Ferritin, ng/ml	1.00	1.000–1.001	0.031
Chitotriosidase, ng/ml	1.06	1.02–1.11	0.008
KL-6, U/ml	1.00	0.999–1.001	0.15
Ferritin ≥ 800 ng/ml	6.06	1.45- 41.4	0.012
Chitotriosidase ≥ 23.5 ng/ml	6.06	1.65–28.5	0.007
KL-6 ≥ 720 U/ml	9.19	1.60–174	0.01

DM: dermatomyositis; CADM: clinically amyopathic dermatomyositis; ILD: interstitial lung disease; CPK: creatine phosphokinase; KL-6: Krebs von den Lungen-6, SP-D: surfactant protein D.

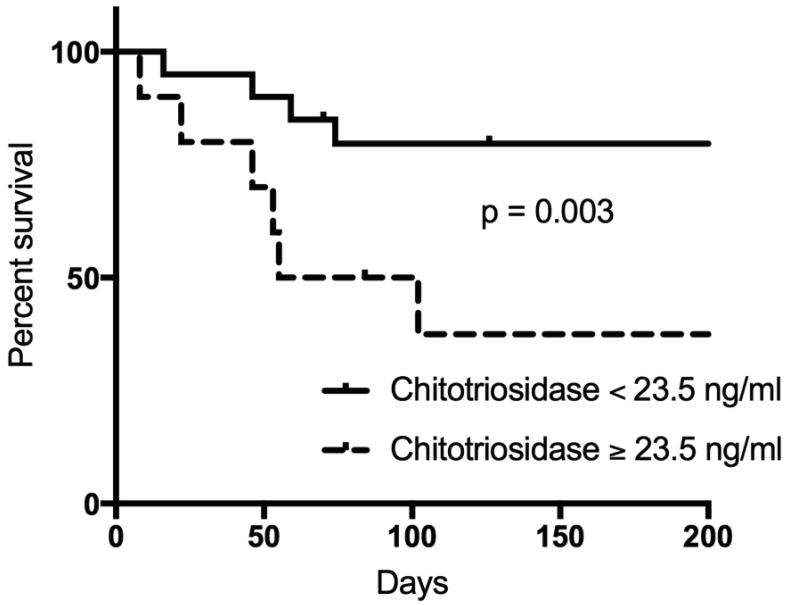

Figure 1. Survival curves for the chitotriosidase^{high} group and chitotriosidase^{low} group. The 30 study patients were divided into the following 2 groups according to the cutoff level of serum chitotriosidase: chitotriosidase^{high} (≥ 23.5 ng/ml, n = 11, dashed line) and chitotriosidase^{low} (< 23.5 ng/ml, n = 19, solid line). The mortality rate is significantly higher in the chitotriosidase^{high} group than in the chitotriosidase^{low} group (60% vs 20%, p = 0.003).

low PaO_2 value at the time of diagnosis were significant predictors of a poor outcome in patients with anti-MDA5–positive DM/CADM-ILD. Using optimal cutoff levels according to ROC curve analyses, we found that chitotriosidase ≥ 23.5 ng/ml, ferritin ≥ 800 ng/ml, and KL-6 ≥ 720 U/ml were significantly associated with a poor prognosis.

The AUC was the largest for serum chitotriosidase among the 3 markers. Moreover, serum chitotriosidase levels were correlated with oxygenation impairment and lung function. The survival rate was poorer in patients with high chitotriosidase levels (≥ 23.5 ng/ml) than in those with low chitotriosidase levels (< 23.5 ng/ml). These findings suggest the clinical significance of serum chitotriosidase as a prognostic biomarker in patients with anti-MDA5-positive DM/CADM-ILD.

