Serum Krebs von den Lungen-6 concentrations reflect severity of anti-melanoma differentiation-associated protein 5 antibody positive dermatomyositis associated interstitial lung disease

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Abstract

Objective

Rapidly progressive interstitial lung disease (RP-ILD) is a major complication of anti-melanoma differentiation-associated protein 5 antibody positive dermatomyositis (anti-MDA5$^+$DM) with a high mortality rate. The aim of the study is to determine whether serum Krebs von den Lungen-6 (KL-6) could be a prognostic biomarker to predict RP-ILD and prognosis in anti-MDA5$^+$DM patients.

Methods

A total of 21 anti-MDA5$^+$DM patients with RP-ILD and 20 anti-MDA5$^+$DM patients without RP-ILD were retrospectively included in this study. Serum KL-6 concentration (pg/mL) was measured using the latex agglutination test.

Results

Serum KL-6 level was higher in RP-ILD patients than those in non-RR-ILD patients (1195.61±872.93 vs. 452.6±465.51 pg/mL, p=0.002). The best cut-off value of KL-6 serum level was 500.9 pg/mL using ROC curve (AUC area = 0.7976, p=0.0011). KL-6 >500.9 pg/mL was an independent risk factor for RP-ILD using multivariate analysis (OR=56.38, 95% CI 5.51-577.504, p=0.001). Serum KL-6 concentrations were significantly higher in dead patients than those in the survivor group (1209.34±840.55 vs. 592.41±667.76, p=0.0033), and higher KL-6 concentration was also an independent risk factor for all-cause death after adjusting confounders (OR = 21.94, 95% CI 3.3-145.73, p=0.001). Anti-MDA5$^+$DM patients with higher KL-6 level displayed a significantly decreased one-year survival rate, as compared with lower KL-6 level (36.36% vs. 89.47%, p=0.0008).

Conclusion

The serum KL-6 levels reflect severity of lung injury and serve as a clinically useful biomarker in detection and monitoring RP-ILD progression in anti-MDA5$^+$DM patients.

Key words

dermatomyositis, melanoma differentiation-associated protein 5 antibody, serum Krebs von den Lungen-6, rapidly progressive interstitial lung disease, mortality

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Introduction

Anti-melanoma differentiation-associated protein 5 antibody positive dermatomyositis (anti-MDA5+DM) is a subset of dermatomyositis that characterised with unique cutaneous features, subtle or no muscle involvement and interstitial lung disease (ILD) (1). ILD is one of the most common manifestation of anti-MDA5+DM with a strong heterogeneity, varying from mild or moderate ILD to a life-threating rapidly progressive interstitial lung disease (RP-ILD). Especially in East Asian, approximately 40% (20-75%) anti-MDA5+DM patients develop RP-ILD (2, 3). Despite aggressive therapy, six-month mortality of anti-MDA5+DM associated RP-ILD is as high as 50-70% (4). To date, there is little information about how the interstitial lung damage evolve in these patients. Identifying biomarkers that associated with severity of lung injury in anti-MDA5+DM patients is essential for early detection and monitoring of RP-ILD, and supporting treatment decision-making.

Krebs von den Lungen-6 (KL-6, also known as mucin-1) is a serum high molecular weight glycoprotein, and tend to highly expressed in injured or regenerating epithelial cells than in normal epithelial cells (5). In normal lungs, KL-6 participates in regulation proliferation and survival of lung fibroblasts (6). In the case of alveolar epithelial lesions, the increased alveolo-capillary permeability resulted in the elevated serum concentration of KL-6 (7). The increased serum KL-6 levels have been found in a majority of ILDs, including idiopathic pulmonary fibrosis, hypersensitivity pneumonitis and multiple connective tissue disease-associated interstitial lung disease (CTD-ILD) (8-11). KL-6 might serve as a useful biomarker in assessment of disease severity, and prediction of treatment outcome and disease progressing (12, 13). Importantly, high KL-6 concentrations have been suggested as a prognostic marker of ARDS and is significantly associated with risk of mortality (14, 15). Recently, KL-6 was reported to be associated with severe status and poor prognosis COVID-19 (16, 17).

