

Original article

Serum CC chemokine ligand-18 predicts lung disease worsening in systemic sclerosis

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Keywords: Systemic sclerosis, interstitial lung disease, chemokines, prognosis

Abstract

Elevated serum CCL18 reflects lung fibrosis activity in systemic sclerosis (SSc) and could be an early marker of lung function worsening. We have therefore sought to evaluate whether serum CCL18 levels at baseline could predict the worsening of lung disease in SSc.

In this prospective study, 83 SSc patients enrolled were analyzed longitudinally over 4 years observation period for the risk of occurrence of combined deleterious events, defined as 10% decrease from baseline of predicted values, of total lung capacity, or forced vital capacity, or death, according to serum CCL18 at inclusion. Receiver operating characteristic (ROC) curve analysis was performed for prediction of event during the first year after inclusion.

The best cut-off of CCL18 for prediction of combined event within the year of follow-up was 187 ng/mL with sensitivity: 53%, and specificity: 96% (area under ROC curve= 0.86; $p < 0.001$). After 33.7 ± 10.8 months of follow-up, a higher rate of disease progression occurred in the group with serum CCL18 levels >187 ng/mL. The adjusted hazard ratio (HR) was 5.36; (95% confidence intervals: 2.44-11.75, $p < 0.001$).

In summary, Serum CCL18 is an accurate predictive biomarker to identify patients with higher risk of subsequent scleroderma lung disease worsening.

word count=196

Introduction:

Systemic sclerosis (SSc) is a chronic autoimmune connective tissue disease characterized by sclerotic changes in skin and internal organs with poor prognosis. Interstitial lung disease (ILD) occurs in more than 50% of SSc patients and became the leading cause of death ¹. It remains a great challenge to develop biomarkers that could identify SSc patients who will have progressive disease with those having slow or stable disease in order to give appropriate treatment and follow-up cares.

CC Chemokine ligand 18 (CCL18) is a chemokine secreted mainly by alternatively activated alveolar macrophages (M2 phenotype) ² by T helper 2 cytokines and plays an important role in the immune-mediated lung fibrosis processes in idiopathic pulmonary fibrosis (IPF) ³ and scleroderma lung disease ^{3, 4}. A high amount of CCL18, produced by alveolar inflammatory cells ⁵, could induce an overproduction of collagen by lung fibroblasts through Sp1 signalling and basal Smad3 activity independently to autocrine transforming growth factor β (TGF- β) ^{6, 7}.

In ILD associated with SSc, although several data strongly suggested that CCL18 reflected an alveolar inflammatory activity, leading up to lung fibrosis and subsequent worsening of lung function. Serum CCL18 fulfilled criteria for a potential biomarker of lung fibrosis activity in ILD associated with SSc ^{3, 4}. Its prognostic value on overtime lung function worsening in patient with SSc has not yet been prospectively assessed in a long term follow-up study. We hypothesized that elevated serum CCL18 level, reflecting the underlying sustained lung inflammatory activity, could herald the subsequent lung function worsening in SSc. We aimed to determine whether the serum CCL18 concentration could provide truly valuable information to identify in SSc patients those at higher risk to have subsequent overtime lung function worsening in a prospective cohort study.

Methods:**Patients:*****Study design:***

Inclusion period of the cohort study was conducted from November 2004 to November 2007, in the department of Internal Medicine, Hospital Saint Antoine, Paris and the follow-up of this time-to-event driven study ended in June 2010. Patients eligible for the study were those who met the following criteria.

Inclusion criteria:

- Patients were considered for inclusion if they were older than 18 years and had a diagnosis of SSc and its subsets according to the American College of Rheumatology criteria⁸, and Leroy's criteria⁹, respectively. Patients with SSc were eligible, irrespective of the values of their forced vital capacity (FVC), diffusing lung capacity for carbon monoxide (DLCO). Patients with ILD as diagnosed by chest high-resolution computed tomography (HRCT) and patients treated by immunosuppressive therapy were also eligible.

Exclusion criteria:

Exclusion criteria were: the presence of recent airway upper tract infection, pneumonia, or other systemic infection in the three last months. Patients with IPF or ILD associated with connective tissue diseases other than SSc were excluded.

