Increased serum levels of sCD40L were associated with rapidly progressive interstitial lung disease in idiopathic inflammatory myopathies

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Abstract

Objective

Whether coagulopathy exists in development of idiopathic inflammatory myopathies associated rapidly progressive interstitial lung disease (IIMs-RPILD) is unclear. In this study, we aimed to investigate soluble CD40 ligand and D-dimer levels in RPILD patients.

Methods

Patients with IIMs-ILD were enrolled and classified as RPILD and stable-ILD group. Clinical data, laboratory examinations including coagulation-associated parameters and the myositis antibodies status, chest high-resolution computed tomography (HRCT) findings and treatment regimens were collected and serum levels of sCD40L were detected by ELISA. Univariable and adjusted multivariable cox regression were performed to identify risk factors for 6-month mortality, and further to select predictors for establishing predictive model for RPILD.

Results

Eighty patients with IIMs-ILD were enrolled and 34 of them were diagnosed as RPILD while 46 as stable-ILD. Multivariable cox regression showed that albumin<32.4 g/L and sCD40L<1658.55 pg/ml were independent risk factors of short-term mortality in RPILD. A SMAD model consisting of serum sCD40L>1054 pg/ml, anti-MDA5 positivity, albumin<32.4 g/L and D-dimer>0.865 mg/L were generated. The odds for RPILD with SMAD score of 0, 1, 2, 3 and 4 were 0, 26.9%, 66.7%, 91.7% and 100%. The 6-month survival stratified by mild (SMAD score 0), moderate (SMAD score 1 and 2) and severe group (SMAD score 3 and 4) were 100%, 79.5% and 20%, respectively.

Conclusion

We established a predictive model for IIMs-RPILD, which provided a clue that coagulopathy might exist in IIMs-RPILD and could help to better treat patients with RPILD. This model awaits further validations.

Key words

IIMs, RPILD, coagulation, sCD40L, prediction model

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Introduction

Interstitial lung disease (ILD) is the major complication that contributes to an increased morbidity and mortality in idiopathic inflammatory myopathies (IIMs) (1-4). As the importance of myositis-specific antibodies (MSAs) and myositis-associated autoantibodies (MAAs) was increasingly recognised, anti-melanoma differentiation-associated protein 5 (MDA5) antibody had attracted much attentions for its association with clinical amyopathic dermatomyositis (CADM), a unique subtype with typical skin lesions with no or mild muscle involvement (5). Rapidly progressive ILD (RPILD) is closely associated with anti-MDA5 positive ADM and represents a life-threatened, conventional therapy-resistant phenotype. Despite early diagnosis and an aggressive treatment, the six-month mortality of MDA5-ILD is up to 50% (6), indicating an unmet need to create a risk prediction model for this fatal condition to guide mechanism-related therapy.

Several risk models have been reported in IIMs-RPILD. A study from multiple centres in Japan created a risk model consisting of anti-MDA5 positivity, the serum levels of Kerbs von den Lungen 6 (KL-6) and C-reactive protein (CRP) to predict mortality of IIM-ILD (7). Another study from Korea constructed a model comprising several factors including fever, lactate dehydrogenase (LDH), age and neutrophils-lymphocytes ratio (8). These prior models were mainly based on the systemic inflammatory parameters. However, to date, few study have investigated whether abnormal coagulation exists in MDA5-ILD.

Soluble CD40 ligand (sCD40L) is a member of the tumour necrosis factor gene superfamily secreted by activated platelets, which can recruit platelets to mediate thrombosis (9). It was considered as a novel mediator participating in platelet activation that was found increased in acute inflammation phase in critical illness including septic shock (10) and COVID-19 associated acute respiratory distress syndrome (ARDS) (11). Also, higher levels of sCD40L were observed to predict acute coronary events and coronary restenosis in patients after percutaneous coronary artery intervention (12). In the current study, we hypothesised that abnormal coagulation played a role in the development of RPILD. IIMs-ILD patients were classified into RPILD and stable-ILD based on the disease behaviours. We measured serological levels of coagulation-associated biomarkers including activated partial thromboplastin time (APTT), D-dimer, thrombin time (TT), prothrombin time (PT) and fibrinogen (FIB), albumin and sCD40L and compared them between the two groups. Then, we investigated whether these biomarkers could make a risk model associated with the short-term mortality in IIMs-RPILD.

