

Serum sCD25 is an indicator for rheumatoid arthritis-associated interstitial lung disease

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Abstract

Objective

CD25 (IL-2R α) is one of IL-2 receptor's polypeptide subunits, and its soluble form is increased in patients with various inflammatory or autoimmune diseases. This study aimed to evaluate the clinical correlation of serum soluble CD25 (sCD25) with interstitial lung disease (ILD) in rheumatoid arthritis (RA) patients.

Methods

294 RA patients, including 72 in the discovery cohort (15 patients with ILD, 57 patients without ILD), 222 in the validation cohort (41 patients with ILD and 181 patients without ILD), and 58 healthy controls (HCs) were recruited. High-resolution computed tomography (HRCT) scan provided evidence and patterns of RA-ILD. Serum sCD25 concentrations were measured by enzyme-linked immunosorbent assay (ELISA). Clinical and laboratory data were recorded and the association with sCD25 was also analysed.

Results

In the discovery cohort, 16 RA-related molecules including cytokines, chemokines and functional soluble cell surface proteins were investigated. The results showed that sCD25 was significantly higher in RA-ILD than in RA-no-ILD group ($p=0.004$). ROC analysis also showed RA-ILD was discriminated with RA-no-ILD by sCD25 (AUC=0.695, 95% CI=0.541-0.849). Logistics regression demonstrated that sCD25 was one of the risk factors of RA-ILD. This result was further confirmed in validation cohort ($p<0.001$). According to the cut-off value in the discovery cohort, the sensitivity and specificity of sCD25 in RA-ILD were 51.2%, 77.3%, respectively. Compared with RA-no-ILD, serum level of sCD25 was also higher in different HRCT patterns including UIP, NSIP and RA-ILA. The ROC curves revealed sCD25 as diagnostic marker in UIP, NSIP and RA-ILA (with AUCs of 0.730, 0.761, and 0.694, respectively, $p<0.05$). The result indicated that sCD25 was a biomarker for RA-ILD subtypes. Although sCD25 was not correlated with HRCT scores, it was significantly higher in consolidation pattern by HRCT.

Conclusion

sCD25 was significantly elevated in RA-ILD (including UIP, NSIP and RA-ILA) compared to RA-no-ILD and HCs, which supports their value as a potential biomarker in RA-ILD screening and assessment.

Key words

interstitial lung disease, sCD25, rheumatoid arthritis, biomarker

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Introduction

Rheumatoid arthritis (RA) is an autoimmune disease, with many extra-articular manifestations (EAMs) due to the chronic, inflammatory, and autoimmune features. Nearly 50% of RA patients suffered from EAMs, including eyes, lungs, skin, heart and nervous system (1-3). EAMs are usually associated with more morbidity and mortality (4). Therefore, diagnosis and appropriate management of RA associated EAMs are necessary for optimal RA treatment. Respiratory involvement of RA is the most common extra-articular manifestations (EAMs). Interstitial lung disease (ILD), ranging from 2 to 10% in RA patients (5-7), was associated with significantly higher morbidity and mortality. Disease prognosis was usually worsened by ILD (8), with median survival time decreased to approximately 3-8 years after ILD diagnosis (8, 9). High-resolution computed tomography (HRCT) scans commonly reveal evidence of RA-ILD, and the most common HRCT patterns of RA-ILD are usual interstitial pneumonia (UIP), non-specific interstitial pneumonia (NSIP) (7) and interstitial lung abnormality (ILA) (10).

A series of serological biomarkers as well as genetic biomarkers of RA-ILD have been reported, including serum uric acid (11), RF-IgA (12), KL-6, CA19-9, CA125, CEA (13), MUC5B gene, RPA3-UMAD1 gene, FAM13A gene, TOLLIP gene and TERT gene (14). Autoantibodies have also been found to be associated with RA-ILD, such as anti-citrullinated alpha-enolase peptide 1 (anti-CEP1) (15), anti-citrullinated heat shock protein 90 (cit-Hsp90) α or β (16); anti-carbamylated proteins: (Anti-FCS, Anti-Fib, Anti-CFFHP and Anti-FCS-IgA); and anti-malondialdehyde-acetaldehyde (anti-MAA) (12,17-18). However, the diagnostic value and prognostic impact of these markers are still unclear. Given the complexity of the diagnosis and prognostic assessment for RA-ILD, exploration of novel biomarkers is still in need to assess this condition more precisely.

CD25, the IL-2 receptor α chain, is one subunit of the high-affinity IL-2 receptor (IL2R), which is comprised of IL-

2R α (CD25), β (CD122), and the common gamma chain (CD132) (19). It is well known that soluble CD25 (sCD25) is generated as a consequence of proteolytic cleavage, mainly from the membrane of activated T cells, and the serum concentrations of sCD25 are associated with the proliferation of activated T cells. High serum sCD25 levels have been reported in patients with various autoimmune diseases (20). Previous studies have shown that sCD25 can act as an early inhibitor of T-cell response related to IL-2 signaling (21). In the experimental autoimmune encephalomyelitis (EAE) model, sCD25 can enhance the Th17 response and exacerbate EAE by prohibiting signalling by sequestering the local IL-2 and IL-2R interaction (22). sCD25 can efficiently bind to secreted IL-2, suggesting its ability to serve as a decoy receptor for IL-2 to play a pathogenic role in autoimmunity development (22).

