Macrophage-mannose receptor CD206 to discriminate connective tissue disease-associated usual interstitial pneumonia from IPF

Xianhua Gui
The Affiliated Drum Tower Hospital of Nanjing University Medical School
Hui Ding
Yixing People's Hospital, Affiliated Jiangsu University
ShenYun Shi
The Affiliated Drum Tower Hospital of Nanjing University Medical School
Jingjing Ding
The Affiliated Drum Tower Hospital of Nanjing University Medical School
Mei Huang
The Affiliated Drum Tower Hospital of Nanjing University Medical School
Jinghong Dai
The Affiliated Drum Tower Hospital of Nanjing University Medical School
Yonglong Xiao
The Affiliated Drum Tower Hospital of Nanjing University Medical School
Min Cao
The Affiliated Drum Tower Hospital of Nanjing University Medical School
Hourong Cai (caihourong2013@163.com)
The Affiliated Drum Tower Hospital of Nanjing University Medical School

Research Article

Keywords: Connective tissue disease, Usual interstitial pneumonia, Idiopathic pulmonary fibrosis, Soluble CD206, Macrophage mannose receptor

Posted Date: March 13th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-284705/v1

Macrophage-mannose receptor CD206 to discriminate connective tissue disease-associated usual interstitial pneumonia from IPF

Xianhua Gui[1,*], Hui Ding[2,*], ShenYun Shi[1,*], Jingjing Ding[1], Mei Huang[1], Jinghong Dai[1], YonglongXiao [1], Min Cao[1,#], Hourong Cai [1,#]

1 Department of Respiratory Medicine, The Affiliated Drum Tower Hospital of Nanjing University Medical School, Nanjing, Jiangsu, China.

2 Department of Respiratory Medicine, Yixing People's Hospital, Affiliated Jiangsu University, Yixing, Jiangsu, China.

* Xianhua Gui, Hui Ding and ShenYun Shi contributed equally to this study.
Correspondence should be addressed to #HourongCai; caihourong2013@163.com

Abstract

Background: Soluble mannose receptor, sCD206, is a marker of alternatively activated macrophages. Activated macrophages play important roles in connective tissue disease (CTD) and lung fibrosis. Interstitial lung disease (ILD) is a common complication of CTD, which leads to poor prognosis, especially in a pattern with usual interstitial pneumonia (UIP). Similarly, idiopathic pulmonary fibrosis (IPF) is also characterized by UIP. Compared with IPF, the prognosis of CTD-associated UIP is tightly correlated with early treatment. In the present study, we aimed to evaluate the clinical significance of sCD206 in discriminating CTD-associated UIP from IPF.

Methods: The serum level of sCD206 was determined in 48 patients with CTD-associated UIP, 54 IPF patients and 27 healthy controls. The clinical significance of sCD206 was also evaluated.

Result: Patients with CTD-associated UIP had a higher level of sCD206 compared with healthy controls and IPF patients ($p<0.001$, $p<0.001$, respectively). A cut-off

value of sCD206 at 523.8 ng/mL could be a useful marker for distinguishing CTD-associated UIP from IPF. Moreover, 15 decedents with CTD-associated UIP exhibited a greater level of sCD206 compared with 33 survivors (p=0.030), and the elevated sCD206 level was associated with a higher mortality rate (log-rank test, p=0.003). Age and gender-adjusted multivariate Cox regression analysis showed that sCD206 (>444.2 ng/mL) was an independent predictor of survival (p= 0.026).

Conclusions: Collectively, the serum level of sCD206 could be a useful marker for distinguishing CTD-associated UIP from IPF and associated with a poor survival of CTD-associated UIP.

Key words: Connective tissue disease; Usual interstitial pneumonia; Idiopathic pulmonary fibrosis; Soluble CD206; Macrophage-mannose receptor

1. Introduction

Connective tissue diseases (CTDs) are a group of diseases with inflammatory, immune-mediated organ damage that can cause a variety of pulmonary manifestations [1]. About 75% of CTD patients may have various patterns of interstitial pneumonia, such as non-specific interstitial pneumonia (NSIP), usual interstitial pneumonia (UIP), organizing pneumonia (OP) and lymphocytic interstitial pneumonia (LIP) [2,3]. CTD-associated UIP is a serious pulmonary complication that is commonly observed in rheumatoid arthritis (RA), systemic sclerosis (SSC), Sjogren syndrome (SS) and systemic vasculitis [4]. Recently, there is no significant difference in terms of the clinical examination and pulmonary function between CTD-associated UIP and idiopathic pulmonary fibrosis (IPF) [5,6]. Moreover, it is difficult to identify CTD-associated UIP and IPF at the initial diagnosis due to the lack of positive serum

immunoantibody markers [7]. It is well accepted that IPF is a rare, progressively debilitating interstitial lung disease characterized by UIP in high-resolution computed tomography (HRCT) and pathology [8]. The median survival time is 3–5 years, which is worse than many cancers [9]. Nevertheless, patients with CTD-associated with UIP would benefit from early treatment compared with IPF. Therefore, it is urgently necessary to identify circulating biomarkers to distinguish CTD-associated UIP from IPF.

It is well known that M2-polarized macrophages are characterized by inhibited secretion of inflammatory cytokines, decreased bactericidal ability and enhanced ability of tissue repair [10,11]. As a mannose receptor, CD206 is primarily expressed on the surface of M2-polarized macrophages, which is involved in immune recognition and adaptive immune response. Therefore, CD206 is used as a marker for activation of M2 macrophages, and soluble forms of CD206 (sCD206) are also found in the serum [12]. It is previously believed that immune disorders play a key role in the pathogenesis of fibrotic disease, especially in macrophage activation [13,14]. In fact, the main infiltration of M2 macrophages in the pulmonary fibrotic region is an important regulator of fibrogenesis [14,15]. Moreover, recent studies have shown that M2 macrophages are implicated in the development and progression of lung fibrosis [16,17].

Recently, some studies have shown that macrophage activation plays a crucial role in the occurrence of autoimmune diseases [18,19]. The elevated sCD206 level has been observed in RA, while it is decreased after treatment of anti-rheumatic drugs [20]. Moreover, macrophages can produce a large amount of inflammatory cytokines

and chemokines, including TNF, IL-1, IL-6, IL-8 and CXCL10, which play an important role in the pathogenesis of RA [21]. Additionally, sCD206 has been proved to be useful for monitoring the progression of dermatomyositis-associated interstitial lung disease (ILD) [22]. CTD-associated UIP has worse prognosis. So far, the precise effect of sCD206 on IPF has been defined, while its role in CTD-associated UIP remains largely unexplored. Therefore, we aimed to investigate the characteristics of sCD206 in CTD-associated UIP and IPF to assess its probable role as the indicator for the discrimination of these two subsets of patients.