Materials and methods

Subjects

This was a retrospective study. Patients with multiple disciplinary team (MDT) diagnosis of IIMs-ILD in the Affiliated Drum Tower Hospital of Nanjing University Medical School from July 2018 to December 2020 were enrolled and all of them signed the informed consent. All the patients were hospitalised due to respiratory symptoms in the Department of Pulmonary and Critical Care Medicine. IIM was diagnosed based on 2017 EULAR/ACR IIM classification criteria (13). Chest HRCT of all patients in supine position were performed and assessed by a thoracic radiologist. ILD was diagnosed when respiratory symptoms combined with radiographic abnormalities in HRCT according to the 2018 ATS guidelines (14). Patients were classified as RPILD and stable ILD group. Rapid progression was defined requiring deterioration of respiratory syndromes or respiratory failure within 1 month combined with new emerging abnormalities in CT progression, with the exclusion of other identified causes such as heart failure or pulmonary embolism etc. None of the patients received prophylactic anticoagulation. Forty-one age-and-gender-matched healthy controls were from the Physical Examination Center of Nanjing University Medical School Affiliated Drum Tower Hospital. This study was approved by the Ethics Committee of Nanjing University Medical School Affiliated Drum Tower Hospital

according to the policy (2022-067-02). All patients signed the written informed consent for clinical data, follow-up and serum samples collection.

Baseline demographic information, clinical characteristics, HRCT findings and laboratory examinations (haemoglobin, white blood cell count, platelet count, creatine kinase, etc.) were collected. Coagulation function parameters were recorded including APTT, D-dimer, TT, PT and FIB. Pulmonary function tests if available were recorded, including forced vital capacity (FVC), FVC % predicted, forced expiratory volume in 1s (FEV1), FEV1% predicted, diffusing capacity of the lung for carbon monoxide (DLCO) and DLCO % predicted.

Detection of biomarkers

Vein blood samples were collected at admission. Serum samples were separated by centrifuge at 3000g for 15 minutes. Sandwich ELISA was adopted to measure the serum level of sCD40L (Multisciences, China). The autoantibodies profiles (anti-MDA5, anti-Mi-2α, anti-Mi-2β, anti-TIF1γ, anti-NXP2, anti-SAE1, anti-Ku, anti-PM-Scl100, anti-PM-Scl75, anti-SRP, anti-RO-52 and anti-ARS (anti-Jo1, anti-PL7, anti-PL12, anti-EJ and anti-OJ) in serum samples were also detected by ELISA. Each sample had a duplicate.

Follow-up data

The vital status was obtained from reviewing the medical records and telephone communications. Clinical outcomes including 1-month and 6-month mortality were recorded. The endpoint was 1st January, 2021. The survival time was calculated from when serum samples were collected to death.

Statistical analysis

For baseline comparison, one-sample Kolmogorov-Smirnov test was performed when needed. Mann-Whitney U-test and Student's t-test were used to compare continuous clinical variables, and continuous variables are expressed as mean ± standard deviations (SD) or median [interquartile range (IQR)]. Chi-square test was performed to compare categorical variables.

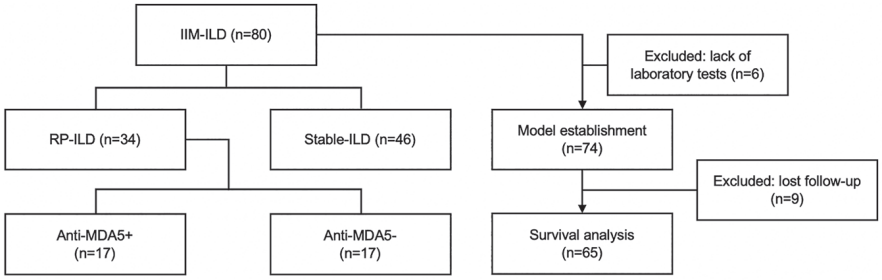

Fig. 1. Flow chart of study design.

Table I. Baseline characteristics and laboratory examination results of significance in patients with IIMs-ILD.