Data collection:*Baseline*

The study was approved by the local ethics committee (CPP Ile de France 5). After written informed consent, patients routinely underwent lung high-resolution computed tomography (HRCT), echocardiogram, and pulmonary function tests (PFTs) ¹⁰. Interstitial lung disease was considered present if pulmonary HRCT demonstrated compatible changes in reticular or air space opacities according to the ATS/ERS Consensus ¹¹. Serum samples were frozen at -80°C until the serum CCL18 measurement by ELISA method.

Follow-up

Survival was obtained from visits and telephone interviews. The changes in PFTs were determined by calculating percent differences of PFTs' measurement between baseline at the time of visit, when CCL18 was measured in the serum and every year or earlier when clinically indicated over a 4-year period. A decrease of more than 10% of predicted value in total lung capacity (TLC) or forced vital capacity (FVC) was considered as significant according to the ATS/ERS criteria ¹¹ and previous report ¹². Patients who had not been seen within 6 months were called to confirm their vitality. Serum CCL18 measurements were also performed at the first following visit.

Measurement of serum CCL18

CCL18 was quantified using a DuoSet ELISA Development kit (R&D Systems Europe, Abingdon, United Kingdom). All serum samples were diluted to 1:200 and measured in duplicate. Intra-assay coefficients of variation of less than 10% were accepted. To insure the reliability of measurement, we assayed CCL18 from 16 healthy volunteers as controls.

Statistical Analysis

First, we assessed the optimal threshold and the diagnostic performance of CCL18 for identifying SSc patients who were going to have subsequent lung disease worsening or to die within the 2 years after inclusion (positive if combined event occurred within 2 years of follow-up and negative if combined event did not occur) by using receiver operating characteristic curves (ROC) analysis.

Second, SSc patients were equally categorized into two groups, patients with serum CCL18 level higher than optimal threshold, and the other patients. Characteristics for group of SSc patients with serum CCL18 equal or lesser than optimal cut-off versus those with levels of serum CCL18 greater were compared by student t-test for continuous parameters and χ^2 tests for categorical measures. Continuous and categorical values were expressed as means \pm SD, and percentage respectively. A p value <0.05 was considered as statistically significant.

To estimate the predictive value of serum CCL18 on lung function outcomes during the whole follow-up, cumulative risks were computed by Kaplan-Meier analysis. We used Cox proportional hazards models, full backward and forward multivariate analysis to assess the risk to develop an overtime lung function worsening for patients whose CCL18 was higher than the best threshold of CCL18, and compared this risk with that of the remaining patients.

Next, we assessed the relationship between change in FVC and variation of serum CCL18 level between baseline and the last visit by linear regression model.

Results:

Characteristics of population at baseline

Eighty five patients with SSc were eligible in this prospective study. Two patients were excluded for pneumonia, and sigmoiditis (Figure 1). Eighty three patients were consecutively included in the study (Table 1). After a mean follow-up of 33.7 ± 10.8 months, 4 SSc patients died, and no patient was lost to follow-up. The composite events, defined as death or decrease of 10% of TLC or FVC, occurred in 43% of patients.

Threshold to identify SSc patients who were going to have subsequent lung disease worsening or to die during the 2 years after inclusion

The baseline serum levels ranged from 33 ng/mL to 649 ng/mL and its distribution in the whole studied population was shown in figure 2. The best cut-off for identifying subsequent lung disease worsening within 2 years was 187 ng/mL (95%CI: 159-218) with an area under ROC curve=0.86 (0.78-0.93); $p < 0.001$ and the sensitivity and specificity were 0.53 (0.35-0.70) and 0.96 (0.85-0.99) respectively (Figure 2). The internal validation of CCL18 cut-off was provided as supplemental data available online (Table S1).

Characteristics of population at baseline according to the best threshold of CCL18 to predict subsequent combined events during the first 2 years after inclusion

We used the best cut-off of CCL18 (at 187 ng/mL) to split the studied population. The composite events, defined as death or decrease of 10% of TLC or FVC, occurred in 91% of patients with CCL18 greater than 187 ng/mL and 27% of those with CCL18 equal or lesser than the best cut-off. There were significantly higher percentages of diffuse form of disease ($p=0.004$), presence of anti-topoisomerase I ($p=0.004$), ILD ($p=0.003$), systolic pulmonary artery pressure higher than 40 mmHg ($p=0.001$), lower level of FVC ($p=0.01$) or DLCO

($p < 0.001$) in the group of SSc patients with high level of CCL18 as compared to those with low level of CCL18. The immunosuppressive therapy including corticosteroids and immunosuppressive agents were comparable between the 2 groups. Further cross sectional analysis of baseline data was provided as supplemental data available online (Cross sectional analysis of the population at baseline, and figure S1).