In this study, we found that the serum sCD25 level was significantly elevated in RA-ILD (including UIP, NSIP and RA-ILA) and was associated with clinical characteristics of RA. Monitoring the serum level of sCD25 may play an important role in the screening of RA-ILD.

Materials and methods

Patients

This is an observational study of 294 patients [72 discovery cohort (15 patients with ILD, 57 patients without ILD), 222 validation cohort (41 patients with ILD and 181 patients without ILD)] who met the 2009 revised American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) criteria for definite RA (23), 58 healthy controls (HCs) and the sera were collected from inpatient or outpatient clinics of the Department of Rheumatology and Immunology, Peking University People's Hospital. Patients were excluded from this study if they were suffering from the occurrence of acute infections in a last month, have Malignant tumour in last 5 years, have other pulmonary diseases, such as pulmonary tuberculosis, other causes ILD and chronic obstructive pulmonary disease, or have other autoimmune disorders or connec-

tive tissue diseases (CTDs) such as SLE, SS, autoimmune hepatitis, Hashimoto's thyroiditis, and so on.

All protocols involving human subjects were approved by the Ethics Committee of the Peking University People's Hospital. Informed consent was obtained from all patients and health volunteers included in the study.

RA patients were separated into 2 cohorts: 72 RA patients (discovery cohort, 15 with high disease activity; 16 with moderate disease activity; 16 with low disease activity and 25 with disease remission) were recruited by Bio-Plex Pro Human Cytokine Assay (multi-marker analysis) or ELISA, and 222 RA patients (validation cohort, including 74 with high disease activity; 87 with moderate disease activity; 27 with low disease activity and 34 with disease remission) were used as validation cohort for detection of sCD25 (Table I).

RA patients were grouped according to the DAS28-ESR. High disease activity was defined as DAS28-ESR >5.1, moderate disease activity was defined as DAS28-ESR >3.2 and ≤5.1, low disease activity was defined as DAS28-ESR >2.6 and ≤3.2 and disease remission was defined as DAS28-ESR ≤2.6 (24).

HRCT pattern of RA-ILD and Fibrosis Score

The evaluation of interstitial lung disease was made according to chest HRCT, all enrolled horizontal subjects underwent chest HRCT scans, and the results was blindly evaluated by one respiratory surgeon and two radiologists (25). According to the HRCT, the most common patterns of RA-ILD are usual interstitial pneumonia (UIP), non-specific interstitial pneumonia (NSIP) (7), interstitial lung abnormality (ILA) is defined as previous indicated CT findings (10, 26-28). UIP pattern was defined as reticular abnormality, honeycombing and traction bronchiectasis, the abnormality is usually basal and peripheral predominance. NSIP is characterised by basilar predominant, ground-glass opacities and absence of honeycombing (29-32).

Fibrosis classes were scored using an arbitrary semiquantitative scale from

Table I. Demographic characteristics of the RA patients.

Index	RA (discovery cohort)	RA (validation cohort)	HC
Age (mean±SD, years)	59.99 ± 15.47	60.73 ± 10.98	60.06 ± 8.46
Male/female	16/56	25/197	12/53
Disease duration(months)	27.40.50 ± 61.29	174.13 ± 140.39	
Tender joints, number	6.39 ± 8.22	7.45 ± 8.02	
Swollen joints, number	5.03 ± 6.66	6.68 ± 7.28	
Rheumatoid nodules	7 /72 (9.7%)	32/222 (14.4%)	
Interstitial lung disease	15/72 (20.8%)	41 /222 (18.5%)	
Renal involvements	7 /72 (9.7%)	6/222 (2.7%)	
Haematological involvements	13/72 (18.1%)	56 /222 (25.2%)	
Metabolic disorders	37/72 (51.4%)	118 /222 (53.2%)	
DAS28 scores	4.33 ± 1.76	4.55 ± 1.76	
ESR (mm/h)	42.21 ± 30.08	38.23 ± 31.15	
CRP (mg/L)	34.32 ± 94.59	21.44 ± 31.19	
RF (IU/ml)	444.39 ± 827.70	456.57 ± 803.89	
CCP(U/ml)	188.42 ± 103.89	162.03 ± 80.25	
IgA (g/L)	3.32 ± 1.74	2.90 ± 1.65	
IgG (g/L)	13.97 ± 4.48	13.43 ± 4.45	
IgM (g/L)	1.41 ± 0.82	2.93 ± 23.35	
C3 (g/L)	1.02 ± 0.24	0.95 ± 0.23	
C4 (g/L)	0.21 ± 0.08	0.20 ± 0.08	

DAS28: 28-joint count Disease Activity Score; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; RF: rheumatoid factor; anti-CCP: anti-citrullinated peptide antibody; IgA: immunoglobulin A; IgG: immunoglobulin G; IgM: immunoglobulin M; C3: complement 3; C4: complement 4.

There were no differences between discovery cohort, validation cohort and healthy controls ($p>0.05$). Descriptive statistics for continuous variables were expressed as mean ± SD, and categorical variables were expressed as numbers with percentages.