	RPILD n=34	stable ILD n=46	*p* value
Gender (male)	20	18	0.081
Age, mean ± SD	55.91 ± 11.156	57.85 ± 12.905	0.485
Smoking, n	9	9	0.465
ASS, n	14	27	0.121
Anti-MDA5, n	17	7	0.001
Anti-RO52, n	26	31	0.375
Co-existing antibody, n	24	25	0.168
Antibody negative, n	1	6	0.229
Mortality, n	15 (n=31)	6 (n=40)	0.002
Respiratory failure, n	33	11	<0.001
Dyspnoea, n	10	2	0.002
Fever, n	22	18	0.024
pH	7.463 ± .037	7.444 ± .033	0.028
pCO_2	34.52 ± 3.88	38.00 ± 6.05	0.004
pO_2	60.5 [53.75, 73.5]	83.06 ± 20.74	<0.001
PaO_2/FiO_2	210.27 ± 57.97	340.44 ± 104.21	<0.001
Neutrophil percentage (%)	82.77 ± 7.85	74.6 [59, 84.8]	0.002
Lymphocyte ($\times 10^9$/L)	0.8 [0.5, 1.43]	1.45 ± 0.75	0.002
Lymphocyte percentage (%)	11.69 ± 6.21	16.7 [10, 29.7]	0.001
IgE (IU/mL)	102 [58, 268]	51 [36.5, 158.5]	0.041
Glucose (mm/L)	6.59 [5.26, 7.86]	5.14 [4.56, 7.43]	0.026
Albumin (g/L)	32.06 ± 3.99	36.30 ± 4.42	<0.001
CKMB (U/L)	19 [9.5, 27]	13 [9, 17]	0.011
aHBDH (U/L)	277 [204, 379]	204 [184.75, 237.75]	0.005
ALT (U/L)	45.75 [23.98, 74.6]	21.15 [15.53, 40.05]	<0.001
AST (U/L)	33.35 [17.58, 70.13]	22.8 [16.23, 33.95]	0.014
LDH (U/L)	427.5 [271.75, 545]	262 [227, 332.25]	0.001
CRP (mg/L)	16.85 [5.18, 43.55]	5.3 [3.85, 10.63]	0.003
ESR (mm/h)	39 [10, 54]	23 [10, 34]	0.016
FVC % predicted	47.92 ± 15.40 (n=13)	67.29 ± 15.24 (n=19)	0.001
FEV1 % predicted	53.89 ± 14.78 (n=12)	70.12 ± 15.17 (n=17)	0.008
DLCO % predicted	36.93 ± 12.73 (n=9)	57.26 ± 15.58 (n=19)	0.002
APTT (seconds)	29.15 ± 4.18 (n=33)	26.99 ± 2.71 (n=43)	0.013

MDA5: anti-melanoma differentiation-associated protein 5; ASS: anti-aminoacyl-tRNA synthetase antibody syndrome; RBC: red blood cells; WBC: white blood cells; PLTs: platelets; CK: creatine kinase; CKMB: creatine kinase isoenzymes MB; ALT: alanine aminotransferase; AST: aspartate aminotransferase; α-HBDH: α-hydroxybutyrate dehydrogenase; LDH: lactate dehydrogenase; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; APTT: activated partial thromboplastin time; TT: thrombin time; PT: prothrombin time; $PaCO_2$: arterial partial pressure of carbon dioxide; PaO_2: arterial oxygen partial pressure; FVC: forced vital capacity; FEV1: forced expiratory volume in 1s; DLCO: diffusing capacity of the lung for carbon monoxide.

The consistent variates are shown as mean ± SD or median [interquartile range]. We used mean (SD) to describe variables of normal distribution and median (range) for variables of abnormal distribution.