The aim of this study was to evaluate KL-6 levels in anti-MDA5+DM patients with ILD, to determine whether KL-6 could be a prognostic biomarker of severity.

Materials and methods

Study population

21 cases of anti-MDA5+DM patients with RP-ILD, and a control group of 20 anti-MDA5+DM patients who did not develop RP-ILD were enrolled in the current study. All patients were hospitalised at the First Affiliated Hospital of Nanjing Medical University, China, from September 2019 to March 2021. The diagnosis of myositis in all patients met the European NeuroMusclar Center (ENMC) criteria or Sontheimer criteria (18, 19). All patients were positive for anti-MDA5 antibodies. Informed consent was obtained from each study participant. The study protocol was approved by the Ethics Committee of the First Affiliated Hospital of Nanjing Medical University Hospital (ID: 2020-SR-265).

Measurement of KL-6 and anti-MDA5 antibodies

Serum KL-6 concentration (pg/mL) was measured using latex agglutination test (Nanopia KL-6, SEKISUI MEDICAL, Japan). Anti-MDA5 antibodies were measured using an immunoblotting method (EUROLINE, EUROIMMUN AG, Germany).

Diagnosis of ILD and RP-ILD

The diagnosis of ILD and RP-ILD was based on both clinical signs and HRCT can features (20, 21). The RP-ILD was defined as the presence of any the following four conditions within one month: 1) dyspnoea or cough symptoms become progressively worse and quality of life is significantly reduced; 2) decreased lung function including forced vital capacity (FVC) decreased by more than 10%, or diffusion capacity for carbon monoxide of the Lung (DLCO) fell over 15% with the decreased FVC; 3) high resolution CT (HRCT) of chest demonstrated that the extent of interstitial pneumonia continues to increase; 4) arterial blood gas analysis suggested respiratory failure or the oxygen partial pressure reduc-

Table I. Clinical manifestations and laboratory features of anti-MDA5+DM patients with RP-ILD and non-RP-ILD.

Parameters	RP-ILD	Non-RP-ILD	*p*-value
Case number	21	20	
Gender, female, no. (%)	13 (61.9%)	13 (65%)	0.837
Age, mean ± SD, years	55.33 ± 11.14	47.45 ± 14.24	0.055
Myasthenia, no. (%)	11 (52.4%)	14 (70.0%)	0.248
Rash, no. (%)	18 (85.7%)	14 (70.0%)	0.627
Gottron's sign, no. (%)	15 (71.4%)	11 (55.0%)	0.275
Heliotrope rash, no. (%)	6 (28.6%)	9 (45.0%)	0.275
V sign, no. (%)	10 (47.6%)	9 (45.0%)	0.867
Shawl sign, no.(%)	4 (19.0%)	6 (30.0%)	0.414
Periungual erythema, no. (%)	3 (14.3%)	4 (20.0%)	0.627
Skin ulcers, no. (%)	1 (4.8%)	1 (5.0%)	0.972
Mechanic's hands, no. (%)	3 (14.3%)	2 (10.0%)	0.675
Arthritis, no. (%)	6 (28.6%)	4 (20.0%v	0.523
ESR, mean±SD, mm/H	48.90 ± 21.70	30.95 ± 18.49	0.007
SF, mean±SD, ng/mL	1651.06 ± 1101.21	861.21 ± 932.17	0.018
KL-6, mean±SD, pg/mL	1195.61 ± 872.93	452.6 ± 465.51	0.002

*Data are presented as mean±SD or case number (percentage). Student's t-test and Pearson's Chi-square test were used to analysis.
Anti-MDA5+DM: anti-melanoma differentiation-associated protein 5 antibody positive dermatomyositis; RP-ILD: rapidly progressive interstitial lung disease; ESR: erythrocyte sedimentation rate; SF: serum ferritin; KL-6: Krebs von den Lungen-6.