2. Materials and Methods

2.1. Study subjects.

Fifty-four IPF patients and 48 CTD associated UIP patients were admitted to Nanjing Drum Tower Hospital between January 2015 and April 2018. Forty-eight patients with CTD included RA: 24 cases, SS: 22 cases, systematic vasculitis: 2 cases. Twenty-seven healthy volunteers from the Center of Physical Examination were included as a control group. IPF was diagnosed according to the diagnostic criteria described by Raghu G [8]. The diagnosis of CTD conform to their respective diagnostic criteria, including the American rheumatic society's diagnostic criteria for RA in 1988 [23], the classification criteria for systematic vasculitis updated at the Chapel Hill conference in 2012 [24], the international classification criteria for SS in 2002 [25]. CTD patients with a pattern of UIP on HRCT were diagnosed according to the guidelines for the American thoracic society and the European respiratory

society [8]. Briefly, the criteria for a diagnosis of UIP were as follows 1) Subpleural and basal distribution; 2) Reticular pattern 3)Honeycombing with or without peripheral traction; Moreover, patients with characteristics inconsistent with UIP, such as upper or middle distribution, distribution along the bronchi, predominant ground-glass opacity, profuse micronodules, scattered cysts, consolidation, diffuse mosaic attenuation, were excluded.

The informed consents to participate in this study were obtained from all subjects. This study was approved by the Ethics Committee at Nanjing Drum Tower Hospital and conducted in accordance with the principles of the Declaration of Helsinki (1989).

2.2. Collection of clinical data.

Clinical data were obtained from the patients' medical records at admission and during follow-up telephone calls. Record of the results of laboratory tests and lung function tests were obtained at the time of diagnosis.

2.3. Measurement of serum sCD206, sCD163 and KL-6.

Serum samples after collection were stored at −80 °C until analysis. sCD206 and sCD163 levels were determined by using human sCD206 and sCD163 enzyme-linked immunosorbent assay kit (Ray-Biotech, Norcross, GA, USA) according to the manufacturer's instructions. KL-6 serum levels were assayed using the KL-6 Kit (Fujire-bio, Inc., Tokyo, Japan) on an automated immunoassay

analyzer LUMIPULSE G1200 (Fujire-bio, Inc., Tokyo, Japan). Written informed consents about serum conservation were obtained from all the subjects.

2.4. Statistical analyses

Continuous variables were expressed as the median [25th to 75th percentiles of the interquartile range (IQR)]. Discrete variables were expressed as counts (percent). Mann-Whitney U test was used for variables among CTD associated UIP, IPF and Controls. The correlations between serum levels of CD206 and clinical variables were analyzed by Spearman's rank correlation technique. Receiver operating characteristic (ROC) curves were drawn, and areas under ROC curves (AUCs) were calculated to determine the value of sCD206 to discriminate between CTD associated UIP and IPF. Survival time was calculated as the time from initial diagnosis until death or the censoring time. To predict mortality of CTD associated UIP patients, univariate analyses were performed with Cox regression analysis. Gender and age as covariates were used for multivariate analysis. Subsequently, the log-rank test was used to compare survival in CTD associated UIP and IPF patients. ROC curves were used to assess the performance of sCD206 as a marker of death in CTD associated UIP. The statistical analyses were performed by using SPSS version 19 (SPSS, Inc., Chicago IL, USA) and Prism version 6 (GraphPad, San Diego, CA, USA). P values lower than 0.05 were considered significant.

3. Results

3.1. Demographic and clinical characteristics of patients with CTD-associated UIP

and IPF.

Table 1 summarizes the baseline characteristics of patients and healthy controls. Data showed that 92.6% of IPF patients were males, while such proportion became 72.9% in patients with CTD-associated UIP. Similar results were found in forced vital capacity (FVC), diffusing capacity of the lung for carbon monoxide (DLco) and arterial oxygen pressure (PaO_2) between the IPF and CTD-associated UIP groups. Patients with CTD-associated UIP had a higher C-reactive protein (CRP) level compared with the IPF patients (Table 1).

Table 1 Baseline characteristics of patients

Clinical Characteristics	CTD-UIP (n=48)	IPF (n = 59)	Controls (n = 30)	p value CTD-UIP VS IPF
Age(years)	68.0(62.0-75.0)	66.0(63.0-75.0)	65.0(63.0-69.3)	0.799
Sex(Male)	35 (72.9%)	54 (91.5%)	26(86.7%)	0.002**
P_aO2	72.5(64.8-78.0)	71.0(65.0-78.0)	NA	0.997
FVC% predicted	69.4(58.6-84.3)	72.1 (62.3-83.6)	NA	0.640
DLco% predicted	43.4(33.1-66.3)	50.0(39.4-67.0)	NA	0.253
LDH (U/L)	248.0(213.0-346.0)	238.0(205.0-278.0)	NA	0.152
CRP (mg/L)	11.8(3.7-38.7)	5.0(3.3-16.4)	NA	0.039*

*p< 0.05,**p< 0.01

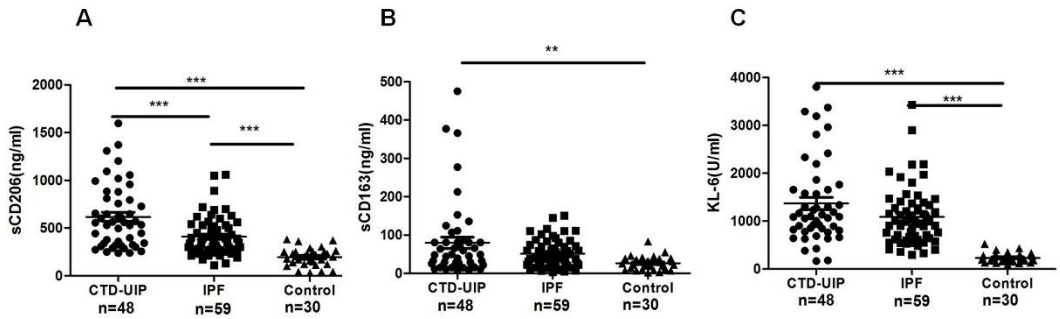
FVC, forced vital capacity. DLco, diffusing capacity of the lung for carbon monoxide; CRP, C-reactive protein; LDH, lactate dehydrogenase;

3.2. Serum concentrations of sCD206, sCD163 and KL-6 in IPF patients.

The serum levels of sCD206 (558.6 [328.6, 807.4] versus 200.0 [120.0, 250.0] ng/mL,

p<0.001), sCD163 (46.8 [20.6, 80.4] versus 25.2 [9.9, 38.8] ng/mL, p=0.005) and KL-6 (1,095.0 [808.0, 1,654.0] versus 181.0 [162.0, 280.0] U/mL, p<0.001) were significantly increased in patients with CTD-associated UIP compared with the healthy controls. The median serum sCD206 level from CTD-associated UIP patients was increased by more than two folds compared with the IPF patients (558.6 [328.6, 807.4] versus 335.0 [267.5, 492.5] ng/mL, p<0.001). However, no obvious differences in serum sCD163 and KL-6 levels were found between the CTD-associated UIP and IPF patients (46.8 [20.6, 80.4] versus 37.9 [22.0, 70.7] ng/mL, p=0.457 and (1,095.0 [808.0, 1,654.0] versus 923.0 [617.0, 1,292.5] U/mL, p=0.059, respectively) (Fig. 1).

