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Serum KL-6 level is a prognostic marker in patients with anti-MDA5 antibody-positive dermatomyositis associated with interstitial lung disease

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Abstract

Objective: This study aimed to investigate the clinical significance of Krebs von den Lungen-6 (KL-6) serum levels in patients with anti-MDA5 antibody-positive dermatomyositis (anti-MDA5$^+$ DM) having interstitial lung disease (ILD), especially in the amyopathic DM phenotype.

Methods: The serum KL-6 level was measured using a chemiluminescence enzyme immunoassay (CLEIA) in patients with anti-MDA5$^+$ DM, including clinically amyopathic dermatomyositis (CADM)-ILD and classic DM-ILD, and healthy donors. The baseline and post-treatment serum KL-6 levels were determined in 39 patients with CADM-ILD who experienced remission or acute exacerbation. The association between laboratory findings, high-resolution computed tomography (HRCT) scores, pulmonary function tests (PFTs), and the predictive value of baseline KL-6 level for death was analyzed.

Results: The serum KL-6 levels were significantly higher in patients with CADM-ILD (1339 ± 1329 U/mL) compared with DM-ILD (642.3 ± 498.4 U/mL) and healthy donors (162.4 ± 54.01 U/mL). The KL-6 levels correlated positively with chest HRCT scores, serum lactate dehydrogenase, serum ferritin levels, and PFTs, but not with erythrocyte sedimentation rate. During follow-up, the post-treatment serum KL-6 levels significantly reduced in the remission/stable group, but increased in the acute exacerbation group. Higher levels of ferritin and KL-6 and HRCT scores were independently associated with poor prognosis. The 1-year survival rate was significantly lower in patients with high KL-6 level than in those with low KL-6 level.

Conclusion: The serum KL-6 levels may be a useful marker for predicting and monitoring ILD in Chinese patients with anti-MDA5$^+$ DM, especially amyopathic DM phenotype.

KEYWORDS

anti-MDA5$^+$ dermatomyositis, clinically amyopathic dermatomyositis, interstitial lung disease, KL-6, serum marker

1 | INTRODUCTION

Inflammatory myopathies (IIM) are a heterogeneous group of disorders. They are classified into four subtypes: dermatomyositis (DM), polymyositis (PM), necrotizing autoimmune myositis (NAM), and inclusion-body myositis (IBM).[1] An intriguing study has recently reported on the histopathologic, immunopathologic, and gene expression differences in muscle biopsy tissue from adult patients with DM with and without circulating anti-MDA5 antibody (Ab).[2] Anti-MDA5$^+$ DM, as an emerging entity with a variable clinical presentation, is drawing much more attention. Anti-MDA5 Ab was originally identified in patients with clinically amyopathic dermatomyositis (CADM) having an increased risk for potentially fatal, rapidly progressive interstitial lung disease (RPILD). CADM, as a unique phenotype of IIM, is defined as having cutaneous manifestations typical of DM, such as heliotrope rashes and Gottron's papules, but without clinically significant muscle involvement.[1] Over the years, CADM has become a well-recognized clinical entity despite its relative rareness and high mortality.[3]

Interstitial lung disease is the most common internal organ involvement affecting the prognosis of patients with anti-MDA5$^+$ DM.[4] Pulmonary involvement in the form of ILD is associated with increased mortality, but ranges from subclinical to rapidly progressive and fatal.[5] Patients with CADM having anti-MDA5 Ab are at high risk of developing RPILD.[4,5] CADM-RPILD is often refractory to intensive therapy, such as high-dose corticosteroids and immunosuppressive agents, resulting in respiratory failure with a 6-month survival rate of 40.8%–54.5%, which is notably lower than that for classic DM-ILD.[4,5] This might be because of the higher prevalence of acute subtype of ILD in patients with CADM.[4] Another main reason for this poor outcome is that a large number of patients who progress rapidly have atypical clinical manifestations and biomarkers at initial diagnosis. Anti-MDA5 Ab, an important tool for supporting initial diagnosis, has been demonstrated to strongly correlate with the risk of developing RPILD in East Asian adults.[6] Nevertheless, their clinical utility still needs to be investigated.[7] In clinical practice, not all patients with anti-MDA5$^+$ DM develop RPILD. The presence of anti-MDA5 Ab is associated with higher mortality in the acute phase of CADM-ILD, but not in the chronic phase.[8] Moreover, the autoantibody does not significantly differ between survivors and deceased patients, indicating that it is not an absolute prognostic indicator.[9] Thus, developing additional biomarkers for predicting disease outcome in patients with anti-MDA5$^+$ DM is essential, especially CADM to improve disease management.