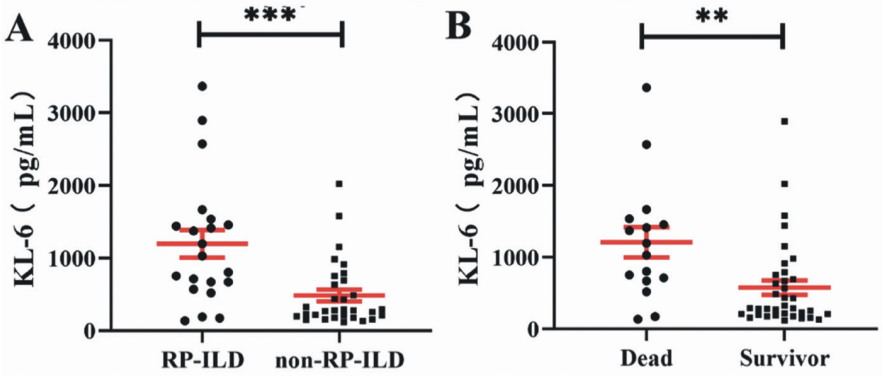

Fig. 1. Serum KL-6 concentration in anti-MDA5+DM patients with different prognosis.
A. The dot plots show the difference in serum KL-6 concentration between anti-MDA5+DM patients with and without RP-ILD; B. Differences in serum KL-6 concentrations at baseline between survivors and deceased patients at 12 months of follow-up.
***p<0.001; **p<0.01.

tion is greater than 10 mmHg, independently determined by senior physicians in the rheumatology department and the respiratory department.

Statistical analysis

Data were entered and self-tested using Microsoft Office Excel 2019, statistically analysed and graph drawn using IBM SPSS Statistics 23.0 and Graphpad Prism 8.4.2. Before the analysis, hypothesis testing was performed using normal probability plots to observe whether the detected values followed normal distribution. Normal distribution measurement data were expressed as mean ± standard deviation, whereas the skewed distribution measurement data were expressed as median. The two sets of measurement data were compared by Student's t-test, while comparison of categorical data was done by Pearson's chi-square test. All transformed dichotomous variables were test by univariate logistic analysis to screen whether it is a related factor of the occur of RP-ILD and death. Furthermore, candidate parameters in univariate logistic analysis based on statistical trend with p<0.1 were included in the following multivariate analysis. P<0.05 was considered statistically significant, and all statistical tests were two-tailed probability tests.

Results

Characteristics of anti-MDA5+DM patients with RP-ILD and non-RP-ILD

A total of 21 patients diagnosed with anti-MDA5+DM with RP-ILD and 20 anti-MDA5+DM patients without RP-ILD were included in this study. In the RP-ILD group, 13 were females, same value as that of the non-RP-ILD group. And the mean age was 55.33±11.14 and 47.45±14.24 years old respectively. There were no statistically significant differences in clinical manifestations between the two groups. However, serum average levels of ESR (48.90±21.70 *vs.* 30.95±18.49 mm/H, p=0.007) and SF (1651.06±1101.21 *vs.* 861.21±932.17 ng/mL, p=0.018) were markedly increased in RP-ILD group as compared with those in non-RP-ILD group (Table I).

Serum KL-6 levels in anti-MDA5+DM patients with RP-ILD and non-RP-ILD

When anti-MDA5+DM patients stratified by ILD status, serum KL-6 level were approximately 2 folds higher in RP-ILD patient than those in non-RR-ILD patients (1195.61±872.93 *vs.* 452.6±465.51 pg/mL, p=0.002) (Fig. 1A). Furthermore, in order to obtain the cut-off value of serum KL-6 concentration for predicting the poor prognosis of anti-MDA5+DM patients, ROC curve analysis was performed. The data showed that 500.9 pg/mL has the highest diagnostic efficiency to distinguish the patients with or without RP-ILD (AUC area=0.7976, 95% CI=0.6524-0.9428; Sensitivity 80.0%; Specificity 85.71%; p=0.0011) (Fig. 2). In order to further clarify the application efficiency of the above cut-off value in anti-MDA5+DM patients with RP-ILD, we next performed the logistic regression analysis. After continuous variables were transformed into dichotomies, the results of univariate analysis showed that age >50 years, ESR >21mm/H and KL-6 >500.9 pg/mL were candidate parameters as possible risk factors for anti-MDA5+DM with RP-ILD. When they were included into the multivariate equation, KL-6 >500.9 pg/mL was an independent risk factor for RP-ILD in anti-MDA5+DM patients (Odd Ratio 56.384, 95% CI