Association between baseline serum CCL18 level and overtime lung function worsening

Progression of lung function decline differed between SSc patients with high and low serum CCL18 levels at baseline. Times to 25% events were significantly shorter in patients with high CCL18 levels (22 months, 95%CI: 14.0-23.5) as compared to those with low CCL18 levels (37 months, 95%CI: 28.7-37.4; logrank $p < 0.001$; figure 3).

In the univariate, Cox proportional hazards model, serum baseline CCL18 level greater than 187 ng/mL was associated with a higher risk of overtime lung function worsening (HR=5.32, 95%CI: 2.70-10.52; $p < 0.001$; table 2). Linear regression analysis using CCL18 as continuous variable to predict subsequent change in FVC was consistent with the Cox proportional hazards model and was available online as supplemental data (Table S2).

In multivariate analysis, when large demographic data, characteristics of disease, serum inflammatory marker, and immunosuppressive therapy were put into the backward and forward multivariate Cox proportional hazards models, serum CCL18 concentration at baseline remained an accurate predictive factor of the subsequent lung disease worsening (HR=5.36, 95%CI:2.44-11.75, $p < 0.001$; table 3). The baseline CCL18 level from 4 SSc patients who died during the follow-up was 139 ng/mL, 228 ng/mL, 230 ng/mL and 649 ng/mL.

Relationship between variation of serum CCL18 level and change in forced vital capacity

After adjustment for FVC at baseline, FVC at follow-up visit was related to CCL18 at baseline but not to its change (serum CCL18 at inclusion minus that at the follow-up visit, table 4). Serum levels from SSc patients with CCL18 higher than the optimal threshold remained higher than 187 ng/mL during follow-up (figure 4).

Discussion:

In this present prospective cohort study, we found for the first time that baseline serum CCL18 level above 187 mg/mL was a strong independent predictive factor of subsequent lung disease worsening in patients with SSc (HR=5.36). CCL18 provided additional information that helps us to identify patient with progressive lung disease in SSc.

In SSc, there are not many relevant biomarkers that could allow us to predict progression of lung fibrosis or survival¹³. Currently, several biological markers such as neutrophilic alveolitis surfactant A or D¹⁴, or cytokines like TNF α ¹⁵, and chemokines like IL8¹⁶, CCL2^{16, 17, 18} and alveolar concentration of nitric oxide^{19, 20} have been shown to be associated with the severity of ILD in SSc. Nevertheless, for each biomarker, long-term follow-up study available showing relevant prognostic value on its progression is lacking. So far FVC^{12, 21} and DLCO²² remained the most relevant predictive factors in ILD. In order to assess scleroderma lung disease which has become the leading cause of death, several physiological lung parameters such as FVC and DLCO have been used as surrogate markers for mortality, or impairment of lung function in clinical trial^{23, 24}. Decreased FVC reflected the severity of lung fibrosis and FVC less than 55% of predicted value predicted increased mortality in 9 years in diffuse form of SSc²⁵. Low DLCO was also a predictor for the occurrence of PAH²⁶ and mortality²⁷. However, patients with more severe impairment of FVC or DLCO were more likely to worsen lung function than others. Unlike physiological lung parameters, biological markers have the advantages to describe the underlying biological activity leading to lung fibrosis independently of the subjectivity of patients-related symptoms and fibrosis-related mechanical changes of lung parenchyma. Herein, the serum level of CCL18 seems to satisfy these requirements to be a good biomarker.

Previous study have shown that high CCL18 was associated with increased pro-fibrotic activity in scleroderma lung disease and high serum level could reflect progressive lung disease³. Consistent with previous findings, we found relationships between elevated CCL18 and severity of scleroderma lung disease, extent of skin fibrosis and systolic pulmonary pressure greater estimated by echocardiogram than 40 mmHg.

Recently, in IPF, Prasse et al.²⁸ reported that serum CCL18 had an accurate prognostic value on disease-related death with a hazards ratio of 8. Although previous studies have proposed potential link between CCL18 and SSc-ILD, we present here the first long-term follow-up data showing that serum level of CCL18 predicted an overtime lung function decline or death. In a large cohort studies, Sin et al²⁹ reported that CCL18 levels was significantly related to age, gender, body mass index, and smoking status. As SSc patients were predominantly female and their mean age was around fifty five years, elevated CCL18 could be biased by age and gender. So, we took account age, gender, body mass index, and smoking status in the multivariate analysis. Serum level of CCL18 was still an accurate predictive marker for an overtime lung function decline or death (HR at 5.36) after adjustment for all these latter factors including duration of disease, baseline FVC and DLCO.