Krebs von den Lungen-6 (KL-6) is a mucin-like glycoprotein expressed predominantly on type II alveolar pneumocytes and bronchiolar epithelial cells. Recently, circulating KL-6 is shown to be a sensitive marker of the activity of various types of ILD.[10,11] Emerging evidence has demonstrated that KL-6 levels correlate with high-resolution computed tomography (HRCT) findings, pulmonary function test (PFT), and some clinical parameters in connective tissue disease (CTD)-ILDs, thus reflecting the severity of CTD-ILD.[12] The poor prognosis of PM/DM-ILD also has a positive correlation with serum KL-6 levels.[12-14]

PM/DM is a highly heterogeneous group of diseases, and anti-MDA5$^+$ DM is a unique subtype closely related to ILD, especially RPILD. Identifying the key biomarkers to guide clinical judgment remains a challenging task. Limited studies have been published on the clinical significance of serum KL-6 levels in anti-MDA5$^+$ DM-ILD. The purpose of the present study was to investigate whether these levels would serve as a useful prognostic marker in anti-MDA5$^+$ DM-ILD.

2 | MATERIALS AND METHODS

2.1 | Study population and protocol

The study included 68 patients with CADM-ILD, 60 patients with classic DM-ILD, and 48 healthy controls from February 2015 to 2018 at the Department of Rheumatology, Renji Hospital, Shanghai, China. CADM and classic DM were diagnosed according to Sontheimer's and Bohan and Peter's criteria, respectively.[15-17] ILD was assessed for pulmonary symptoms, such as cough, production of sputum, dyspnea, pleurisy, hemoptysis, and the findings of HRCT and PFT. Patients were considered to have ILD when they showed HRCT findings compatible with ILD (nodular, reticulonodular, linear or ground-glass opacities; consolidations; irregular interface; honeycombing; or traction bronchiectasis). No evidence of heart failure was found in both clinical and echocardiographic examinations associated with the onset of ILD in any of the patients. Cultures of sputum, blood, and urine were examined for bacteria and fungi, and were found to be negative in all patients. Age, sex, diagnosis, medical and past history, duration of disease, and treatment regimen of patients were recorded. HRCT was performed using a LightSpeed CT750 HD scanner (GE Healthcare) to image 1.0- to 1.5-mm sections at 10-mm intervals during the end inspiration from the lung apices to the bases. The lung function parameters were recorded (MasterScreen Pneumo, Jaeger-Toennies), including forced vital capacity (FVC), diffusing capacity of the lung carbon monoxide (DLCO), total lung capacity (TLC), and ratio of forced expiratory volume in 1 second to FVC (FEV1/FVC). Healthy volunteers with no known diseases were used as controls.

All the patients' samples were collected at baseline, and the patients with CADM-ILD were followed up after treatment. Thirty-nine of sixty-eight patients donated blood for further analysis to evaluate KL-6 levels after treatment. The whole protocol is summarized in Figure 1. Samples were obtained from the Renji Hospital Biobank, Shanghai Jiao Tong University School of Medicine. The study protocol was approved by the Ethics Committee of Renji Hospital (ID: 2013-126), Shanghai, China. Informed consent was obtained from the study participants. This study was conducted according to the Declaration of Helsinki.

2.2 | Definition of the remission and exacerbation groups

During the follow-up period, 39 patients with CADM-ILD were assigned to either the remission/stable group or the exacerbation group

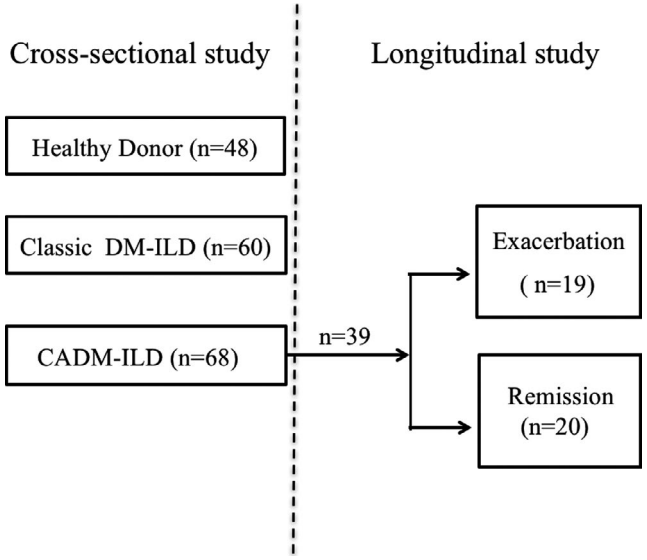

FIGURE 1 Schematic of the study design. Patients with CADM and classic DM who fulfilled Sontheimer's and Bohan and Peter's criteria, respectively, were enrolled in the study. Patients with CADM-ILD were followed up after treatment, whose clinical and laboratory data were both collected at baseline and after treatment. Among them, thirty-nine patients consented to donate blood for further analysis, whose samples were collected before and after treatment