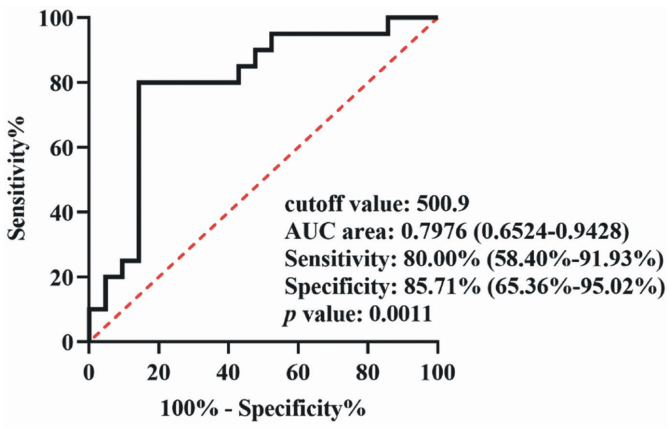

Fig. 2. ROC curve of serum KL-6 concentration in anti-MDA5+DM patients. ROC curve shows the area under the curve (AUC) of serum KL-6 concentration in anti-MDA5+DM patients based on whether the patient has developed RP-ILD.

Table II. Logistic analysis of RP-ILD influenced by characteristics of anti-MDA5+DM patients.

RP-ILD	Unadjusted		Adjusted	
	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Gender, female	1.393 (0.395-4.917)	0.607		
Age, > 50 years	3.018 (0.845-10.776)	0.089	5.499 (0.551-54.912)	0.147
Myasthenia	0.471 (0.131-1.702)	0.471		
Rash	0.500 (0.042-5.990)	0.584		
Gottron's sign	2.045 (0.561-7.455)	0.278		
Heliotrope rash	0.489 (0.134-1.782)	0.278		
V sign	1.111 (0.325-3.796)	0.867		
Shawl sign	0.549 (0.129-2.339)	0.417		
Periungual erythema	0.667 (0.129-3.442)	0.628		
Skin ulcers	0.950 (0.055-16.293)	0.972		
Mechanic's hands	1.500 (0.223-10.077)	0.677		
Arthritis	2.267 (0.481-10.680)	0.301		
ESR, > 21 mm/H	5.542 (0.983-31.249)	0.052	4.670 (0.367-59.348)	0.235
SF, > 336.2 ng/mL	3.000 (0.626-14.371)	0.169		
KL-6, > 500.9 pg/mL	24.000 (4.649-123.904)	<0.001	56.384 (5.505-577.504)	0.001

*Binary logistical regression analysis was used in regression equation. Age >50 years, ESR >21 mm/H and KL-6 >500.9 pg/mL were put into the multivariate regression analysis.
Anti-MDA5+DM: anti-melanoma differentiation-associated protein 5 antibody positive dermatomyositis; RP-ILD: rapidly progressive interstitial lung disease; ESR: erythrocyte sedimentation rate; SF: serum ferritin; KL-6: Krebs von den Lungen-6.

5.505-577.504, *p*=0.001) (Table II).

Finally, we conducted a Kaplan-Meier analysis of RP-ILD by the occurrence time. All patients who did not developed RP-ILD were also followed for a minimum of 12 months. The result showed that the 1-year RP-ILD incidence rate significantly higher in patients whose serum KL-6 concentration above 500.9 pg/mL, compared with lower serum KL-6 level (81.82% *vs.* 15.79%, *p*<0.0001) (Fig. 3A).