The rate of progressive form in this present study seemed higher than that reported in previous large cohort studies^{21, 30}. As compared with previous studies, patients from the present study seemed to have more severe disease, as evidenced by the presence of severe organs involvement in most patients and 4 deaths during the first year after inclusion (4.8%). The fact that our Center is a national reference receiving the most severe cases of SSc might account for the high rate of combined outcomes in the present study.

We showed the robustness of CCL18 optimal threshold for prediction of lung disease worsening in SSc by an internal validation. However, external validation by an independent cohort is needed prior to recommending intervention studies targeting CCL18.

The variation of serum CCL18 obtained by repeated measurement did not provide more information on change in FVC during history of progression of lung disease. However, the fact that serum levels from patients with CCL18 higher than the optimal cut-off still remained higher than this latter during follow-up may reflect the continuous deterioration of lung function, even in those patients loss of lung volume due to lung fibrosis had already begun.

Our findings showed that serum CCL18 had a good predictive value to predict worsening of lung disease in patients with SSc and it might therefore be used as a surrogate biomarker to identify at risk SSc patients. Further studies are needed to show whether therapies may have an influence on CCL18 levels.

Statement of interest:

The authors have no conflicts of interest to disclose

Support statement

This study was supported by the Legs Poix (Chancellerie des Universités de Paris, France) and the Association des Sclérodermiques de France (28260 Sorel-Moussel, France).

Word count: abstract: 194, text: 2311;

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Table 1: Baseline characteristics of patients

	All SSc patients (n=83)	CCL18 > 187 ng/ml (n=21)	CCL18 ≤ 187 ng/ml (n=62)	<i>p</i>
Age (years)	53.5 ± 12.2	56.4 ± 12.5	52.6 ± 12.0	0.23
Female, n (%)	73 (88.0)	16 (76.6)	57 (91.9)	0.13
Duration of disease (years)	11.6 ± 11.1	11.1 ± 9.8	11.6 ± 11.5	0.84
Diffuse, n (%)	35 (42.2)	15 (71.4)	20 (32.3)	0.004
Modified Rodnan skin score	8.6 ± 6.8	12.5 ± 7.9	7.3 ± 5.9	0.01
Anti-centromere antibody	42 (50.6)	6 (28.6)	36 (58.1)	0.02
Anti-topoisomerase I antibody	37 (44.6)	15 (71.4)	22 (35.5)	0.004
History of smoking, n (%)	19 (22.9)	5 (23.8)	14 (22.5)	0.85
ILD, n (%)	46 (55.4)	18 (85.7)	28 (45.2)	0.003
Baseline FVC (% pred)	93 ± 20	84 ± 16	96 ± 21	0.01
Baseline FEV ₁ (% pred)	90 ± 17	82 ± 15	93 ± 19	0.02
Baseline DLCO (% pred)	62 ± 16	50 ± 10	65 ± 16	<0.001
Baseline Creatinine serum level (μmol/L)	82.4 ± 22.1	81.4 ± 23.5	82.8 ± 21.8	0.82
Baseline Fibrinogen (g/L)	3.2 ± 0.8	3.8 ± 1.2	3.1 ± 0.7	0.02
Baseline sPAP (mmHg)	32.9 ± 10.8	37.7 ± 7.4	31.2 ± 4.4	0.01
sPAP>40 mmHg	13 (15.6)	9 (42.9)	4 (6.4)	0.001
Immunosuppressive treatment, n (%)	15 (18.1)	6 (28.6)	9 (14.5)	0.26

ILD: interstitial lung disease, % of pred: percentage of predicted value, FVC: forced vital capacity, FEV₁: forced expiratory volume in one second, DLCO: diffusing capacity of carbon monoxide, sPAP: Systolic pulmonary artery pressure assessed by echocardiogram.