0 to V (14,33,34). According to the consensus criteria for fibrosis, honeycomb cysts was a sufficient criterion, other fibrosis criteria are subpleural irregular opacities, septal lines, curvilinear opacities, subpleural ground glass opacity and parenchymal bands, etc.

HRCT fibrosis score of RA-ILD was evaluated blindly by one respiratory surgeon and two radiologists (35) and the average value was calculated as the final score, which was evaluated the severity of pulmonary interstitial fibrosis.

Clinic and Laboratory Indexes and organ or system involvement

Clinical data were recorded using electronic data processing, included demographic information (age, sex, history of smoking, disease duration, number of tender joints, and number of swollen joints, organ involvements) and systemic inflammation [erythrocyte sedimentation rate (ESR) (positive if more than 20 mm/h for female and 15 mm/h for male); C-reactive protein (CRP) (positive if more than 8 mg/L)]. Plasma levels of anti-cyclic citrullinated peptide antibody (anti-CCP) (positive if more than 20 U/ml), rheumatoid factor

(RF) (positive if more than 20 IU/ml), immunoglobulins (IgG, IgM and IgA) and complements (C3, C4) were also recorded (36, 37).

Rheumatoid nodules: body surface nodes associated with rheumatoid arthritis. Haematological involvements: a) anaemia: haemoglobin level (female <110 g/L, male <120 g/L), b) Abnormal blood cell count associated with RA: leukopenia (white blood cell count <4,000/mm³). Infection: occurrence of organ or systems infection associated with RA (35). Renal involvements: a) persistent proteinuria >0.5 g/day, b) raised serum creatinine >1.5 mg/dL; Metabolic disorders were defined as presence of one or more of the following three medical conditions: 1) hypertension, *i.e.* systolic blood pressure (SBC) ≥140 mmHg, or diastolic blood pressure (DBP) ≥90 mmHg, or previously diagnosed; 2) dyslipidaemia, *i.e.* fasting triglyceride (TG) ≥1.7 mmol/L, or fasting high density lipoprotein cholesterol (HDL-c) <0.9 mmol/L; 3) hyperglycaemia, *i.e.* fasting blood-glucose (FBG) ≥6.1 mmol/L, or 2h postmeal glucose (PG) ≥7.8 mmol/L, or previously diagnosed (35, 38).