Fig.1. Serum sCD206, sCD163 and KL-6 levels in CTD-associated UIP, IPF patients and normal controls

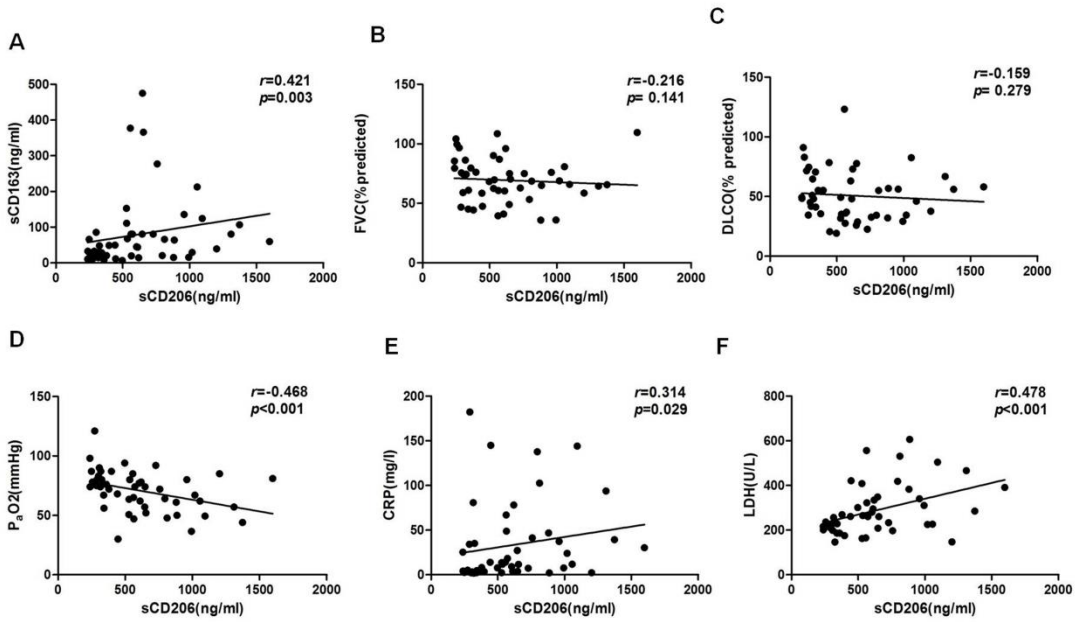


Serum concentrations of sCD206 (A), sCD163 (B) and KL-6 (C) levels in patients with CTD-associated UIP, IPF patients and controls. P-values were determined by Mann Whitney U test. CTD: connective tissue disease; UIP: usual interstitial pneumonia; IPF: idiopathic pulmonary fibrosis; sCD206: Soluble CD206; sCD163: Soluble CD163.

3.3. Correlations between sCD206 concentration and study variables at baseline.

Correlation analyses showed that the serum sCD206 level in CTD-associated UIP patients was not associated with lung function (FVC% or DLco%). The serum sCD206 level was positively associated with sCD163, and the inflammatory markers CRP and lactate dehydrogenase (LDH), but negatively correlated with PaO_2 (Fig. 2).

Fig.2. Correlations between sCD206 concentration and study variables



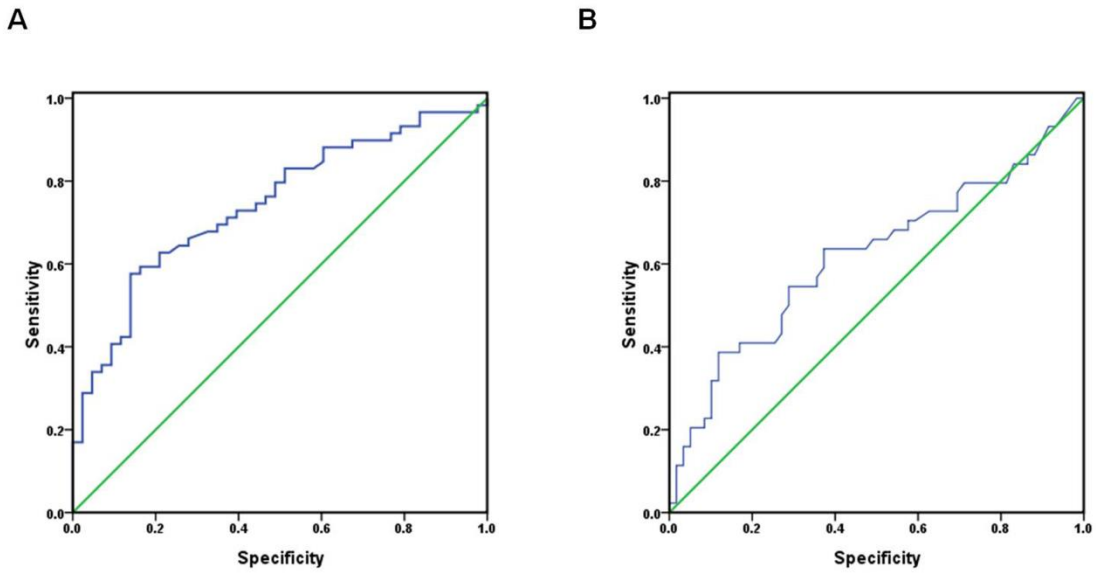
The statistical comparisons of sCD206 and variables were performed using Spearman's rank correlation coefficient. FVC, forced vital capacity. DLco, diffusing capacity of the lung for carbon monoxide; CRP, C-reactive protein; LDH, lactate dehydrogenase;

3.4. Serum sCD206 as the discriminative feature between CTD -associated UIP and IPF.

We calculated the optimal cut-off value of sCD206 for distinguishing CTD-associated UIP and IPF patients by using receiver operating characteristic

(ROC) analysis. The cut-off value of serum sCD206 was 523.8 ng/mL (sensitivity 60.3%, specificity 79.6%). The area under the ROC curve (AUC) for sCD206 in distinguishing CTD-associated UIP from IPF was 0.728 (95% CI, 0.631-0.826; p<0.001). The level of CRP was also elevated in patients with CTD-associated UIP, while serum sCD206 was a better biomarker of CTD-associated UIP compared with CRP (AUC 0.633, p=0.024) (Fig. 3).

Fig.3. ROC curve analyses for all the patients according to the serum sCD206 levels.