Table 2: Univariate analysis assessing the risk of overtime lung functions worsening

	Hazard Ratio (95% CI)	p*
CCL18 > 187 ng/ml	5.32 (2.70-10.52)	<0.001
Age (as continuous, per year)	1.02 (0.98-1.05)	0.19
Female	0.53 (0.24-1.20)	0.11
Diffuse form of SSc	2.09 (1.08-4.05)	0.03
Anti-centromere antibody	0.61 (0.31-1.18)	0.14
Anti-topoisomerase I antibody	1.62 (0.84-3.13)	0.15
History of smoking	0.81 (0.35-1.85)	0.62
Duration of disease (as continuous, per year)	0.99 (0.95-1.02)	0.46
Presence of ILD	2.40 (1.15-4.99)	0.02
DLCO (as continuous, per % of pred)	0.96 (0.94-0.98)	0.001
FVC (as continuous, per % of pred)	0.99 (0.97-1.00)	0.11
sPAP>40 mmHg	3.19 (1.55-6.56)	0.002
Fibrinogen (as continuous, per g/L)	1.41 (1.00-2.00)	0.05
Immunosuppressive treatment	2.18 (1.04-4.56)	0.04

SSc: Systemic sclerosis, CI: confidence interval, ILD: interstitial lung disease, % of pred: percentage of predicted value, FVC: forced vital capacity, DLCO: diffusing capacity of carbon monoxide, sPAP: Systolic pulmonary artery pressure assessed by echocardiogram, * p log rank test

Table 3: Risks of overtime lung functions worsening in patients with systemic sclerosis according to optimal cut-off

	Hazard ratio (95%CI)	<i>p</i>
CCL18 > 187 ng/mL	5.36 (2.44-11.75)	<0.001
Duration of disease (per year)	0.97 (0.93-1.01)	0.10
DLCO (per % of pred)	0.97 (0.95-1.00)	0.04

CI: Confidence interval, SSc: Systemic sclerosis, % of pred: percentage of predicted value, DLCO: diffusing capacity of carbon monoxide, sPAP: Systolic pulmonary artery pressure assessed by echocardiogram. Full backward analysis provided a final model that we also obtained using the forward multivariate analysis. In the initial multivariate analysis model, we input for adjustment age and duration of disease and all other parameters with a p value <0.20 in univariate analysis (*i. e.* gender, form of SSc, presence of interstitial lung disease, forced vital capacity, presence of systolic PAP ≥ 40 mmHg, fibrinogen, immunosuppressive therapy, and forced vital capacity).

Table 4: Forced vital capacity at follow-up visit according CCL18 at baseline and its variation between inclusion and the following visit

	$\beta \pm SE$	p
Intercept	9.417 \pm 7.057	0.19
FVC at baseline *	0.952 \pm 0.061	<0.0001
Serum CCL18 level at baseline**	-0.035 \pm 0.014	0.01
Variation of CCL18 levels [#] **	-0.016 \pm 0.012	0.16

FVC: forced vital capacity, * by additional unit 1% of predicted value, ** by additional ng/ml, [#] serum CCL18 at follow-up visit minus that at baseline