Survival analysis was performed in RPILD group. Receiver-operating characteristic (ROC) analysis was conducted to calculate the cut-off value of continuous variables, which could be transited into binary variables for further analysis. Cox regression model was used to assess the prognostic utility of variates for 6-month mortality. Kaplan-Meier curves and log-rank test

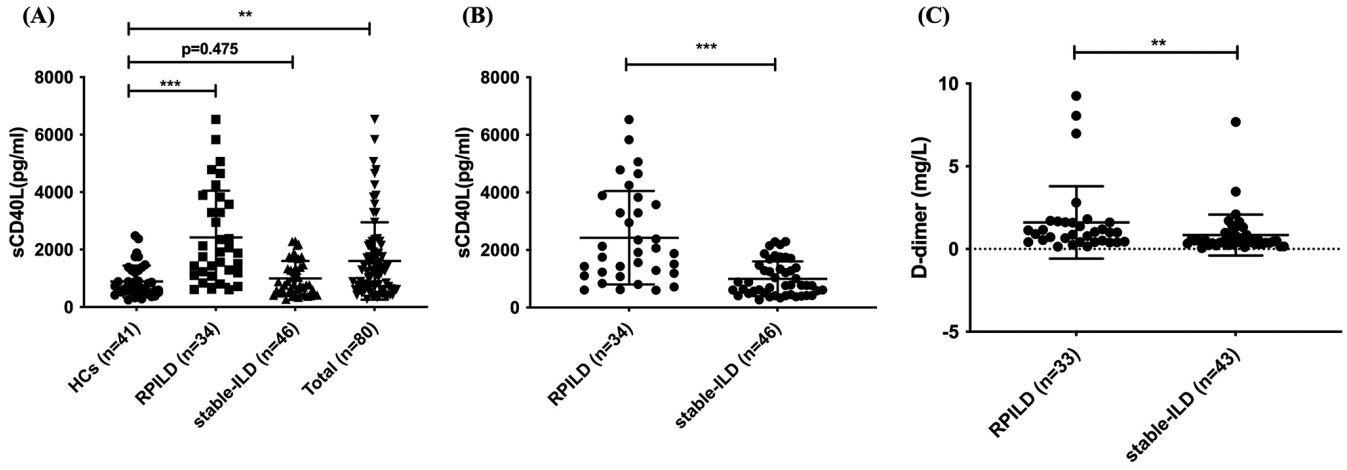

Fig. 2. Serum levels of sCD40L in patients with IIMs-ILD.
A: Serum levels of sCD40L among patients with IIMs-ILD and healthy controls. **B**: Serum levels of sCD40L between RPILD and stable-ILD.
C: Plasma levels of D-dimer between RPILD group and non-stable-ILD.
**p*<0.05, ***p*<0.01, ****p*<0.001

was performed to evaluate the association between biomarkers levels and mortality.

Finishing the cox regression analysis above, we chose risk factors with $p<0.05$ as potential predictors for rapid progression and were included into the predictive model for RPILD. Multivariate logistic regression was applied to assess significance of all elements. ROC analysis was then conducted to evaluate the diagnostic utility of the model for RPILD. Survival analysis was also conducted to compare the prognosis of subgroups in IIMs-ILD patients based on the model. *P* values lower than 0.05 was considered to be of significance. All statistical analysis was performed by SPSS 22.0 and Graphpad Prism 9.3.1.

Table II. Predictive factors for 6-month mortality in RPILD group.

Predictive factors	*p*-value	Hazard ratio	95% CI
Univariate (adjusted)			
sCD40L<1658.55 (pg/ml)	0.008	46.913	2.693, 817.130
Anti-MDA5	0.013	211.498	3.129, 14295.275
Albumin <32.4 g/L	.007	12.743	2.024, 80.237
Multivariate			
Albumin <32.4 g/L	0.012	16.606	1.861, 48.166
sCD40L <1658.55 (pg/ml)	0.020	48.278	1.864, 1250.364

The predictive values of each factor were assessed by cox regression model.

Results

Patient characteristics

During the study period, there were 80 patients diagnosed with IIMs-ILD enrolled (Fig. 1). They were 38 males and 42 females, with mean age of 57.02±12.16 years old (ranged from 22 to 87). Among them, 34 patients were classified as RPILD, whose mean age was 55.91±11.156 years old (ranged from 22 to 87), 46 were stable ILD with mean age of 57.85±12.905 years old (ranged from 26 to 86). As summarised in Table I, patients with RPILD were more likely to have fever ($p=0.024$), dyspnoea ($p=0.002$) and respiratory failure ($p<0.001$). Unsurprisingly, anti-MDA5 positivity was significantly more frequent in RPILD group (17/34, $p=0.001$).