Predictive value of KL-6 for death in anti-MDA5+DM patients

In the current study, the all-cause mortality rate of all anti-MDA5+DM patients was 39.02%. All deaths occurred in RP-ILD group due to respiratory failure. And our data showed KL-6 serum concentrations were significantly higher in dead patients than the survivor group (1209.34±840.55 *vs.* 592.41± 667.76, *p*=0.0033) (Fig. 1B).

Using the above logistic regression statistical strategy, we found that serum KL-6 concentration >500.9 pg/mL was also a strong independent risk factor for all-cause death in anti-MDA5+DM patients after adjusting confounders (odds ratio 21.943, 95% CI 3.304–145.732, *p*=0.001) (Table III).

The Kaplan-Meier survival curve showed patients with serum KL-6 level >500.9 pg/mL displayed a significantly decreased one-year survival rate, as compared with lower KL-6 level (36.36% *vs.* 89.47%, *p*=0.0008) (Fig. 3B).

Discussion

In the current study, high KL-6 levels were an independently risk factor for RP-ILD in anti-MDA5+DM patients. KL-6 levels were also a prognostic factor for the mortality in these patients. ILD, especially RP-ILD, is a major complication in anti-MDA5+DM patients. These findings indicate that serum KL-6 can act as a surrogate marker for disease progression and disease severity in anti-MDA5+DM patients with RP-ILD.

KL-6 produced by damaged or regenerating type II alveolar epithelial cells. KL-6 levels are widely used as biomarkers for the diagnosis, severity assessment and prognosis prediction of various ILDs patients, such as hypersensitivity pneumonitis, idiopathic pulmonary fibrosis (IPF) and pulmonary Langerhans' cell histiocytosis (9, 22). Specifically, IPF patients with baseline plasma KL-6 levels ≥2.5 ng/mL had a higher risk of nintedanib-related hepatic injury and on-treatment acute-exacerbation (23). Several reports also revealed that serum KL-6 levels were higher in multiple CTD-ILD including rheumatoid arthritis, scleroderma and polymyositis/dermatomyositis-associated ILD (24-26).

Our interest was aroused by the observation that KL-6 was correlated with prognosis in ARDS and severe COVID-19 pneumonia (17, 27, 28). In accordance with these findings, our study revealed, serum KL-6 levels were significantly increased in anti-MDA5+DM with RP-ILD patients, compared with the non-RP-ILD group. Moreover, with a cut-off value of 500.9 pg/ml used, serum KL-6 can help distinguish RP-ILD patients from anti-MDA5+DM patients. Patients with high levels of serum KL-6 tend to have a lower one-year survival rate. Thus, similar with the effect of KL-6 in ARDS or COVID-19, our date suggested that serum KL-6 levels reflected the severity of lung injury, and was able to predict RP-ILD progression in anti-MDA5+DM patients.

RP-ILD is a life-threaten complication in anti-MDA5+DM. Early diagnosis and treatment are the keys to improve prognosis. Several serum risk factors including anti-MDA5 antibodies titre,