(Figure 1). The remission/stable group comprised patients whose symptoms were relieved after treatment. The exacerbation group comprised patients with acute exacerbation of interstitial pneumonia (AE-IP), including those with acute interstitial pneumonia (AIP) and acute exacerbation of non-AIP. AIP was defined as a RPILD showing deterioration within 1 month. Non-AIP was defined as a slowly progressive ILD that gradually deteriorated over more than 1 month. AE-IP was defined based on the criteria for acute exacerbation of idiopathic pulmonary fibrosis (IPF) proposed by the Japanese guidelines.[18]

2.3 | HRCT scoring system

The HRCT examinations were performed within 5 days (median, 3 days; range, 0-5 days) after the onset of symptoms. The HRCT scores were graded on a scale of 1 to 6 based on the classification method.[19] The images were reviewed in a random order by two radiologists with 12 and 18 years of experience. They were not informed about the diagnosis or clinical course of the patients.

2.4 | Measurement of KL-6 level

Peripheral blood samples were collected from patients at the time of diagnosis and after treatment, and then centrifuged at 500 *g* for 10 minutes. Separated serum samples were stored at −80°C for KL-6 quantification. All sample assays were performed at the same time. The serum KL-6 level was measured by chemiluminescence enzyme immunoassay (CLEIA) using a KL-6 antibody kit (LUMIPULSE G1200, Fujirebio) according to the manufacturer's protocol.

2.5 | Measurement of anti-MDA5 antibody

The anti-MDA5 antibody was measured by the enzyme-linked immunosorbent assay using recombinant MDA5 antigen (rMDA5) in Dr Kuwana's laboratory.[20] In brief, 96-well plates were coated with purified rMDA5 (0.5 μg/mL) at 4°C overnight, followed by incubation with patients' serum diluted at 1:250. Optical absorbance was read at 450 nm in a microplate reader. The cutoff level was 8.0 U/mL.

2.6 | Statistical analysis

Data were analyzed using the GraphPad Prism 6.0 statistical software (GraphPad). Descriptive statistics were presented as mean ± standard deviation (SD) for variables with normal distributions and as medians with minimum and maximum values for variables with non-normal distributions. The Student *t* test was used to evaluate normally distributed data. Nonparametric tests were used for non-normally distributed data. The Mann-Whitney *U* tests were used to compare between the groups. Analysis of variance and Kruskal-Wallis tests were used to compare the mean and median values of the groups, respectively. Spearman's rank correlation coefficient was used to test correlations between the groups. The receiver operating characteristic (ROC) curve was used to obtain the optimum cutoff level. Comparisons of ROC curves were performed using SPSS (version 20.0). Survival analysis was performed using the Kaplan-Meier method. A P value < .05 was accepted as statistically significant.

3 | RESULTS

3.1 | Characteristics of the study population

A total of 68 patients diagnosed with CADM-ILD (mean age ± SD, 50.02 ± 9.34 years; female/male ratio, 51/17), 60 patients with classic DM-ILD (mean age ± SD, 54.57 ± 15.2 years; female/male, 39/21), and 48 healthy controls (mean age ± SD, 41.5 ± 8.9 years; female/male, 28/20) were included in the study. Age, gender, disease duration, laboratory data, HRCT score, and therapy for all patients are summarized in Table 1. The results demonstrated statistically significant differences among the groups with regard to CK and lactate dehydrogenase (LDH). The HRCT score was higher in CADM than in classic DM, but the differences were not significant. Also, no significant differences in age, gender ratio, disease duration, therapy, ferritin, and other laboratory data were observed among the groups.

3.2 | Baseline serum levels of KL-6 in patients with anti-MDA5$^+$ DM

The serum KL-6 levels were significantly higher in patients with CADM-ILD than in those with classic DM-ILD (1339 ± 1329 U/mL vs 642.3 ± 498.4 U/mL, P = .0002) and healthy controls (162.4 ± 54.01 U/mL, P < .0001; Figure 2A).