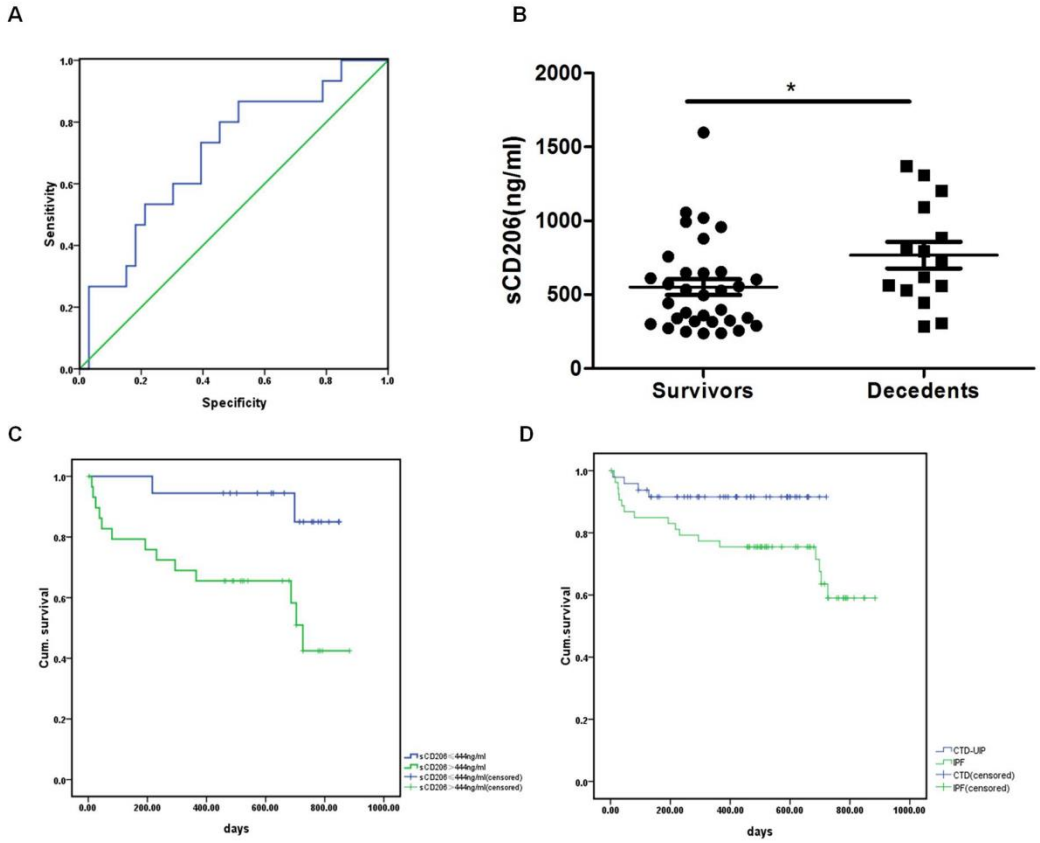
ROC curve analyses for all the patients according to the serum sCD206 levels. The AUC for serum sCD206 were statistically obvious in the differentiation of CTD associated UIP subjects (p<0.001, cut-off value 523.8 ng/ml) ,which was better than CRP levels (p=0.024, cut-off value 23.9 mg/l).

3.5. *Prognostic value of sCD206 in patients with CTD-associated UIP and survival*

analyses.

ROC analysis was used to evaluate the optimal cut-off value of sCD206 to predict the mortality of CTD-associated UIP. In the ROC analysis, the AUC for sCD206 was 0.697 (95%CI, 0.537-0.857) with the cut-off value of 444.2 ng/mL. The sensitivity and specificity were 86.7% and 60.3%, respectively (Fig. 4A). Of the 48 subjects with CTD, 15 died, and 33 survived. The serum sCD206 level was higher in decedents compared with survivors (726.9 [528.9, 1,094.1] versus 495.8 [316.9, 650.7] ng/mL, p=0.030) (Fig. 4B). Compared with patients with a sCD206 level higher than 444.2 ng/mL, those with a level lower than 444.2 ng/mL (n=15) had a better survival (n=33) (log-rank, p= 0.003) (Fig. 4C). Moreover, the mortality of IPF patients was higher compared with the patients with CTD-associated UIP (log-rank, p= 0.003) (Fig. 4D). In addition, univariate and multivariate analyses adjusted with age and gender were used to evaluate the mortality risk for the patients with CTD-associated UIP. Among the variables, elevated serum levels of CRP, LDH and sCD206 but not KL-6 were obviously associated with a poor outcome in patients with CTD-associated UIP. Besides, decreased FVC% and PaO_2 were also prognostic factors of markedly worse outcomes (Table 2).

Fig.4. Prognostic value of sCD206 in patients with CTD-associated UIP and survival analyses



ROC curve analyses for CTD-associated UIP patients to predict the mortality of CTD-associated UIP (A); The serum concentrations of sCD206 were higher in decedents than survivors with CTD-associated UIP (B); Kaplan-Meier curves of patients with CTD-associated UIP according to serum sCD206 (C); Kaplan-Meier curves showed that mortality was lower in patients with CTD-associated UIP than in those with IPF by log-rank test (D).

Table 2 Prediction for survival in CTD-UIP patients

Variables	Univariate analysis			Multivariate analysis		
	HR	95% CI	p-value	HR	95% CI	p-value
Age, yr	1.035	0.970-1.103	0.299			
Gender,	2.452	0.887-6.773	0.084			
P a O2	0.965	0.937-0.993	0.016*	0.954	0.925-0.983	0.002**
FVC%	0.955	0.918-0.994	0.023*	0.954	0.920-0.988	0.009**
DLco%	0.969	0.932-1.008	0.118	0.966	0.933-0.999	0.056
CRP	1.015	1.006-1.025	0.001**	1.019	1.009-1.029	< 0.001***
LDH	1.008	1.004-1.012	< 0.001***	1.009	1.005-1.014	< 0.001***
sCD206 (continuous)	1.001	1.000-1.003	0.030*	1.001	1.000-1.003	0.048*
sCD206 (>444.2ng/ml)	0.18	0.040-0.801	0.024*	0.183	0.041-0.820	0.026*
sCD163	0.997	0.990-1.004	0.403	0.997	0.990-1.004	0.385
KL-6 (continuous)	1.000	0.999-1.000	0.320	1.000	0.999-1.000	0.322

*p< 0.05,**p< 0.01,***p< 0.001

FVC, forced vital capacity. DLco, diffusing capacity of the lung for carbon monoxide; CRP, C-reactive protein; LDH, lactate dehydrogenase;

4. Discussion

In this study, we evaluated the clinical importance of alternatively activated macrophage marker CD206 in patients with CTD-associated UIP. We found that patients with CTD-associated UIP had a higher serum sCD206 level compared with IPF patients. In particular, the higher level of sCD206 (>523.8 ng/mL) was helpful in distinguishing patients with CTD-associated UIP from those with IPF. Statistically, elevated sCD206 was closely associated with a higher risk of mortality. We also confirmed an improved survival for patients with CTD-associated UIP. Additionally, elevated serum levels of CRP and LDH as well as worse lung function (FVC%) could also be used to evaluate the prognosis of CTD-associated UIP. Collectively, these

results provided clinical implications of alternatively activated macrophages in the disease differentiation of CTD-associated UIP and IPF, and evaluations of its related marker CD206 might predict the mortality in patients with CTD-associated UIP.

The relatively macrophage-specific receptor CD206 is primarily expressed by macrophages, while its expression may also be found in dendritic cells and endothelial cells [26], which is a commonly used marker of macrophages both in vivo and in vitro [27]. Several studies have revealed that the elimination of macrophages in lupus rats significantly alleviates the activity of lupus nephritis [28]. Moreover, in renal tissues of patients with type IV lupus nephritis, high levels of macrophages predict a poor prognosis [29]. Similarly, the activation of macrophages is also involved in the pathogenesis of IPF.