Laboratory tests of significance were all described in Table I. At baseline, $PaCO_2$ ($p=0.004$), PaO_2 ($p<0.001$), PaO_2/FiO_2 ($p<0.001$), lymphocyte ($p=0.002$), lymphocyte percentage ($p=0.001$), the level of albumin ($p<0.001$), FVC ($p=0.010$), FVC %predicted ($p=0.001$), FEV1 %predicted ($p=0.008$), DLCO ($p=0.013$),DLCO%predicted($p=0.002$) were significantly reduced in RPILD group than those in stable group, while the values of pH (p=0.028), neutrophil percentage ($p=0.002$), IgE ($p=0.041$), glucose ($p=0.026$), CKMB ($p=0.011$), aHBDH ($p=0.005$), ALT ($p<0.001$), AST ($p=0.014$), LDH ($p=0.001$), CRP ($p=0.003$) and ESR ($p=0.016$) were significantly higher in patients with RPILD than those in stable-ILD group.

Comparison of coagulopathy between RPILD with stable-ILD

Serum levels of sCD40L were significantly increased in patients with IIMs-ILD than those in healthy controls (1604.93±1345.39 *vs*. 889.98±553.58 pg/mL, $p=0.002$), and significantly higher in RPILD group than stable-ILD group (2424.40±1623.81 *vs*. 999.24±601.41 pg/mL, $p<0.001$). No significance was observed when comparing between stable-ILD and healthy controls ($p=0.475$). Similarly, plasma levels of D-dimer in RPILD were significantly higher (1.61±2.18 *vs*. 0.85±1.24 mg/L, $p=0.006$) than those in stable-ILD group. (Fig. 2) With regard to thrombosis reaction, activated partial thromboplastin time (APTT, $p=0.013$) of RPILD patients were prolonged than in stable-ILD patients (Table I).

Subgroup analysis in anti-MDA5 and non-MDA5-RPILD

We further performed subgroup analysis in RPILD between anti-MDA5 and non-MDA5 groups. As presented in Supplementary Table S1, MDA5-RPILD patients were more likely to

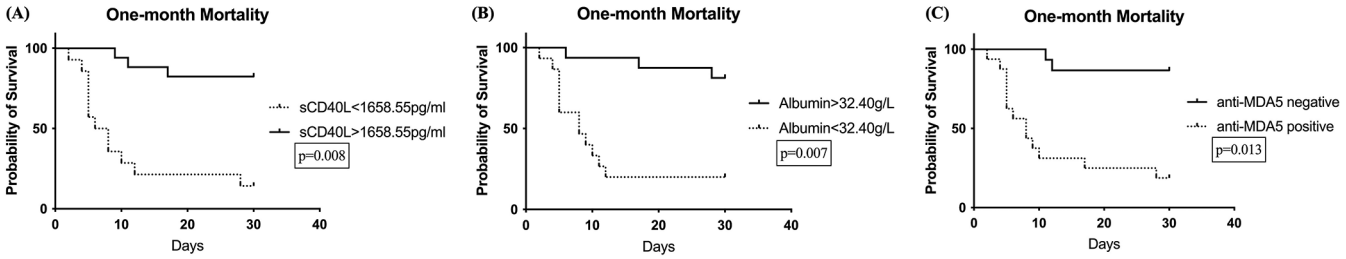

Fig. 3. Survival curves in RPILD group.
The cut-off value of sCD40L and albumin levels were determined by receiver operating characteristic (ROC) analysis. Log-rank test was performed to analyse the significance of two Kaplan-Meier curves. Time was represented in days.

have rashes (p=0.010), compared to those with non-MDA5-RPILD. Blood analysis showed that albumin (p=0.013), RBC (p=0.023), WBC (p=0.004), neutrophils (p=0.009), lymphocytes (p=0.001), C3 (p=0.016) and PLT (p=0.002) were lower in anti-MDA5-positive RPILD, while C4 (p=0.048), IgE (p=0.036), APTT (p=0.002) and TT (p=0.008) were higher than non-MDA5 group. Coagulation tests showed that sCD40L levels of patients with anti-MDA5 positivity were significantly lower (1726.39 ± 1214.09 *vs*. 3122.41±1711.42 pg/mL, p=0.010) than those negative. Notably, the mortality of patients with MDA5-RPILD was significantly higher than that of non-MDA5-RPILD (p<0.001).