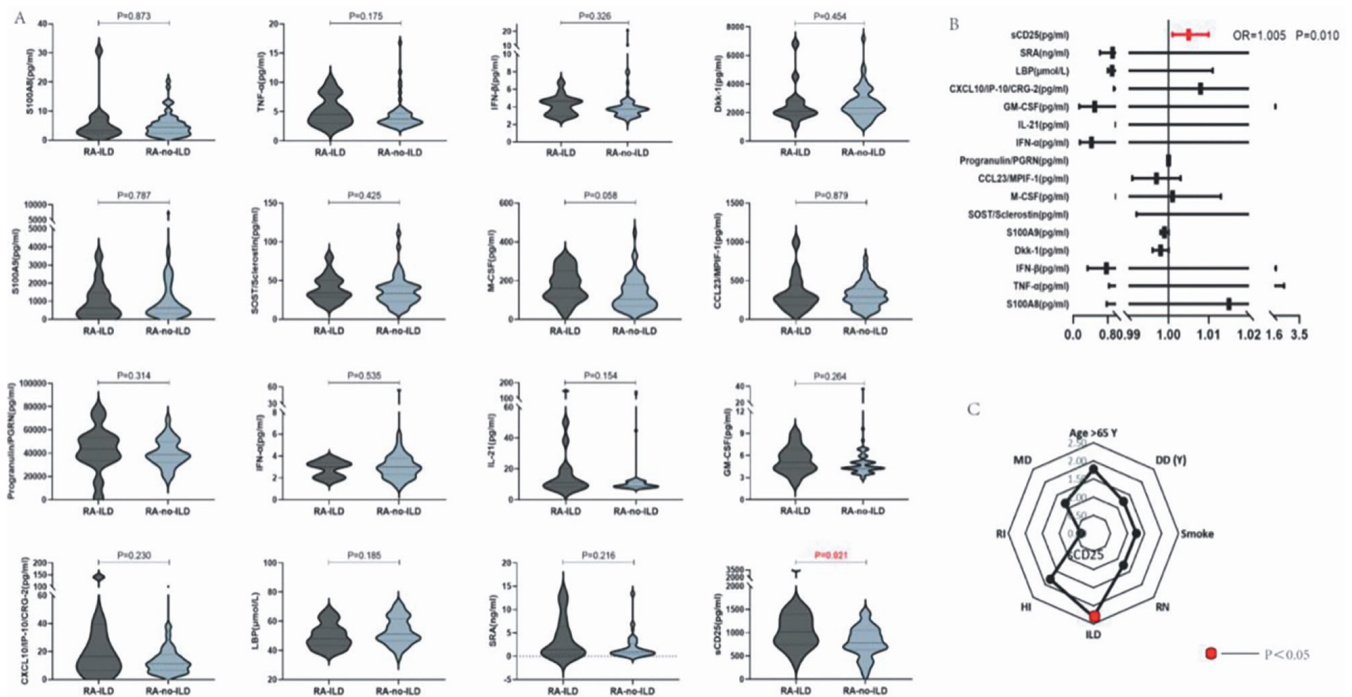


Fig. 1. Up-regulation of sCD25 in sera of RA-ILD patients. A: Comparison of 16-cytokines/chemokines in RA patients with or without ILD. B: Logistics regression of 16-cytokines/chemokines in RA-ILD balancing sex, age and disease duration. C: Radar chart of sCD25 comparing age, history, smoke, rheumatoid nodules, ILD, haematological involvement, renal involvement, and metabolic disorder in RA patients. *p*-value of sCD25 was statistical significance and is marked in red. RA: rheumatoid arthritis; RA-ILD: RA with interstitial lung disease; RA-no-ILD: RA without interstitial lung disease; DD (Y): disease duration (years); RN: rheumatoid nodules; ILD: interstitial lung disease; HI: haematological involvement; RI: renal involvement; MD: metabolic disorder.

Measurement of the serum cytokine levels

The blood sampling for the serum cytokine level analysis was performed at the same time of the clinical evaluation. The same blood samples are used for serum cytokine analysis and the laboratory tests for RA. According to the manufacturer's instructions, the serum cytokine levels of S100A8, TNF- α , IFN- β , Dkk-1, S100A9, SOST/Sclerostin, M-CSF, CCL23/MIPF-1, Progranulin/PGRN, IFN- α , IL-21, GM-CSF, CXCL10/IP-10/CRG-2 were measured by Bio-Plex Pro Human Cytokine 27-plex Assay (BIO-RAD) and sCD25, LBP and SRA were measured by enzyme-linked immunosorbent assay (ELISA).

Measurement of the serum sCD25

Sera from RA patients stored at -80°C until use. There were determined with BIO-RAD and ELISA. All measurements of sCD25 were centralised at the same time in one single laboratory. Serum samples were diluted 1: 3 in sample dilution buffer, absorbance

at 450 nm and 570 nm was measured with a microplate reader and the measurements and data analyses were performed twice independently.

Statistical analysis

Data analyses were performed using SPSS 25.0 (SPSS Inc., IBM, USA) and GraphPad Prism 7.0 (GraphPad Software, San Diego, CA, USA) for Windows. The data were presented as the as the mean \pm standard deviation (SD) or median (interquartile range). The continuous variables were compared by the student's t-test and Mann-Whitney U-test. Binary logistic regression was used to assess the association between categorical clinical variables and the levels of sCD25. Spearman's rank correlation test analysis was applied to calculate the correlations between two variables. Receiver operating characteristic (ROC) curve analysis was applied to evaluate the sensitivity and specificity for the indicative power of sCD25. The optimal cut-off point of sCD25 is selected according to the maximum of

the Youden index. All statistical analyses with *p*-value <0.05 was considered statistically significant.

Results

Serum sCD25 is elevated in patients with RA-ILD

In order to identify potential markers which was correlated with RA-ILD, 16 molecules including cytokines, chemokines and functional soluble cell surface proteins were investigated. The 16 candidate markers were compared in the discovery cohort composed of RA patients with ILD (RA-ILD, n=15) and RA patients without ILD (RA-no-ILD, n=57). Level of sCD25 was significantly elevated in patients with RA-ILD (*p*=0.021, Fig. 1A). Moreover, the elevated sCD25 was also significantly associated with interstitial lung disease by binary regression analysis, after balancing age, sex, disease duration [odds ratio (OR)=1.005, 95% CI=1.001–1.010, *p*=0.022] (Fig. 1B). Multivariate analysis showed that, in comparison with the commonly used RA biomarkers CRP,