TABLE 1 Baseline characteristics of patients with CADM and DM/PM

Variables	All patients (n = 128)	CADM (n = 68)	Classic DM (n = 60)	*P* value
Age (year)	50.90 ± 10.75	50.02 ± 9.34	54.57 ± 15.2	.1563
Gender (female, %)	94 (70.31)	51 (75)	39 (65)	.073
Disease duration (month)	16.98 ± 49.69	15.75 ± 9.27	48.25 ± 33.27	.374
Baseline blood test				
WBC ($\times 10^9$/L)	7.66 ± 3.61	7.21 ± 3.51	9.61 ± 3.50	.48
GPT (U/L)	54 (32.5, 92.25)	51.5 (30.25, 81.5)	83.5 (39.5, 130)	.482
SCr (mmol/L)	45.98 ± 11.82	46.6 ± 11.81	43.3 ± 12.06	.394
BuN (mg/L)	5.33 ± 1.80	5.16 ± 1.70	6.07 ± 2.07	.113
HRCT score (%)	146.39 ± 32.71	148.5 ± 34.49	138.6 ± 24.0	.313
LDH (U/L)	289 (239, 410)	282 (226, 373)	506(405.5, 806.25)	.004*
CK (U/L)	53.5 (32.25, 152)	47 (26.25, 81.75)	1311.5 (208, 4543.75)	.007*
Ferritin (ng/mL)	676.62 ± 492.7	689.76 ± 492.01	606.96 ± 516.96	.630
ESR (mm/h)	30.97 ± 21.60	30.5 ± 21.24	32.92 ± 23.89	.731
CRP (mg/dL)	3.3 (3.28, 5.29)	3.3 (3.28, 5.17)	3.29 (3.16, 22.83)	.227
ANA (1:80), (n, %)	31 (45.59)	21 (42.86)	10 (50)	0.060
Jo-1 (n, %)	2 (2.94)	0	2 (10)	.166
Therapy				
Prednisolone or methylprednisolone (n, %)	125 (97.7)	68 (100)	57 (95)	.823
Immunosuppressive therapy				
MMF (n, %)	27 (21.26)	9 (13.23)	18 (30)	.112
CsA (n, %)	50 (39.06)	35 (51.47)	15 (25)	.051
FK506 (n, %)	18 (14.06)	18 (26.47)	0	1.000
AZA (n, %)	19 (14.84)	4 (7.35)	15 (25)	.097

Note: The dosage of prednisolone was 1-5 mg/kg.

Abbreviations: ANA, anti-nuclear antibody; AZA, azathioprine; BuN, blood urea nitrogen; CADM, Clinically amyopathic dermatomyositis; CK, creatine kinase; CRP, C-reactive protein; CsA, cyclosporine A; DM, dermatomyositis; ESR, erythrocyte sedimentation rate; FK506, tacrolimus; GPT, glutamic-pyruvate transaminase; LDH, lactate dehydrogenase; MDA-5, antibody anti-melanoma differentiation-associated gene 5 antibody; MMF, mycophenolate mofetil; PM, polymyositis; SCr, serum creatinine concentration; WBC, white blood cell.

*P < 0.05

3.3 | Changes in serum KL-6 levels in response to therapy in the exacerbation and remission/stable groups of patients with anti-MDA5$^+$ CADM

The present study focused on patients with CADM-ILD because of their high mortality. The patients with CADM were followed up to December 31, 2018. The median follow-up time was 23.6 months. During the follow-up period, the KL-6 levels significantly increased from 2420 ± 2239 to 3262 ± 2124 U/mL (*P* = .048; Figure 2B) in the exacerbation group (n = 19) suffering from AE-IP, but decreased from 1156 ± 624.7 to 734.5 ± 331.8 U/mL (*P* = .0010) in the remission/stable group (n = 20). A significant difference was observed both at baseline and after treatment between the two groups (*P* = .0219, *P* < .0001, respectively). KL-6 levels demonstrated significant differences between the remission/stable and exacerbation groups in the follow-up period (*P* = .0219). A significant difference was found at the baseline between the two groups (*P* < .0001; Figure 2B). This suggested that the serum KL-6 level could serve as a sensitive marker for predicting and evaluating CADM disease severity and response to treatment.

3.4 | Correlation of serum KL-6 levels with laboratory findings, HRCT scores, and PFTs

Previous studies demonstrated that ferritin and HRCT scores closely correlated with 1-year survival rates.[21] In the present study, the KL-6 levels showed a moderate positive correlation with