It has been well documented that pulmonary fibrosis is considered as a histopathological feature of epithelial cell damage and activation, fibroblast proliferation and excessive extracellular matrix formation in lung parenchyma [30,31]. Macrophages play a vital role in the pathogenesis of IPF through the production of specific factors and biological activities [17]. In particular, M2 macrophages are recognized to associate with the progressive phase of lung fibrosis in mice and humans [17,32]. For example, CC chemokine ligand18 (CCL18), a product of M2 macrophages, promotes collagen production by lung fibroblasts in patients with pulmonary fibrosis [33]. Moreover, high levels of M2 cytokine CCL18 are significantly predictive for the risk of acute exacerbation in IPF patients [34].

Similarly, fibrotic mediators TGF-β and PDGF released by M2 macrophages play an important role in migration, accumulation of myofibroblast and collagen deposition, which boost the progression of lung fibrosis [35]. In addition, IL10 can accelerate bleomycin-induced lung fibrosis, which may be involved in fibrocyte recruitment and M2 macrophage activation via the CCL2/CCR2 axis [36]. Based on these findings, macrophage activation not only plays a role in CTD, but also in the occurrence and development of pulmonary fibrosis. However, we speculated that serum sCD206 could be used as an inflammatory marker in patients with CTD-associated UIP. Here, we firstly showed that the sCD206 level in CTD-associated UIP patients was higher compared with IPF patients, which was positively associated with sCD163, CRP and LDH levels, but negatively correlated with PaO_2. In addition, the cut-off value of serum sCD206 level obtained from ROC curves could be used to distinguish these two subsets of patients. Although results from some studies have suggested that the KL-6 level is useful in predicting the IPF outcomes [37,38], the specificity of KL-6 has not been evaluated for CTD-associated UIP. In the present study, the serum KL-6 level in patients with CTD-associated UIP was similar to that in IPF patients. In contrast, KL-6 was unable to distinguish CTD-UIP from IPF in our study. The CRP level was also higher in patients with CTD-associated UIP, while no obvious correlation was found between the CRP and other clinical parameters. We hypothesized that the activity of CTD might affect the CRP level, which could not be used to differentiate the etiology of UIP.

Previous study has demonstrated that individuals with CTD-associated pulmonary

fibrosis have a better survival compared with IPF patients [39,40]. Consistent with the published reports, we also found that the mortality of patients with CTD-associated UIP was lower compared with IPF patients. In addition, patients with a serum sCD206 level above the cut-off value (444 ng/mL) had an obviously shorter survival than those with a lower value. Lastly, the multivariate analysis demonstrated that sCD206 remained a predictor of survival even after the adjustment for age and gender.

Overall, we assessed the role of serum sCD206 in differentiating CTD-associated UIP from IPF, and its prognostic significance in patients with CTD-associated UIP was also evaluated. However, there are several limitations in our present study. First, our ability to conduct a serial analysis of sCD206 was limited due to the rarity of IPF population. Second, there was a lack of long-term follow-up for the prognosis of patients to confirm the results. A longitudinal study in a larger population would be helpful for further research. Third, the pathogenesis of M2 macrophages was not clearly clarified in the occurrence of CTD-associated UIP, and further basic research is still necessary to reveal the specific mechanism.

Data Availability

All data used to support the finding of this study are available from the corresponding author upon request.

Ethical approval and consent to participate

This study was approved by the Ethics Committee of Nanjing Drum Tower Hospital

of Medical School of Nanjing University (No.31/93, 84/93, 29/01). Written informed consent was obtained from all subjects in the study protocol.

Conflicts of interest

The authors have declared that no conflict of interest exists.

Authors' Contributions

Hourong Cai conceived and designed the study. Xianhua Gui, Hui Ding, ShenyunShi and JingjingDing collected and analyzed the data. Meihuang and JinghongDai contributed to analysis tools. Xianhua Gui, MinCao wrote the paper. All authors reviewed the manuscript critically and agreed upon publication. Xianhua Gui, Hui Ding and Shenyun Shi contributed equally to this work.

Acknowledgement

This work was supported by National Natural Science Foundation of China (Grant No. 81970063), Mr Hourong Cai;

References

1 Fischer, A. & du Bois, R. Interstitial lung disease in connective tissue disorders. *Lancet (London,England)* **380**,689-698, doi:10.1016/s0140-6736(12)61079-4 (2012).

2 Vij, R. & Strek, M. E. Diagnosis and treatment of connective tissue disease-associated interstitial lung disease. *Chest* **143**, 814-824, doi:10.1378/chest.12-0741 (2013).

3 Kim, E. J. *et al.* Usual interstitial pneumonia in rheumatoid arthritis-associated interstitial lung disease. *The European respiratory journal* **35**, 1322-1328, doi:10.1183/09031936.00092309 (2010).

4 Wallace, B., Vummidi, D. & Khanna, D. Management of connective tissue diseases associated interstitial lung disease: a review of the published literature. *Current opinion in rheumatology* **28**, 236-245, doi:10.1097/bor.0000000000000270 (2016).

5 White, E. S. *et al.* Plasma Surfactant Protein-D, Matrix Metalloproteinase-7, and Osteopontin Index Distinguishes Idiopathic Pulmonary Fibrosis from Other Idiopathic Interstitial Pneumonias. *American journal of respiratory and critical care medicine* **194**, 1242-1251, doi:10.1164/rccm.201505-0862OC (2016).

6 Flaherty, K. R. *et al.* Fibroblastic foci in usual interstitial pneumonia: idiopathic versus collagen vascular disease. *American journal of respiratory and critical care medicine* **167**, 1410-1415,doi:10.1164/rccm.200204373OC (2003).

7 Wuyts, W. A. *et al.* Differential diagnosis of usual interstitial pneumonia: when is it truly idiopathic? *European respiratory review : an official journal of the European Respiratory Society* **23**, 308-319, doi:10.1183/09059180.00004914 (2014).

8 Raghu, G. Diagnosis of Idiopathic Pulmonary Fibrosis. An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline. **198**, e44-e68, doi:10.1164/rccm.201807-1255ST (2018).

9 Nalysnyk, L., Cid-Ruzafa, J., Rotella, P. & Esser, D. Incidence and prevalence of idiopathic pulmonary fibrosis: review of the literature. *European respiratory review : an official journal of the European Respiratory Society* **21**, 355-361, doi:10.1183/09059180.00002512 (2012).

10 Stein, M., Keshav, S., Harris, N. & Gordon, S. Interleukin 4 potently enhances murine macrophage mannose receptor activity: a marker of alternative immunologic macrophage activation. *The Journal of experimental medicine* **176**, 287-292, doi:10.1084/jem.176.1.287 (1992).

11 Doyle, A. G. *et al.* Interleukin-13 alters the activation state of murine macrophages in vitro: comparison with interleukin-4 and interferon-gamma. *European journal of immunology* **24**, 1441-1445, doi:10.1002/eji.1830240630 (1994).

12 Nielsen, M. C. *et al.* The macrophage-related biomarkers sCD163 and sCD206 are released by different shedding mechanisms. *Journal of leukocyte biology* **106**, 1129-1138, doi:10.1002/jlb.3a1218-500r (2019).

13 Duffield, J. S., Lupher, M., Thannickal, V. J. & Wynn, T. A. Host responses in tissue repair and fibrosis. *Annual review of pathology* **8**, 241-276, doi:10.1146/annurev-pathol-020712-163930 (2013).