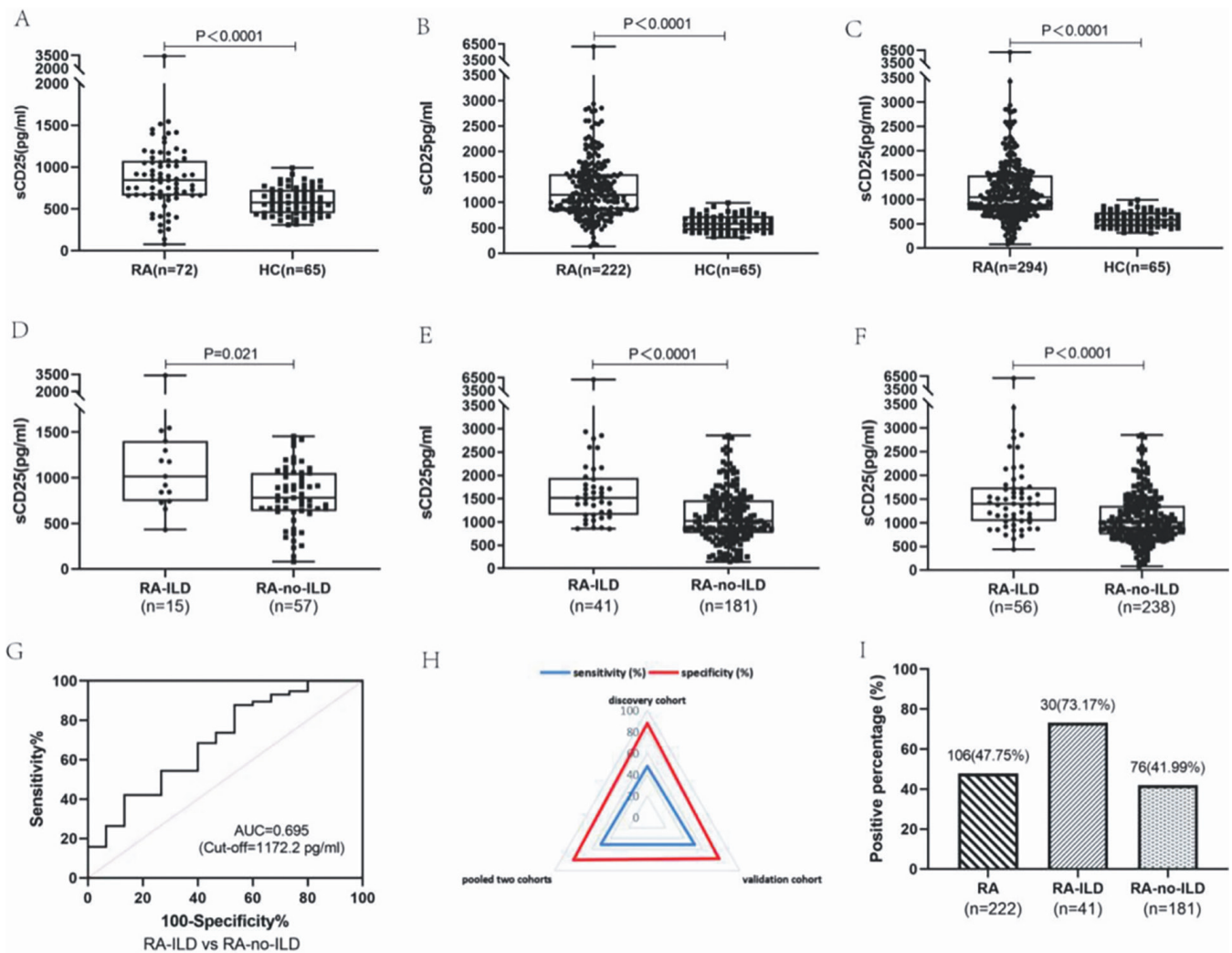


Fig. 2. Comparison of sCD25 in RA-ILD and controls.

A-C: Serum sCD25 level of RA compared with healthy controls, in discovery cohort (A), validation cohort (B) and merged cohort (C).

D-F: Serum sCD25 level of RA-ILD compared with RA-no-ILD, in discovery cohort (D), validation cohort (E) and merged cohort (F); G: ROC curve of sCD25 to discriminate RA-ILD and RA-no-ILD in discovery cohort; H: Sensitivity and specificity of sCD25; I: Percentage of sCD25 positive patients in RA, RA-ILD and RA-no-ILD groups, respectively, in validation cohort.

CCP and RF, only sCD25 significantly associated with RA-ILD (OR=1.003, 95%CI=1.001-1.004, $p=0.001$, Supplementary Table S1). Next, we evaluated sCD25 levels in patients with other organ involvement of RA such as rheumatoid nodule, kidney involvement, haematological disorders and metabolic disorders. It showed that sCD25 was only correlated with RA-ILD but not other organ disorders. ($p=0.021$, Fig. 1C). We further examined the performance of sCD25 in a validation cohort with 41 RA-ILD patients and 181 RA-no-ILD patients. Compared with healthy controls, sCD25 was significantly elevated in both discovery and validation RA cohorts, as well as in the merged cohort (Fig. 2A-B-C, $p<0.001$).

Among the 222 patients with available HRCT data (41 RA-ILD, 181 RA-no-ILD), sCD25 was compared between RA-ILD and RA-no-ILD, the serum sCD25 levels were significantly higher in patients with RA-ILD (Fig. 2D for the discovery cohort; 2E for the validation cohort; 2F for the merged cohort; $p<0.001$).

To further clarify whether sCD25 could be an indicator for RA patient with ILD, ROC analysis was performed to discriminate RA-ILD and RA-no-ILD. The ROC curve of sCD25 was built in discovery cohort with AUCs of 0.695 (Fig. 2G). The cut-off value was 1172.20 pg/ml, with sensitivity of 46.7% and specificity of 87.7% in the discovery cohort and 51.2% sensitivity and 77.3% speci-

ficity in the validation cohort (Fig. 2H). The percentage of sCD25 positive patients in RA-ILD group was 73.17%, which was significantly higher than RA-no-ILD (41.99%) and total RA groups (47.75%) (Fig. 2I, $p<0.01$, $p=0.003$).

Correlation of sCD25 with RA clinical manifestations

We compared sCD25 levels in RA patients with different clinical manifestations and found that sCD25 levels were significantly increased in RA patients with ILD incidence ($p<0.001$), as well as patients with higher DAS28, ESR, CRP and RF ($p<0.001$, 0.001, 0.012, <0.001 , 0.001, Fig. 3A). The correlation between serum sCD25 levels and clinical characteristics were also evalu-

ated by Spearman's correlation analysis. As shown in Figure 3B, sCD25 displayed positive correlation with ESR ($r=0.433$, $p=0.003$), CRP ($r=0.575$, $p<0.001$), DAS28 ($r=0.352$, $p=0.018$), RF ($r=0.361$, $p=0.015$), CCP ($r=0.404$, $p=0.007$) and C3 ($r=0.335$, $p=0.037$).