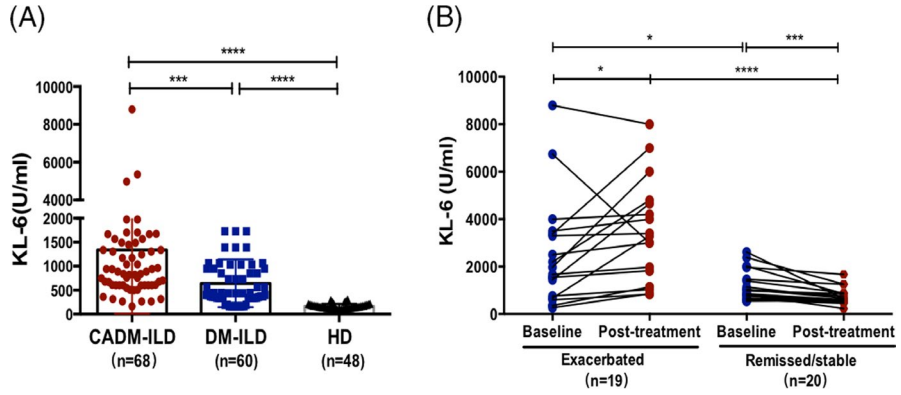

FIGURE 2 Serum levels of KL-6 in patients with anti-MDA5$^+$ DM. (A), Elevated serum levels of KL-6 in patients with CADM-ILD. Serum levels of KL-6 from patients with CADM-ILD (●, n = 68), DM-ILD (■, n = 60), and HD (▲, n = 48) are shown. Serum KL-6 levels were significantly higher in patients with CADM-ILD than in those with classic DM-ILD (1339 ± 1329 U/mL vs 642.3 ± 498.4 U/mL, $P = .0002$) and HD (162.4 ± 54.01 U/mL, $P < .0001$). All standards and samples were assayed in technical replicates, and readings were performed in duplicate. The serum value for each individual is represented as a single point. Horizontal lines represent the means ± SD. $^{***}P < .05$, $^{****}P < .0001$. (B), Changes in serum KL-6 levels in patients with CADM-ILD according to therapy outcome. More attention was paid to CADM-ILD because the unique subtype had higher KL-6 level and mortality. Thirty-nine patients with CADM-ILD were followed up whose samples were collected at baseline and after treatment. During the follow-up period, KL-6 levels significantly increased from 2420 ± 2239 to 3262 ± 2124 U/mL ($P = .048$) in the exacerbation group (n = 19) suffering from AE-IP, but decreased from 1156 ± 624.7 to 734.5 ± 331.8 U/mL ($P = .0010$) in the remission/stable group (n = 20). A significant difference was found both at baseline and after treatment between the two groups ($P = .0219$, $P < .0001$, respectively). The serum value for each individual is plotted as a single point. Values represent the means of replicate assays. $^{*}P < .05$, $^{***}P < .001$, and $^{****}P < .0001$. CADM-ILD, clinically amyopathic dermatomyositis-related interstitial lung disease; DM-ILD, dermatomyositis-related interstitial lung disease; HD, healthy donors

the HRCT score ($r = .4200$, $P = .0005$), LDH ($r = .5439$, $P = .0001$), and ferritin ($r = .2708$, $P = .0165$; Figure 3A–C). However, no significant correlation was observed between serum KL-6 and erythrocyte sedimentation rate (ESR) ($r = .2057$, $P = .0660$; Figure 3D). The results demonstrated a moderate negative correlation between serum KL-6 levels and applied PFTs: TLC ($r = -.3963$, $P = .0368$), FVC ($r = -.3084$, $P = .0357$), and DLCO ($r = -.3976$, $P = .0268$; Figure 4A–C). However, no significant correlation existed between serum KL-6 and FEV1/FVC ratio ($r = -.0542$, $P = .7723$; Figure 4D).

3.5 | Predictive value of KL-6 for death in the early stage of anti-MDA5$^+$ DM

During the follow-up period, 18 patients died from respiratory failure. All these patients received aggressive and systemic immunosuppressive therapy. No infectious cases were included. Next, the patients were divided into death or survival group according to their 1-year survival. To further evaluate the relevant laboratory findings and HRCT score in terms of survival, univariate and multivariate

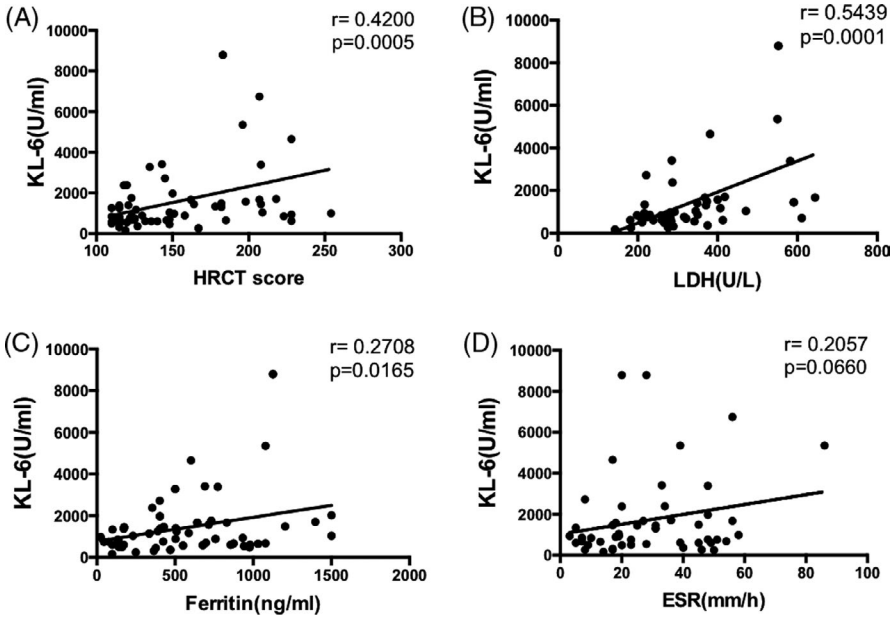

FIGURE 3 Correlations among serum KL-6 levels, laboratory findings, and HRCT scores in patients with CADM-ILD. (A) KL-6 levels had a moderate positive correlation with the HRCT score ($r = .4200$, $P = .0005$), (B) LDH ($r = .5439$, $P = .0001$), and (C) serum ferritin ($r = .2708$, $P = .0165$), (D) but no significant correlation was found between the serum levels of KL-6 and ESR ($r = .2057$, $P = .0660$). ESR, Erythrocyte sedimentation rate; HRCT, high-resolution computed tomography; LDH, lactate dehydrogenase