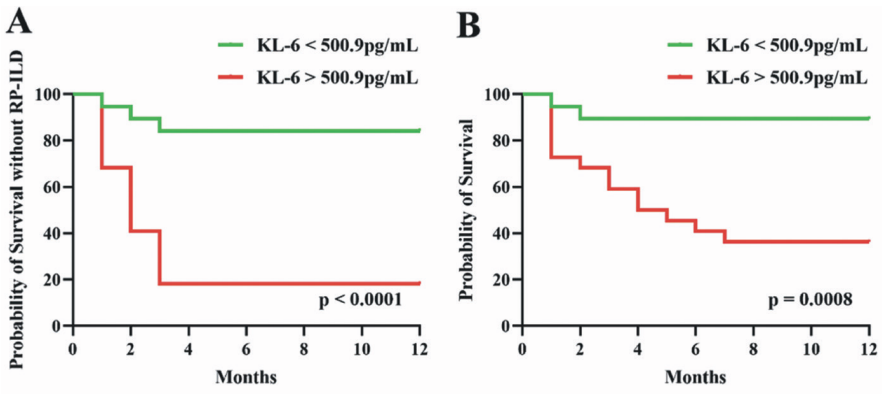

Fig. 3. Kaplan-Meier analysis of poor prognosis based on serum KL-6 concentration.
A: Kaplan-Meier non-RPILD survival curves for Whether serum KL-6 concentration is elevated using 500.9 pg/mL as the cut-off value.
B: Kaplan-Meier survival curves for Whether serum KL-6 concentration is elevated using 500.9 pg/mL as the cut-off value.

Table III. Logistic analysis of death influenced by characteristics of anti-MDA5+DM patients.

Death	Unadjusted		Adjusted	
	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Gender, female	1.383 (0.384-4.981)	0.620		
Age, >50 years	2.500 (0.688-9.084)	0.164		
Myasthenia	0.723 (0.201-2.605)	0.620		
Rash	0.292 (0.024-3.515)	0.332		
Gottron's sign	4.000 (0.910-17.579)	0.066	7.213 (1.169-44.491)	0.033
Heliotrope rash	1.067 (0.291-3.916)	0.923		
V sign	0.843 (0.239-2.975)	0.790		
Shawl sign	0.593 (0.129-2.738)	0.504		
Periungual erythema	0.571 (0.097-3.376)	0.537		
Skin ulcers	1.600 (0.093-27.547)	0.746		
Mechanic's hands	1.048 (0.155-7.079)	0.962		
Arthritis	0.731 (0.154-3.461)	0.693		
ESR, >21 mm/H	2.882 (0.514-16.150)	0.229		
SF, >336.2 ng/mL	3.062 (0.544-17.230)	0.204		
KL-6, >500.9 pg/mL	14.875 (2.708-81.695)	0.002	21.943 (3.304-145.732)	0.001

*Binary logistical regression analysis was used in regression equation. Gottron's sign and KL-6 >500.9 pg/mL were put into the multivariate regression analysis.
Anti-MDA5+DM: anti-melanoma differentiation-associated protein 5 antibody positive dermatomyositis; RP-ILD: rapidly progressive interstitial lung disease; ESR: erythrocyte sedimentation rate; SF: serum ferritin; KL-6: Krebs von den Lungen-6.

CRP, LDH, SF and surfactant protein D levels are implicated in the development of RP-ILD (26, 29-31). In the current study, univariate logistic analysis indicated ESR and KL-6 levels are significantly associated with RP-ILD risk. Given ESR or CRP, LDH and SF are non-specific serum marker for ILD during disease course, the increased serum concentration of KL-6 might provide a direct evidence of lung injury in anti-MDA5+DM patients with ILD. Indeed, it is difficult to predict or assess the disease severity on single factors. In a MCK model that using KL-6 levels combined CRP levels and anti-MDA5 antibody status could well predict prognosis in patients with PM/DM-ILD (26). Considering cost-effectiveness, serum KL-6 level could be a simple easy-used warning marker for monitoring the severity of ILD. We proposed that frequently KL-6 measurements combined with HRCT and arterial blood gas analysis might provide the best clues as to acuteness and severity of disease exacerbation in anti-MDA5+DM patients.

This study had several limitations. First, anti-MDA5+DM is a rare autoimmune disease, resulting in a relatively small sample used in current the study. Second, FVC and DLCO values have been reported as risk factors for RP-ILD and poor prognosis in patients with anti-MDA5+DM (32), lung function tested were not performed in all patients. Third, although all patients followed up, we did not obtain blood donation from all patients, which making us failed to monitor the dynamic changes of KL-6 or correlate KL-6 levels with treatment response. These results are need to further validation in a large and prospective cohort.

High serum KL-6 levels reflect severity of lung injury and can help identify RP-ILD patients in anti-MDA5+DM, and serum KL-6 could serve as a clinically useful biomarker in detection and monitoring disease progression anti-MDA5+DM with RP-ILD.

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