Elevated serum sCD25 indicated exacerbated disease severity in RA patients with ILD

To further identify the clinical relevance of sCD25 in RA patients with ILD, according to the sCD25 cut-off value (1172.20 pg/ml), RA-ILD patients were grouped into sCD25-positive and sCD25-negative groups, and their clinical characteristics were compared. As shown in Table II, RA-ILD patients with positive sCD25 was more prone to have high disease activity and higher ESR, CRP level. The HRCT patterns of sCD25 positive patients were mainly consisted of UIP and NSIP, while sCD25 negative patients were primarily ILD (Suppl. Table S2). Furthermore, there were more systemic involvements in sCD25 positive patient including rheumatoid nodules, haematological involvements, metabolic disorders, however the differences were not statistically significant (Table II).

We then investigated the association of sCD25 and clinical manifestations in RA patients with ILD and without ILD separately. In RA-ILD patients, sCD25 displayed positive correlation with disease activity index, including tender joint count, swollen joint count, DAS28, ESR and CRP (Fig. 4A-B). Similar correlation was also observed in RA without ILD patients, but the correlation coefficients (Fig. 4A-B) were weaker than in RA-ILD group. It was interesting that RF was correlated with sCD25 in RA-ILD, but was not correlated with sCD25 in RA-no-ILD patients. The correlation of anti-CCP with sCD25 was also weaker in RA-no-ILD group than in RA-ILD group.

The association of sCD25 with different RA-ILD patterns

The most common HRCT patterns were UIP and NSIP. We compared the serum level of sCD25 with different HRCT patterns including UIP and