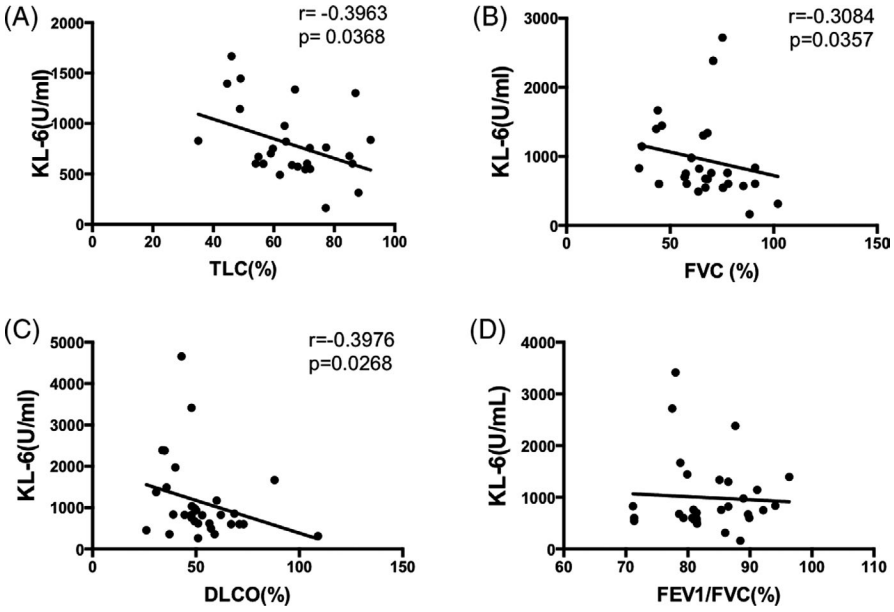

FIGURE 4 Correlation between serum KL-6 levels and PFTs in patients with CADM-ILD. A significant inverse correlation was found between serum KL-6 levels and applied PFTs: (A) TLC (r = −.3963, P = .0368), (B) FVC (r = −.3084, P = .0357), and (C) DLCO (r = −.3976, P = .0268), but (D) no significant correlation was observed between KL-6 serum levels and FEV1/FVC (r = −.0542, P = .7723). Percentage values are plotted as a single point. DLCO, diffusing capacity of the lung carbon monoxide; FEV1/FVC, forced expiratory volume in 1 s/forced vital capacity; FVC: forced vital capacity; TLC, total lung capacity

analyses were performed (Table 2). KL-6, ferritin, and HRCT score were found to be independent risk factors for mortality. In brief, higher baseline KL-6, ferritin level, and HRCT score predicted poor prognosis. Accordingly, the ROC analysis revealed that a cutoff level for survival was calculated as 792 U/mL, showing the best sensitivity (75%) and specificity (54.5%); the area under the curve (AUC) value was 0.685. Interestingly, when KL-6 and ferritin/HRCT score were combined, the AUC value reached 0.911 (Figure 5A). This meant that the combined predictive value of KL-6 and ferritin/HRCT score might be higher than that of KL-6 alone.

Finally, patients were classified as low and high KL-6 level groups based on the cutoff level of 792 U/mL. The Kaplan-Meier survival curve showed that the patients with higher serum KL-6 had significantly lower survival rates than those with lower serum KL-6 (66.7% vs 90%, log-rank test, P = .0274) (Figure 5B).

4 | DISCUSSION

This study was novel in investigating the KL-6 levels in patients with anti-MDA5$^+$ DM-ILD. As expected, the serum KL-6 levels were significantly elevated in patients with CADM-ILD compared with those with classic DM-ILD. Serum KL-6 reflected the severity of ILD, as KL-6 levels significantly and positively correlated with

TABLE 2 COX proportional hazards regression models to predict the mortality of patients with anti-MDA5$^+$ CADM