Identifying risk factors for predicting short-term mortality in RPILD patients

In total, 9 patients, were lost for follow-up, of whom 3 patients were with RPILD. The mean follow-up time of RP-ILD group was 6.09±5.43 (range 1 to 24) months. The median time from onset to diagnosis of rapid progression was 30 days (ranged from 9 to 180 days). There were 21 non-survivors with a mean survival of 22.33 ± 41.55 days from samples collection. Fifteen of them were diagnosed RPILD, 13 non-survivors were with anti-MDA5 positivity. The 6-month mortality in RPILD group (15/31, 48.4%) was significantly higher than that in stable-ILD (6/40, 10%).

Survival analysis was performed in RPILD group. All non-survivors in RPILD group died within 1-month from the serum collected. After adjustment for age, sex, smoking history, myositis associated autoantibodies and medication (Table II), univariate cox regression exhibited serum levels of sCD40L<1658.55pg/ml (p=0 .008, HR 46.913, 95% CI 2.693, 817.130), anti-MDA5 positivity (p=0.013, HR 211.498, 95% CI 3.129, 14295.275) and albumin<32.4 g/L (p=0.007, HR 12.743, 95% CI 2.024, 80.237) were risk factors of 1-month mortality. Log-rank tests also supported the results above. (Fig. 3) Multivariable cox regression showed albumin<32.4 g/L (p=0.012, HR 16.606, 95% CI 1.861, 148.166) and sCD40L<1658.55pg/ml (p=0.020, HR 48.278, 95% CI 1.864, 1250.364) remained risk factors after adjustment.

Table III. Association of parameters with RPILD occurrence.

Predictive factors	p-value	Odds ratio	95% CI
Univariate			
sCD40L>1054 pg/ml	0.018	0.999	0.999, 1.000
D-dimer >0.865 mg/L	0.026	1.221	1.024, 1.055
Anti-MDA5	0.001	11.554	2.565, 52.048
Albumin <32.4 g/L	<0.001	0.748	0.643, 0.870
aHBDH >297 (U/L)	0.006	1.003	1.001, 1.005
Multivariate			
sCD40L >1054 pg/ml	0.011	0.999	0.998, 1.000
Anti-MDA5	<0.001	1340.553	26.086, 68891.854
Albumin <32.4 g/L	0.001	1.232	1.089, 1.394
D-dimer >0.865 mg/L	0.006	1.544	1.132, 2.107

The predictive values of each factor were assessed by logistic regression.

Establishing SMAD model predicting IIMs-RPILD

To establish a clinical model closely associated with the development of RPILD, we selected risk factors for short-term mortality in RPILD identified from cox regression in RPILD above, albumin<32.4 g/L and anti-MDA5 positivity as potential predictors. Meanwhile, adjusted univariate logistic regression identified sCD40L>1054 pg/ml and D-dimer>0.865 mg/L as potential predictors for RPILD (Table III).

Then we finally constructed the SMAD model based on sCD40L>1054 pg/ml (S), anti-MDA5 positivity (M), albumin<32.4 g/L (A) and D-dimer>0.865 mg/L (D). The SMAD score was defined as the number of selected predictors. Clinical data of 74 patients in total were available for model construction, among which 41 with stable-ILD and 33 with RPILD. The odds of RPILD in patients with SMAD score of 0, 1, 2, 3 and 4 were 0, 26.9%, 66.7%, 91.7% and 100%. (Table IV) ROC curve of SMAD score for RPILD showed an AUC of 0.890, while AUC of sCD40L and D-dimer individually for RPILD was 0.808 and 0.686, respectively (Supplementary Fig S1).

SMAD model also showed prognostic value for long-term survival. Data of 65 patients were available for survival analysis. We clarified all the IIMs-ILD patients into three subgroups according to SMAD score, which were mild (SMAD score 0, n=11), moderate (SMAD score 1 and 2, n=39) and severe (SMAD score 3 and 4, n=15). Up to the date at which all patients were followed up over 6 months, the survival rate of mild, moderate and se-

Table IV. SMAD model and the specificity for RPILD prediction.