14 Wynn, T. A. & Vannella, K. M. Macrophages in Tissue Repair, Regeneration, and Fibrosis. *Immunity* **44**, 450-462, doi:10.1016/j.immuni.2016.02.015 (2016).

15 Gibbons, M. A. *et al.* Ly6Chi monocytes direct alternatively activated profibrotic macrophage regulation of lung fibrosis. *American journal of respiratory and critical care medicine* **184**, 569-581, doi:10.1164/rccm.201010-1719OC (2011).

16 Gordon, S. & Martinez, F. O. Alternative activation of macrophages: mechanism and functions. *Immunity* **32**, 593-604, doi:10.1016/j.immuni.2010.05.007 (2010).

17 Zhang, L. *et al.* Macrophages: friend or foe in idiopathic pulmonary fibrosis? *Respiratory research* **19**, 170, doi:10.1186/s12931-018-0864-2 (2018).

18 Zhang, W. *et al.* AIM2 facilitates the apoptotic DNA-induced systemic lupus erythematosus via arbitrating macrophage functional maturation. *Journal of clinical immunology* **33**, 925-937, doi:10.1007/s10875-013-9881-6 (2013).

19 Zhang, W., Xu, W. & Xiong, S. Blockade of Notch1 signaling alleviates murine lupus via blunting macrophage activation and M2b polarization. *Journal of immunology (Baltimore, Md. : 1950)* **184**, 6465-6478, doi:10.4049/jimmunol.0904016 (2010).

20 Heftdal, L. D., Stengaard-Pedersen, K. & Ørnbjerg, L. M. Soluble CD206 plasma levels in rheumatoid arthritis reflect decrease in disease activity. **77**, 385-389, doi:10.1080/00365513.2017.1331462 (2017).

21 Hamilton, J. A. & Tak, P. P. The dynamics of macrophage lineage populations in inflammatory and autoimmune diseases. *Arthritis and rheumatism* **60**, 1210-1221, doi:10.1002/art.24505 (2009).

22 Horiike, Y. *et al.* Successful classification of macrophage-mannose receptor CD206 in severity of anti-MDA5 antibody positive dermatomyositis associated ILD. *Rheumatology (Oxford, England)* **58**, 2143-2152, doi:10.1093/rheumatology/kez185 (2019).

23 Arnett, F. C. *et al.* The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis and rheumatism* **31**, 315-324, doi:10.1002/art.1780310302 (1988).

24 Jennette, J. C. *et al.* 2012 revised International Chapel Hill Consensus Conference Nomenclature of

Vasculitides. *Arthritis and rheumatism* **65**, 1-11, doi:10.1002/art.37715 (2013).

25 Vitali, C. *et al.* Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. *Annals of the rheumatic diseases* **61**, 554-558, doi:10.1136/ard.61.6.554 (2002).

26 Martinez-Pomares, L. The mannose receptor. *Journal of leukocyte biology* **92**, 1177-1186, doi:10.1189/jlb.0512231 (2012).

27 Andersen, M. N. *et al.* The novel biomarker of alternative macrophage activation, soluble mannose receptor (sMR/sCD206): Implications in multiple myeloma. *Leukemia research* **39**, 971-975, doi:10.1016/j.leukres.2015.06.003 (2015).

28 Chalmers, S. A. *et al.* Macrophage depletion ameliorates nephritis induced by pathogenic antibodies. *Journal of autoimmunity* **57**, 42-52, doi:10.1016/j.jaut.2014.11.007 (2015).

29 Cheunsuchon, B., Incharoen, P., Chawanasuntorapoj, R., Chanchairujira, T. & Shayakul, C. Glomerular macrophage is an indicator of early treatment response in diffuse proliferative lupus nephritis. *Journal of the Medical Association of Thailand = Chotmaihet thangphaet* **96 Suppl 2**, S246-251 (2013).

30 King, T. E., Jr., Pardo, A. & Selman, M. Idiopathic pulmonary fibrosis. *Lancet (London, England)* **378**, 1949-1961, doi:10.1016/s0140-6736(11)60052-4 (2011).

31 Wuyts, W. A. *et al.* Differential diagnosis of usual interstitial pneumonia: when is it truly idiopathic? *European respiratory review : an official journal of the European Respiratory Society* **23**, 308-319, doi:10.1183/09059180.00004914 (2014).

32 Stahl, M. *et al.* Lung collagens perpetuate pulmonary fibrosis via CD204 and M2 macrophage activation. *PloS one* **8**, e81382, doi:10.1371/journal.pone.0081382 (2013).

33 Prasse, A. *et al.* A vicious circle of alveolar macrophages and fibroblasts perpetuates pulmonary fibrosis via CCL18. *American journal of respiratory and critical care medicine* **173**, 781-792, doi:10.1164/rccm.200509-1518OC (2006).

34 Schupp, J. C. *et al.* Macrophage activation in acute exacerbation of idiopathic pulmonary fibrosis. *PloS one* **10**, e0116775, doi:10.1371/journal.pone.0116775 (2015).

35 Murray, L. A. *et al.* TGF-beta driven lung fibrosis is macrophage dependent and blocked by Serum amyloid P. *The international journal of biochemistry & cell biology* **43**, 154-162, doi:10.1016/j.biocel.2010.10.013 (2011).

36 Sun, L. *et al.* New concepts of IL-10-induced lung fibrosis: fibrocyte recruitment and M2 activation in a CCL2/CCR2 axis. *American journal of physiology. Lung cellular and molecular physiology* **300**, L341-353, doi:10.1152/ajplung.00122.2010 (2011).

37 Ishikawa, N., Hattori, N., Yokoyama, A. & Kohno, N. Utility of KL-6/MUC1 in the clinical management of interstitial lung diseases. *Respiratory investigation* **50**, 3-13, doi:10.1016/j.resinv.2012.02.001 (2012).

38 Yokoyama, A. *et al.* Prognostic value of circulating KL-6 in idiopathic pulmonary fibrosis. *Respirology (Carlton, Vic.)* **11**, 164-168, doi:10.1111/j.1440-1843.2006.00834.x (2006).

39 Navaratnam, V. *et al.* Does the presence of connective tissue disease modify survival in patients with pulmonary fibrosis? *Respiratory medicine* **105**, 1925-1930, doi:10.1016/j.rmed.2011.08.015 (2011).