Variables	Per unit for HR	HR (95% CI)	*P* value
Univariate analysis			
Age	1 y	1.022 (0.983-1.052)	.812
Gender (male/female)	Male	0.602 (0.293-1.728)	.421
Ferritin	1 ng/mL	1.001 (1.000-1.002)	.003*
CRP	1 mg/L	1.020 (0.853-1.232)	.563
ESR	1 mm/h	1.126 (1.029-1.229)	.831
GPT	1 U/L	1.008 (0.991-1.019)	.334
SCr	1 μmol/L	0.950 (0.921-1.016)	.230
LDH	95% CI	1.006 (1.002-1.009)	.004*
HRCT score	5	1.168 (1.021-1.323)	.031*
KL-6	1 U/mL	1.122 (1.031-1.227)	.012*
Multivariate analysis			
KL-6	1 U/mL	1.090 (0.932-1.241)	.041
HRCT score	5	1.181 (1.004-1.321)	.013
Ferritin	1 ng/mL	1.004 (1.000-1.009)	.001

Abbreviations: CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; GPT, glutamic-pyruvate transaminase; HRCT, high-resolution computed tomography; KL-6, Krebs von den Lungen-6; LDH, lactate dehydrogenase; SCr, serum creatinine concentration.

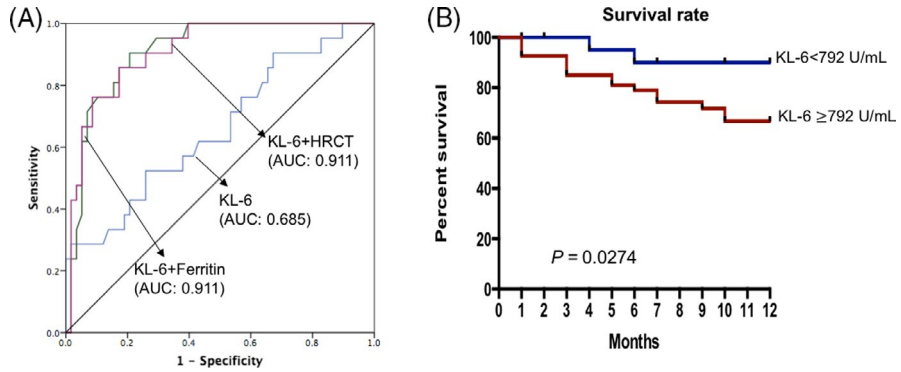

FIGURE 5 (A) Prognostic analysis for patients with CADM-ILD. ROC curves for predicting CADM-ILD were generated, and the corresponding AUC values were calculated. The cutoff value for serum KL-6 was also obtained. (B) The Kaplan-Meier survival curve showed the survival rate according to initial the KL-6 level. Relative to those with initial serum KL-6 <792 U/mL, patients with initial serum level KL-6 ≥792 U/mL showed a significantly shorter 1-y survival rate (66.7% vs 90%, *P* = .0274). Comparisons were performed using the log-rank test

ferritin, LDH, and HRCT scores, but inversely correlated with FVC, TLC, and DLCO percentages. KL-6 levels during the follow-up period decreased in the remission/stable group, but increased in the exacerbation group. Importantly, the serum KL-6 level ≥792 U/mL predicted poor prognosis in an early stage of anti-MDA5$^+$ DM-ILD. These findings indicated that KL-6 might be involved in the pathophysiological process of ILD in anti-MDA5$^+$ DM, especially in patients with CADM, and had certain predictive value.

The increased KL-6 levels are derived from damaged or regenerating type II alveolar epithelial cells in the lower respiratory tract and may trigger transforming growth factor-β (TGF-β) signaling and fibrosis, which in turn reflects lung damage.[22] Changes in serum KL-6 concentration may reflect the state of lung tissues in various inflammatory ILDs.[13] Although surfactant protein (SP)-A, SP-D, monocyte chemoattractant protein-1 (MCP-1), matrix metalloproteinase-7, and CCL-18 have been reported as biomarkers of ILD, KL-6 was identified as the most effective.[22] KL-6 has a putative role in the pathophysiology of ILD because it acts as a chemoattractant for fibroblasts.[23] Hence, it was concluded that CADM-ILD might be associated with more severe lung damage than classic DM-ILD. This explained the higher mortality of patients with CADM-ILD.

The findings of this study were consistent with those of previous studies. The clinical features of classic DM-ILD and CADM-ILD were compared in a Japanese retrospective study. KL-6 was higher in patients with CADM-ILD (1460 ± 1200 U/mL) than in those with DM-ILD (978 ± 485 U/mL).[4] Kubo and Fathi et al found KL-6 levels to be inversely correlated with FVC and DLCO in Japanese and Caucasian patients with PM/DM, respectively.[14,24] Bandoh et al reported increased serum KL-6 levels in patients with interstitial pneumonia associated with PM/DM; the levels correlated with the clinical course.[13] However, a follow-up study was not conducted nor an in-depth analysis of its predictive value was made. Therefore, the present study just filled the gap.