Risk score	Stable-ILD, n (%)	RPILD, n (%)	Odds of RPILD (%)
0	16 (39.1)	0 (0)	0
1	19 (46.3)	7 (21.2)	26.9
2	5 (12.2)	10 (30.3)	66.7
3	1 (2.4)	11 (33.3)	91.7
4	0 (0)	5 (15.2)	100

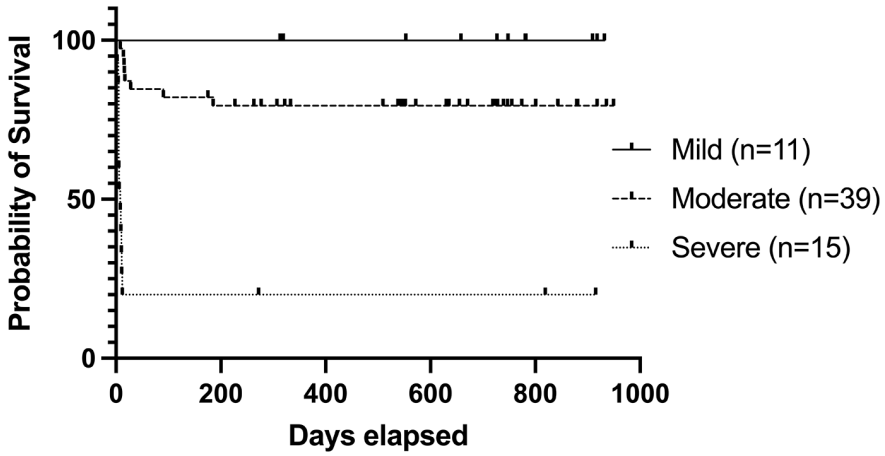

Fig. 4. Survival analysis of IIMs-ILD based on SMAD score.
Patients with IIMs-ILD were classified into mild (SMAD Score 0), moderate (SMAD Score 1 and 2) and severe (SMAD Score 3 and 4).

vere group was 100% (11/11), 79.5% (31/39) and 20% (3/15), respectively (Fig. 4).

Discussion

In this study, we considered the pathophysiology of IIMs-RPILD from a novel perspective and investigated the association of two biomarkers (sCD40L and D-dimer) with RPILD. The current study demonstrated that sCD40L, albumin, D-dimer and presence of anti-MDA5 constituted a risk model (SMAD score) for reflecting state of RPILD progression and predicting long-term survival in patients with IIMs-ILD. The study provided a clue that abnormal coagulation needs to be noticed in RPILD.

Notably, we found the presence of anti-MDA5 antibody is an independent risk factor of short-term mortality in RPILD, further in SMAD model. MDA5 was encoded by the IFIH1 gene, functioning as a key intracellular sensor of dsRNA viral replicative intermediates or by-products (15). Previous literatures have suggested a strong association of anti-MDA5 antibody with the occurrence of RPILD(16). In a non-Asian cohort, occurrence rate of RPILD in patients with anti-MDA5 positivity ILD was 29.6 (32/108) (17). Interestingly, patients with MDA5-RPILD exhibited clinical characteristics similar to coronavirus disease 2019 (COVID-19) including the biology pattern of "cytokine storms", the high-resolution computed tomography (HRCT) appearances, the potential effects of high-dose corticosteroids and anti-IL-6 receptor antibody biologics, and so on (18, 19). The inflammatory cytokines storms that clarified the differences in pathophysiology between anti-MDA5-ILD and other subtypes of IIMs-ILD have been highlighted (20). In this current study, a higher neutrophil percentage, CRP, IgE and ESR levels were observed in patients with RPILD, suggesting an acute inflammation response in this condition. The risk models for IIMs-ILD have been widely explored. Li *et al*. established a multiparametric model consisting of fever, periungual erythema, elevated CRP, anti-MDA5 antibody and anti-Ro-52 antibody (21). The FLAIR score was a mortality risk model for ADM-associated ILD including ferritin, LDH, anti-MDA5 antibody, HRCT imaging score and RPILD (22). These models were constructed mainly focusing on the inflammatory characteristics of the disease (Suppl. Table S2).