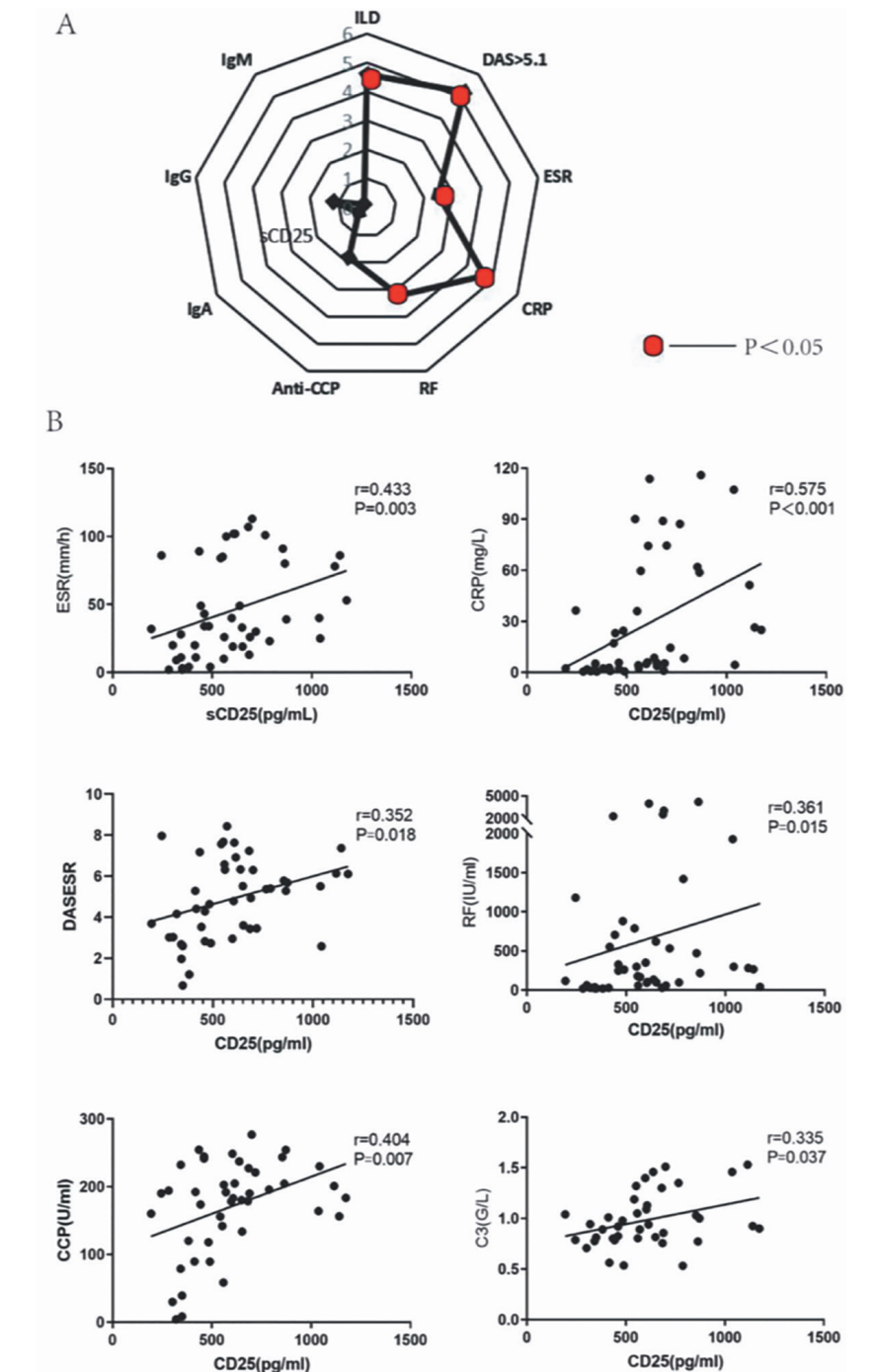


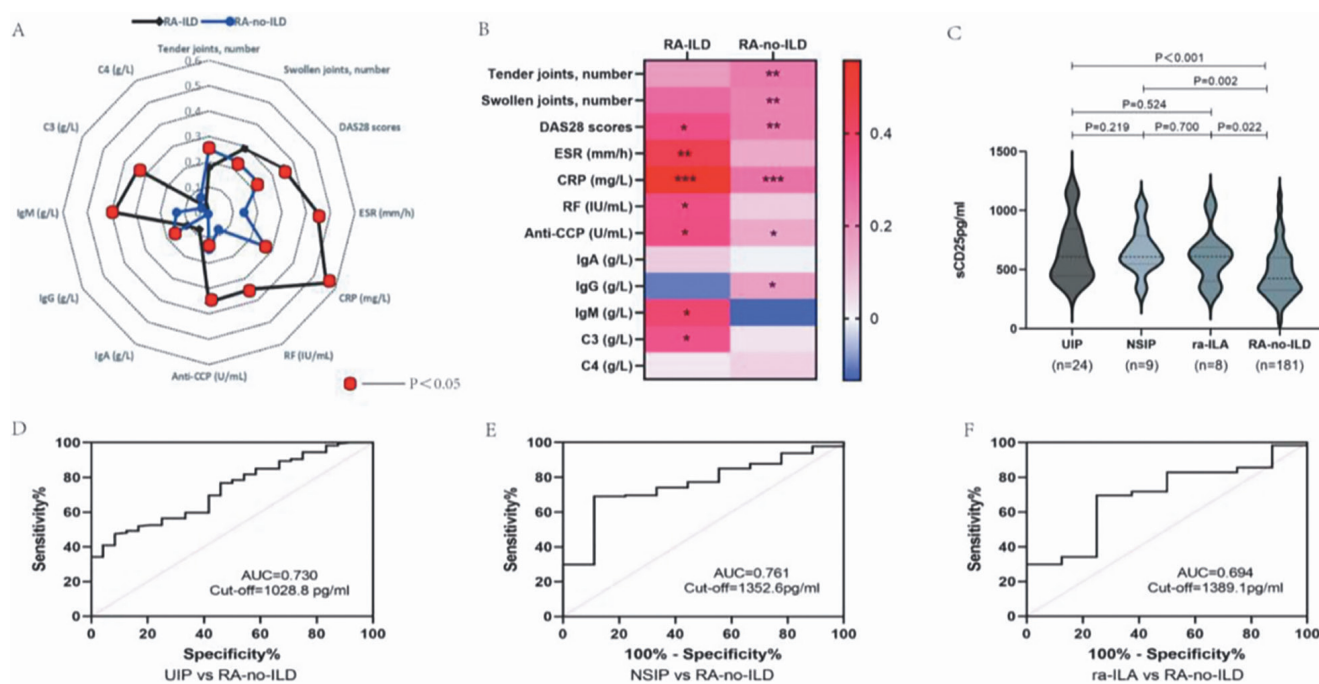
Fig. 3. Correlation of sCD25 and clinical, laboratory manifestations in validation cohorts. **A:** Radar chart of sCD25 in ILD, DAS28, ESR, CR, RF, CCP, IgA, IgG, IgM groups, in the validation cohorts, black lines inside the circle represent p-value. Statistical significance was marked in red dots. **B:** Correlation analysis of sCD25 and RA laboratory characteristics, including ESR, CRP, DAS28, RF, CCP and C3.

NSIP. The result showed that sCD25 were higher in all of the RA-ILD subtypes compared with RA-no-ILD control (UIP: 718.98 ± 437.10 pg/ml, NSIP: 626.27 ± 216.10 pg/ml, RA-ILA: 490.70 ± 248.83 pg/ml vs. RA-no-ILD: 524.43 ± 327.42 pg/ml), with p-values less than 0.05 (Fig. 4C). However, there

were no difference of sCD25 between the UIP, NSIP and RA-ILA groups. ROC curves were generated to analyse the sensitivity and specificity of sCD25 to discriminate RA-ILD from RA-no-ILD (Fig. 4D-F). The ROC curves of sCD25 in UIP, NSIP, RA-ILA revealed sCD25 as diagnostic marker with AUCs

Table II. Sensitivity, specificity, accuracy and area under the ROC curve (AUC) of sCD25 cut-off values in RA-ILD.