Because the KL-6 level may be affected by various lung conditions, the appropriate cutoff value for a specific condition needs to be drawn based on a comparison with adequate control.[12] Serum KL-6 concentrations lower than 275.1 U/mL indicated the absence of ILD.[12] In patients with cancer, 500 U/mL was reported as a cutoff value for diagnosing ILD.[25] Lee et al[12] reported that the corresponding patients with CTD and without ILD had a mean serum KL-6 level of 256 U/mL, and those with grade 1 ILD had a mean serum KL-6 level of 414 U/mL after adjusting for age, sex, and type of CTD. In a previous study, patients with IPF having initial KL-6 level of 1000 U/mL had a poor prognosis.[26,27] Compared with patients with initial KL-6 <1000 U/mL, those with initial KL-6 ≥1000 U/mL tended to have a higher frequency of acute exacerbation.[26] In the present study, the ROC curves supported an initial KL-6 level of 792 U/mL as the optimal cutoff point for discriminating between survivors and nonsurvivors during the follow-up observation. The result was in accordance with the previous study. Indeed, KL-6 was able to predict the progression of IIM-ILD.[28] These findings suggested that initial serum KL-6 levels helped predict anti-MDA5$^+$ CADM-ILD progression.

KL-6 level has previously been used to measure disease severity. In the present study, not only HRCT score and ferritin but also LDH positively correlated with KL-6 level. LDH may reflect lung cell damage, although cell destruction in other organs, such as liver and muscle, may also influence LDH levels.[29] Similarly, Yokoyama et al found using univariate analysis that LDH and KL-6 levels significantly correlated with prognosis in IPF, but multivariate analysis revealed only KL-6 as a predictor of prognosis.[26]

Besides, KL-6 may have a substantial role in evaluating ILD among patients with CTD, including those with IIM.[12] First, considering the cost-effectiveness and radiation hazard, KL-6 measurement by simple blood test would be a good alternative to chest HRCT for evaluating the current status of ILD in rheumatology clinics.[12] Second, serial measurement of KL-6 level can be a monitoring method for the exacerbation of ILD. Chest HRCT can provide objective evidence of exacerbation with overt clinical symptoms, but the optimal time interval between CT scans in asymptomatic patients with ILD was difficult to determine. Hu et al[30] reported that the post-treatment serum KL-6 levels significantly reduced in patients with improved ILD, but markedly increased in patients with exacerbated ILD. In this study, KL-6 levels decreased in the exacerbation group after

treatment, but increased in the remission/stable group. Elevated KL-6 levels could indicate the possibility of ILD, helping to predict outcomes and evaluate treatment efficacy. A recent study reported a patient with anti-MDA5$^+$ CADM-ILD successfully treated with aggressive immunotherapy and followed up using lung ultrasound and KL-6.[31] This indicated that the combined use of lung ultrasound and KL-6 might be a safe, helpful way for the assessment and follow-up of ILD.[31] The ROC curves in this study supported that AUC was better when KL-6 was combined with ferritin or HRCT score compared with KL-6 alone. The combined use of the three indicators improved the sensitivity for anti-MDA5$^+$ CADM-ILD. Regular, such as yearly, chest HRCT combined with more frequent KL-6 measurements can be an ideal protocol for the surveillance of exacerbated anti-MDA5$^+$DM-ILD.[12] However, more accurate data for disease prediction might require a study involving a prospective cohort.

This study had several limitations. First, the existence of patients with CADM is now widely accepted, but formally validated inclusion criteria are still lacking. Sontheimer's criteria were used in this study, which are often cited.[32] Second, the study was limited by the small sample size because CADM is rare. In a population-based study, the overall incidence of CADM was 2.08 per 1 000 000.[33] Third, a total of 68 patients were included in the observation, and actually, they were all followed up. However, 39 patients consented to donate blood for further analysis, whereas we did not obtain consents from the other 29 patients, who were unwilling to be drawn blood because of non-medical reasons. Large scale study is indeed needed. Finally, the retrospective design and the inclusion of patients from a single center might have caused potential selection bias. These limitations might have introduced an ascertainment bias that affected the results.

5 | CONCLUSIONS

In summary, the KL-6 level in the serum appears to be a promising, clinically applicable marker for predicting and evaluating ILD in Chinese patients with anti-MDA5$^+$ DM, and serial KL-6 measurements may be useful for assessing prognosis. A large-size clinical study is warranted to verify the results.

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CONFLICT OF INTEREST

The authors declare no competing interests.

AUTHORS' CONTRIBUTIONS

YanYe carried out the CLEIA experiment, analyzed the data, and drafted the manuscript. Ran Wang collected the clinical data and serum samples. Qiong Fu and Qiang Guo participated in the design of the study and revised the manuscript. Chunde Bao conceived the study, designed and participated in experiments, helped with coordination, analyzed data, and drafted, edited, and revised the manuscript. All authors read and approved the final manuscript.

ETHICAL APPROVAL

The study was approved by the Ethics Committee of Renji Hospital, Shanghai, China (ID: 2013-126). Informed consent was obtained from all the study participants.

DATA AVAILABILITY

Data used in this study can be obtained from the author on request.

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