Furthermore, the amplified inflammation response induced epithelial cells damage and platelet activations, which subsequently activated coagulation cascade, had been well discussed during the acute inflammation process (23). Soluble CD40 ligand is released by a variety of cell types, including epithelial cells, fibroblasts, endothelial cells, and platelets. It exhibited proinflammation and procoagulant effects via promoting platelet aggregation and triggers activation of NF-κB signalling pathway (24). The persistently higher circulating sCD40L levels were observed in patients with sepsis and was associated with an increased mortality (25). Moreover, Higher levels of sCD40L were observed to predict acute coronary events and coronary restenosis in patients after percutaneous coronary artery intervention (12). Higher serum sCD40L levels were also associated with the severity of endotheliopathy in patients with COVID-19, particularly those acquiring intensive care (26). In addition, serum albumin was reported to predict portal vein thrombosis (PVT) when manifesting lower levels (27). In this study, we firstly found that serum sCD40L and D-dimer levels were significantly higher in patients with RPILD compared with stable group. We also found that serum albumin was reduced in RPILD group, especially in patients with anti-MDA5 positivity. However, in RP-ILD group, sCD40L levels of patients with anti-MDA5 positivity were significantly lower than those negative. Meanwhile, APTT and D-dimer in RP-ILD group were significantly higher compared to stable-ILD, while D-dimer was positively predictive to 1-month mortality of RP-ILD, indicating the dysregulation of coagulation pathways participating in rapid progression of ILD. Also, lower PLTs together with higher APTT and TT in anti-MDA5-positive patients indicated abnormal coagulation state in this subgroup. We suspected the immune response induced by anti-MDA5 antibody might influence

platelets activity to limit the secretion of biomarkers, which suggested that the mechanism of rapid progression of ILD is of diversity including cytokine storm, abnormal coagulation, immune response related to anti-MDA5 antibody and other unknown reasons. Our study provided biochemical evidence that coagulopathy might be observed in IIMs-RPILD.

The management of IIMs-RPILD remains a great challenging. A triple treatment consisting of high-dose glucocorticoids, tacrolimus, and cyclophosphamide were recommended as the first line therapy for MDA5-ILD (19). Recently, tofacitinib, a Janus kinase (JAK) inhibitor, had showed an excellent efficacy in patients with early-diagnosis of MDA5-ADM-ILD in a single-centre, open-label clinical trial(28). Another study enrolling five anti-MDA5 $^{+}$-ILD patients who failed to respond to conventional therapy and were given additional tofacitinib, three of them survived while two died (29). Furthermore, combination treatment increased the risk of opportunistic infections, in particularly viral infection (30), which leading the disease conditions more complicated. In addition, rituximab and plasma exchange had been reported efficient in refractory MDA5-ILD cases. But most of these studies came from a single centre with limited cases. Well-designed random controlled clinical trial is still warranted. Whether anticoagulation therapy should be taken into consideration in severe cases with IIMs-RPILD? Further risk stratification therapy is worth exploring.

The current study has some limitations that should be acknowledged. First, serum samples of non-ILD IIM patients were not available in our department, which limited the comparison of sCD40L levels between IIM-ILD and non-ILD IIM patients. Next, the study was conducted in a single ILD-institution with a relatively small sample size for a rare autoimmune disease. Future studies consisting of multiple-centre and large samples are still warranted. The SMAD model in this study was associated with RPILD and mortality but it was not capable for progression prediction. The SMAD model conducted in the study requires validation in a larger cohort from other centres. Also, the cross-sectional study was not capable to demonstrate longitudinal changes of serum biomarker levels.

In conclusion, we established the SMAD score including sCD40L, anti-MDA5 antibody, albumin and D-dimer, which was associated with RPILD in IIMs-ILD. Based on the SMAD model, Patients with IIMs-ILD were clarified into mild, moderate and severe subgroups, which had survival of 100%, 79.5% and 20%, respectively. This investigation extended the previous observations and implicated the potential role of abnormal coagulation in the pathogenesis of IIMs-ILD. Ongoing trial is needed to validate our findings. More importantly, to determine how such biomarkers can inform clinical decisions-anticoagulation in the management of IIMs-ILD.

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