Figures

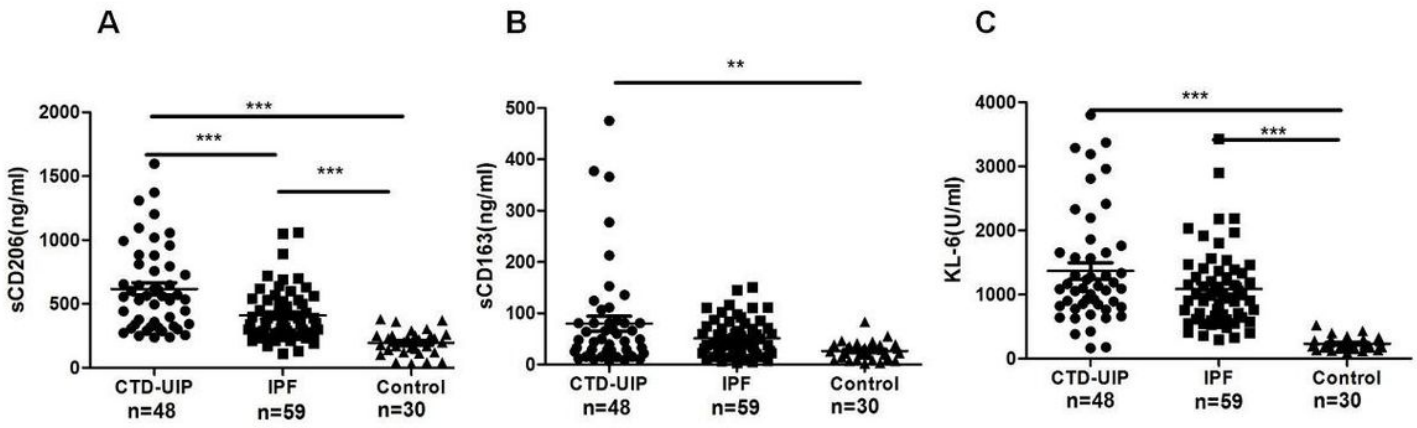


Figure 1

Serum sCD206 sCD 163 and KL 6 levels in CTD associated UIP , IPF patients and normal controls. Serum concentrations of sCD206 (sCD 163 (B) and KL 6 (C) levels in patients with CTD associated UIP , IPF patients and controls . P values were determined by Mann Whitney U test. CTD: c onnective tissue disease; UIP: u sual interstitial pneumonia; IPF: i diopathic pulmonary fibrosis; sCD206 Soluble CD206 sCD 163: Soluble CD 163.

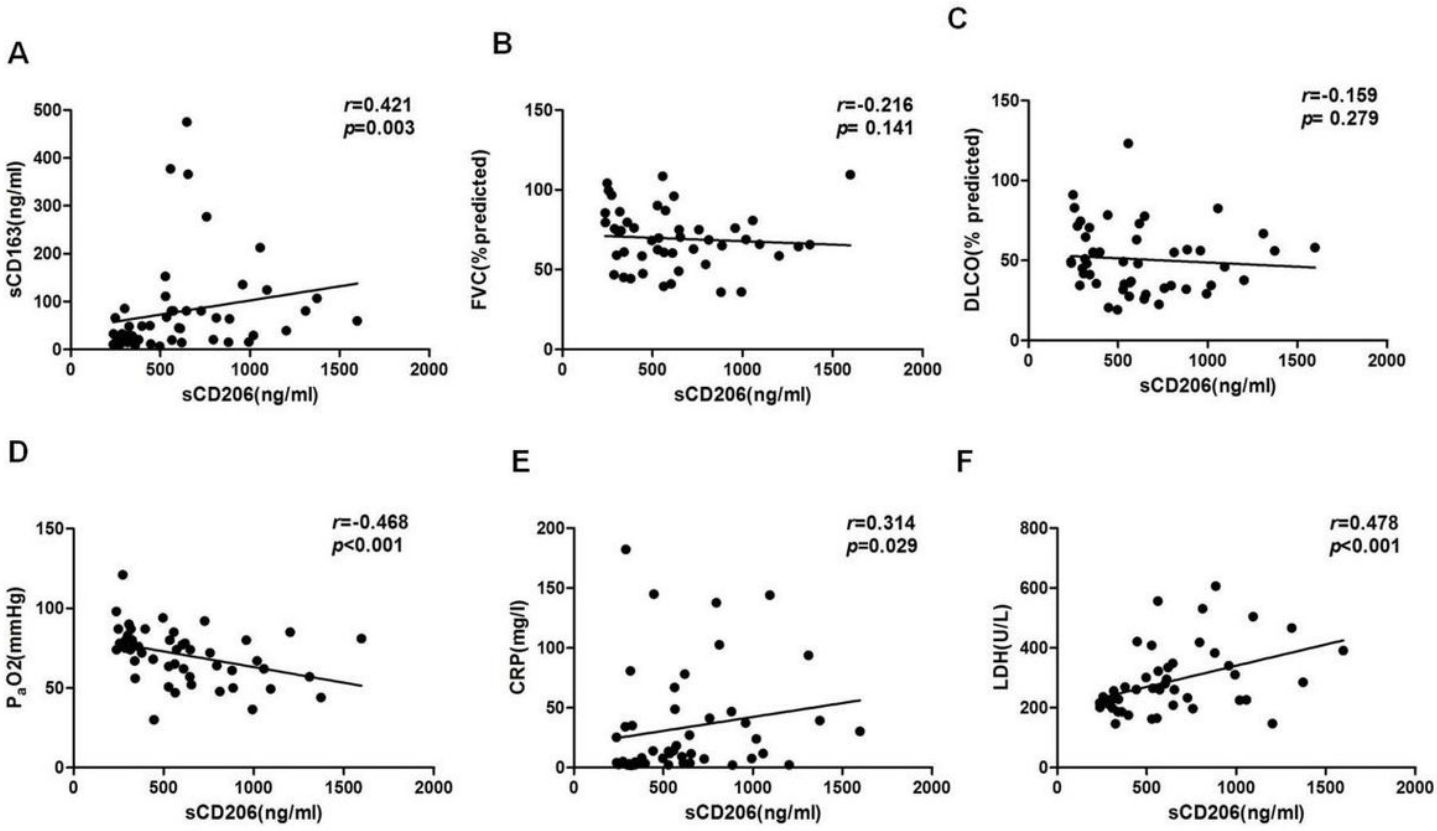


Figure 2

Correlations between sCD206 concentration and study variables. The statistical comparisons of sCD206 and variables were performed using Spearman's rank correlation coefficient. FVC, forced vital capacity. DLco, diffusing capacity of the lung for carbon monoxide; CRP, C reactive protein; LDH, lactate dehydrogenase;