To our knowledge, this is the first study to show that the serum chitotriosidase level was higher in patients with anti-MDA5–positive DM/CADM-ILD, particularly those with a fatal outcome, than in healthy controls and in anti-ARS–positive PM/DM/CADM-ILD, and that the serum chitotriosidase level was associated with a poor prognosis in anti-MDA5–positive DM/CADM-ILD. Chitotriosidase is produced by monocyte-derived macrophages, lung macrophages, neutrophils, and epithelial cells in the lungs and intestine[35,36,37]. Its expression is upregulated by Toll-like receptor stimulation, interferon (IFN)-γ, tumor necrosis factor (TNF)-α, and granulocyte–macrophage colony-stimulating factor (GM-CSF)[35,38]. Anti-MDA5–positive DM/CADM is partially considered a type of MAS, in which uncontrolled activation of macrophages and T lymphocytes and marked increases in the levels of circulating cytokines [including TNF-α, interleukin (IL)-1β, IL-6, IL-18, IFN-β, and GM-CSF] are involved in the pathogenesis[39,40]. Elevated serum chitotriosidase levels might reflect the pathophysiology of anti-MDA5–positive DM/CADM-ILD, including the excessive production of proinflammatory cytokines and activation of alveolar macrophages and neutrophils in the lungs. Further investigations involving lung biopsy or bronchoalveolar lavage are required to elucidate the underlying pathophysiology of anti-MDA5–positive DM/CADM-ILD.

Serum chitotriosidase levels might reflect disease severity in patients with anti-MDA5–positive DM/CADM-ILD. Indeed, the present study demonstrated that serum chitotriosidase levels were negatively correlated with PaO_2 and %FVC in patients with anti-MDA5–positive DM/CADM-ILD. Lee, *et al* showed that plasma chitotriosidase activity and chitotriosidase protein expression in lung specimens were significantly higher in patients with systemic sclerosis–associated ILD than in healthy controls and that chitotriosidase activity was negatively correlated with %FVC[37]. Additionally, they used animal models and demonstrated that bleomycin-induced pulmonary fibrosis was significantly reduced in chitotriosidase null mice and enhanced in chitotriosidase overexpression transgenic mice, indicating that chitotriosidase enhanced disease severity and could be a therapeutic target for pulmonary fibrosis. Interestingly, a study involving a bleomycin-induced lung injury model, using human MUC1-expressing mice, demonstrated that increased serum KL-6 levels were associated with the degree of lung injury and disruption of the alveolar-capillary barrier[41]. In our present study, serum KL-6 levels, as well as serum chitotriosidase levels and ferritin levels, were significantly higher in the nonsurvivor group than in the survivor group. This suggests that KL-6 levels reflect the extent of lung injury in anti-MDA5–positive DM/CADM-ILD. Given that an elevated KL-6 level may reflect disease severity for both anti-MDA5–positive DM/CADM-ILD and bleomycin-induced lung injury, partial similarity of pathological condition between anti-MDA5–positive DM/CADM-ILD and bleomycin-induced lung injury might exist. Further studies are necessary to determine the detailed pathological conditions in anti-MDA5–positive DM/CADM-ILD.

Few studies have evaluated potential biomarkers in patients with anti-MDA5–positive DM/CADM-ILD[16,42]. Gono, *et al* showed that serum ferritin levels ≥ 828 ng/ml and alveolar–arterial oxygen difference ≥ 32 mmHg were associated with a poor prognosis in rapidly progressive ILD patients with anti-MDA5–positive DM[16]. Consistent with these findings, our study demonstrated that serum ferritin levels ≥ 800 ng/ml, a low PaO_2 value, and a high serum chitotriosidase level were significantly associated with a poor prognosis in patients with anti-MDA5–positive DM/CADM-ILD. Serum ferritin is a key biomarker of MAS, and thus, high serum ferritin levels might be attributable to the systemic activation of macrophages, similar to high chitotriosidase levels, which might be a key feature in the pathogenesis of anti-MDA5–positive DM/CADM-ILD.