Index	Cut-off	AUC	95%CL	p-value	Sensitivity	Specificity	Youden's index
RA-ILD vs. RA-no-ILD							
Discovery cohort (n=72)	1172.2	0.695	0.541-0.849	0.021	46.7%	87.7%	0.344
Validation cohort (n=222)					51.2%	77.3%	0.285
Pooled two cohort (n=294)					50.0%	79.8%	0.298
UIP vs. RA-no-ILD							
UIP vs. RA-no-ILD	1028.8	0.730	0.638-0.823	<0.0001	91.7%	47.5%	0.392
NSIP vs. RA-no-ILD							
NSIP vs. RA-no-ILD	1352.6	0.761	0.629-0.892	0.008	88.9%	69.1%	0.580
RA-ILA vs. RA-no-ILD							
RA-ILA vs. RA-no-ILD	1389.1	0.694	0.528-0.861	0.063	75.0%	69.6%	0.446

**Fig. 4.** Association of sCD25 with RA-ILD.

A-B: Association of sCD25 with clinical characteristics in RA-ILD vs. RA-no-ILD, by Radar chart (A) and heat map (B).

C: Comparison of sCD25 level among RA-ILD with HRCT subtypes.

D-F: ROC of sCD25 in discrimination of UIP (D), NSIP (E) and RA-ILA from RA-no-ILD.

of 0.730, 0.761, and 0.694, respectively ($p < 0.05$, Table II), indicating that sCD25 might be useful biomarkers for RA-ILD. The sensitivity and specificity were shown in Table II. After adjusting for sex, age, history, disease activity, the serum levels of sCD25 [odds ratio (OR) = 1.005, 95% CL = 1.001-1.010] were still significantly associated with RA-ILD by binary logistic regression ($p = 0.01$, OR = 1.005). The same trend was observed in UIP (OR = 1.002, $p = 0.001$) and NSIP (OR = 1.001, $p = 0.016$).

Of the 56 RA with HRCT data available, consolidation, ground glass attenuation, and traction bronchiectasis were frequently observed. As shown in Supplementary Figure S1, sCD25 was significantly higher in consolidation pattern by Mann-Whitney U-test ($p = 0.009$). How-

ever, sCD25 was not correlated with HRCT score ($r = 0.195$, $p = 0.270$, data not shown) which indicated that sCD25 might play roles in RA-ILD patients with consolidation pattern.

Discussion

ILD may be the most frequent pulmonary manifestation of RA patients (5), and has become one of the leading causes of death in individuals with RA. In the present study, we screened 16 cytokines and bone metabolic factors, and found that sCD25 was correlated with RA-ILD. Compared with RA-no-ILD, sCD25 was significantly higher in RA-ILD, especially in UIP and NSIP. Serum sCD25 was also higher in HRCT featured by consolidation but not in other HRCT features. Previous study has dem-

onstrated that sCD25 was elevated in RA patients with high disease activity. It is the first time sCD25 was demonstrated to be correlated with ILD in RA.

Cytokines and bone metabolic factors are related with disease activity and progression of RA. According to previous researches (39-53), we screened 16 cytokines and bone metabolic factors as markers for RA. All of the 16 markers were applied in Bio-Plex Pro Human Cytokine 27-plex Assay (BIO-RAD), including S100A8, TNF- α , IFN- β , Dkk-1, S100A9, SOST/Sclerostin, M-CSF, CCL23/MPIF-1, Progranulin/PGRN, IFN- α , IL-21, GM-CSF and CXCL10/IP-10/CRG-2. Cell surface proteins including sCD25, LBP and SRA were measured by enzyme-linked immunosorbent assay (ELISA).

Interleukin-2 (IL-2) is one of the most important regulators of immune responses (54). CD25 (IL-2R α) is one of IL-2 receptor's polypeptide subunits. The soluble IL-2R α (sIL-2R α), associated with the proliferation of activated T cells (55, 56), is increased in inflammatory diseases, autoimmune diseases and different cancer types. IL-2R α did not participate in receptor signalling directly, instead it enhanced the receptors affinity for IL-2. It has been proposed to have immune-inhibitory and immunostimulatory effect. Associations between genetic variants in IL2RA and RA have been described (57). sIL-2R is generally regarded as a marker of T-lymphocyte activation; however, the cellular function of sIL-2R is still not clear. It has been reported that other types of immune cells, including monocytes, dendritic cells, and B lymphocytes may release sIL-2R as well (58-60). The present study provided clues about the function of sCD25 in RA-ILD.

Serum concentration of sCD25 (61) has also been reported to be elevated in RA, especially in activated disease. Our data showed that in RA-ILD group, sCD25 was correlated with disease activity index, including DAS28, ESR, CRP and swallow joint count. But in the RA without ILD group, the correlation of sCD25 with disease activity index was not as tight as in RA-ILD group. The mechanism might be that sCD25 induces immune cell activation, leading to disease activation and pulmonary inflammation (62, 63).

We further compared sCD25 level and HRCT subtypes. Consolidation in HRCT was correlated with sCD25, but the HRCT Fibrosis Score was not. It might be that sCD25 was more prone to be aggregated in inflammation but not fibrosis site. Our study was a cross sectional study and the included number of patients with HRCT was limited. Further study with enlarged RA-ILD was needed to further confirm relationship between sCD25 and RA-ILD subtype. A prospective cohort is also needed to analyse whether sCD25 could be a prognostic factor in RA-ILD.

In conclusion, sCD25 was elevated in RA-ILD and its subtypes. Serum sCD25 may be a potent serum biomarker for evaluating ILD in RA.

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