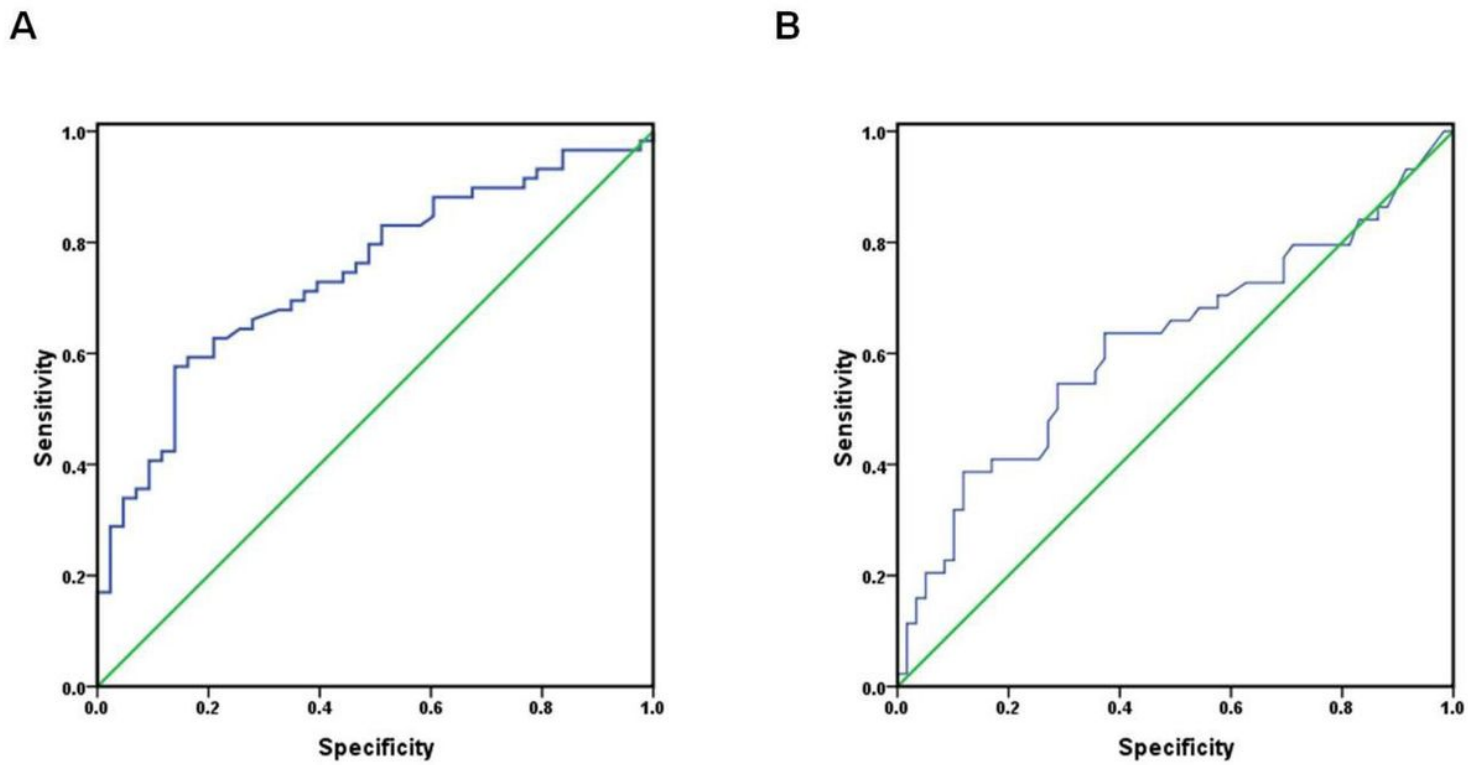


Figure 3

ROC curve analyses for all the patients according to the serum sCD206 levels. ROC curve analyses for all the patients according to the serum sCD206 levels. The AUC for serum sCD206 were statistically obvious in the differentiation of CTD associated UIP subjects ($p<0.001$, cut off valu e 523.8 ng/ml) ,which was better than CRP levels ($p=0.024$, cut off value 23.9 mg/l).

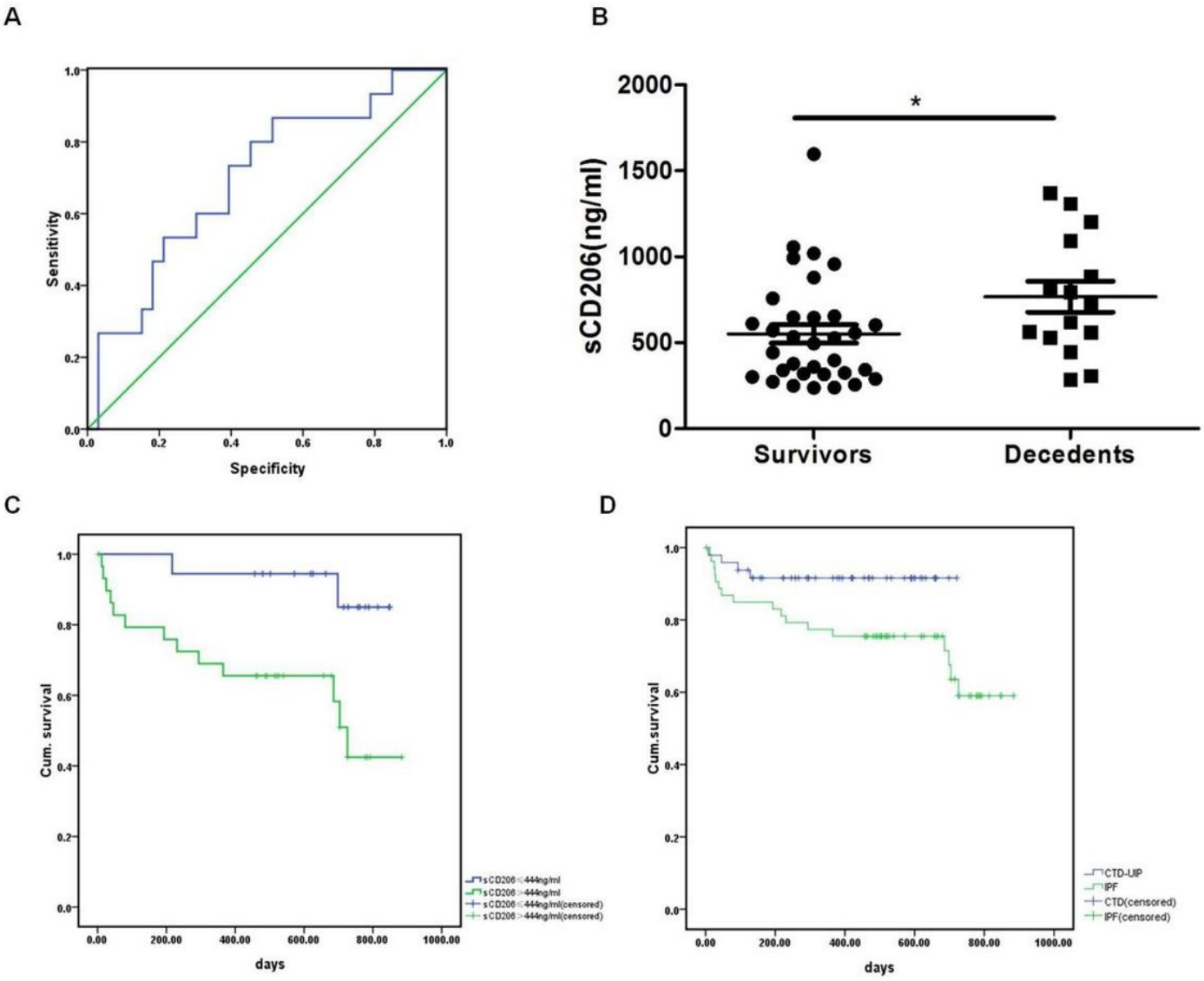


Figure 4

Prognostic value of sCD206 in patients with C T D associated UIP and survival analyses. ROC curve analyses for CTD associated UIP patients to predict the mortality of CTD associated UIP (A); The serum concentrations of sCD 206 were higher in decedents than survivors with CTD associated UIP (B); Kaplan Meier curves of patients with CTD associated UIP according to serum sCD206 (C Kaplan Meier curves show ed that mortality was low er in patients with CTD associated UIP than in those with IPF by l og rank test (D).