In our study, serum chitotriosidase levels showed no significant correlation with serum ferritin levels in patients with anti-MDA5–positive DM/CADM-ILD (Table 3). Cellular sources and activators of chitotriosidase may not be identical to those of ferritin, although both are produced by activated macrophage. Chitotriosidase is produced by macrophages, neutrophils, and epithelial cells under inflammatory conditions[35,36,37], while ferritin can be secreted by various cell types such as lymphocytes as well as macrophages[43,44]. Pathological conditions of anti-MDA5–positive DM/CADM-ILD are complicated and require further determination. This may affect the diversity of elevated level of serum chitotriosidase and ferritin. Moreover, the range of serum ferritin levels was much greater (107–12701 ng/ml) than chitotriosidase levels (0–53.2 ng/ml). Further, our study population was relatively small, which may have influenced the results of our statistical analyses. Because both serum chitotriosidase and ferritin levels were higher in the nonsurvivor group than the survivor group, further investigations are warranted to validate the correlation between serum chitotriosidase levels and serum ferritin levels in anti-MDA5–positive DM/CADM-ILD.

KL-6, a glycoprotein secreted by type II alveolar pneumocytes and bronchiolar epithelial cells, has been identified as a serum biomarker for monitoring a variety of ILD conditions, including PM/DM-ILD[45,46,47]; however, its prognostic value in patients with anti-MDA5–positive DM/CADM-ILD

has not been evaluated. In our study, we showed that KL-6 levels were higher in the nonsurvivor group than in the survivor group and that KL-6 levels ≥ 720 U/ml were associated with a poor prognosis. On comparing chitotriosidase, ferritin, and KL-6 as prognostic biomarkers in patients with anti-MDA5–positive DM/CADM-ILD, we found that the AUC of serum chitotriosidase was larger than those of serum ferritin and KL-6, and the AIC index of chitotriosidase model was lower than those of ferritin and KL-6 models. This suggests that serum chitotriosidase is a better prognostic predictor in patients with anti-MDA5–positive DM/CADM-ILD. Large-scale prospective observational studies are warranted to validate prognostic significance of serum markers in patients with anti-MDA5–positive DM/CADM-ILD.

Our study had several limitations. First, the number of study patients was small. Because of the small sample size (10 deaths among 30 patients), the findings of the multivariate analysis involving survival should be carefully interpreted. Second, the treatment regimen was not consistent among the study patients. The frequency of combination therapy, including corticosteroids, calcineurin inhibitors (cyclosporine or tacrolimus), and IV CYC, was higher in the nonsurvivor group than in the survivor group, suggesting that the disease activity among patients in the nonsurvivor group was severe although intensive treatments were administered. Finally, this study was retrospective, and the observation period varied among the patients. Studies have demonstrated the utility of new potential biomarkers, such as serum progranulin, serum YKL-40, and soluble CD163[48,49,50], in patients with PM/DM-ILD; however, all those studies were retrospective with a relatively small number of patients. Larger prospective studies are needed to further assess the prognostic factors and validate the prognostic significance of serum chitotriosidase in patients with anti-MDA5–positive DM/CADM-ILD.

We found that a high serum chitotriosidase level at the time of diagnosis was significantly associated with a poor outcome in patients with anti-MDA5–positive DM/CADM-ILD. Using the optimal cutoff levels of serum chitotriosidase, ferritin, and KL-6, we showed that these markers could predict a poor prognosis. The AUC of serum chitotriosidase was larger than the AUC of serum ferritin and KL-6. Further, serum chitotriosidase levels were correlated with oxygenation impairment and lung function, suggesting that serum chitotriosidase may be a promising noninvasive prognostic biomarker in patients with anti-MDA5–positive DM/CADM-ILD.

ACKNOWLEDGMENT

The authors thank C. Karasawa and Y. Morita from Medical and Biological Laboratory Co. Ltd., Tokyo, Japan, for assistance in the measurement of serum chitotriosidase levels. The authors also thank Dr. Kazutaka Mori from the Department of Respiratory Medicine, Shizuoka City Shimizu Hospital, Shizuoka, Japan, for his assistance in statistical analyses.

ONLINE SUPPLEMENT

Supplementary material accompanies the online version of this article.

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