Figure legends

Figure 1: Population of the study

Figure 1: Flow of patients with systemic sclerosis in study

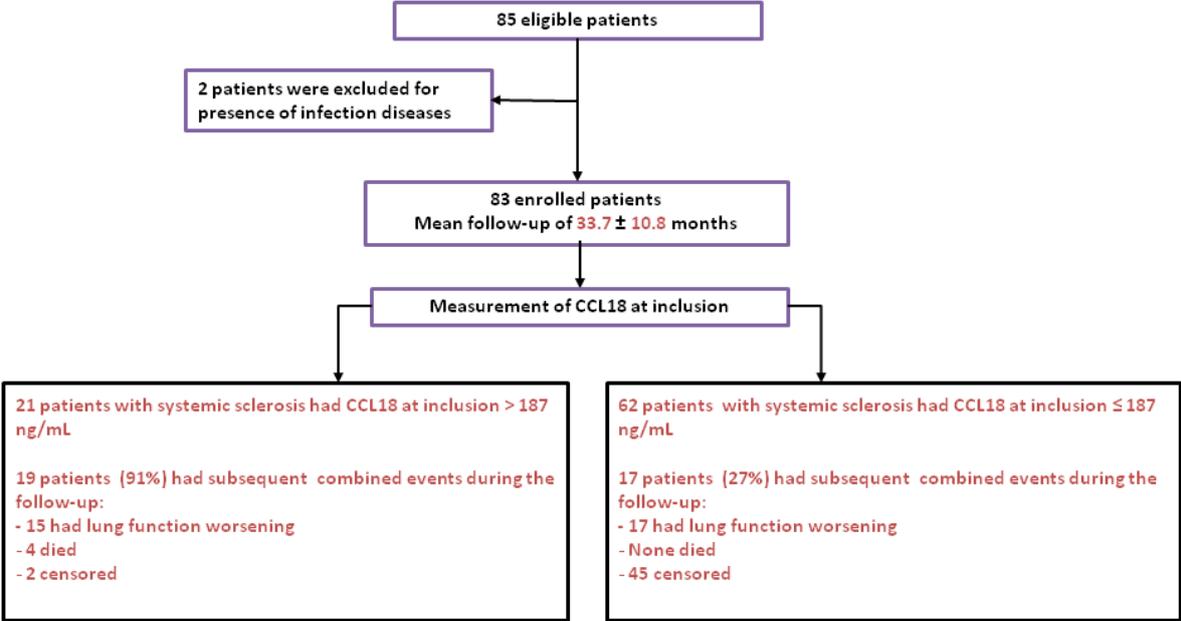


Figure 2: Distribution of baseline CCL18 serum level in patients with systemic sclerosis

SSc: systemic sclerosis; SSc patients with stable lung disease and those with subsequent lung disease worsening were represented by grey bars [█], and black bars [█], respectively. The best threshold of CCL18 (187 ng/mL) and its 95% confidence interval (159-218 ng/mL) were represented by the black line [—] and small dotted lines [.....], respectively.

Figure 2

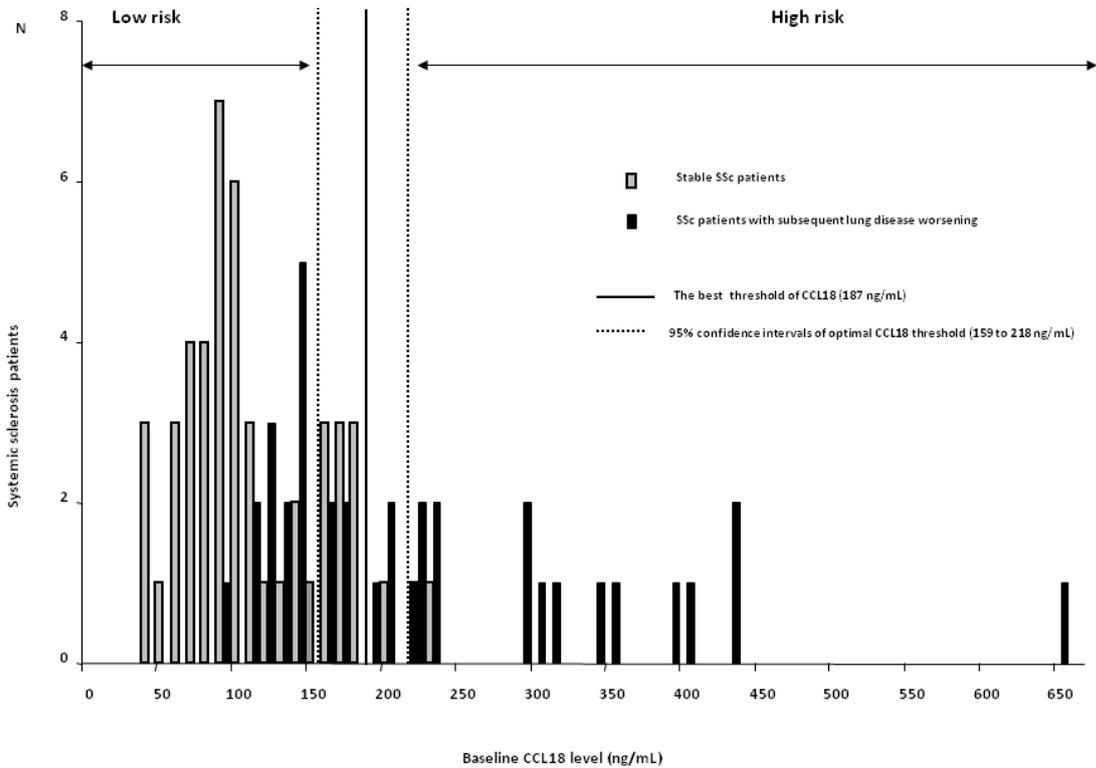


Figure 3: Kaplan Meier analysis grouped by baseline serum CCL18 level

The black line represents the group of SSc patients with baseline serum CCL18 level above the best threshold of CCL18 (187 ng/mL) and the dotted line the group of SSc patients with baseline serum CCL18 level equal or below the best threshold.

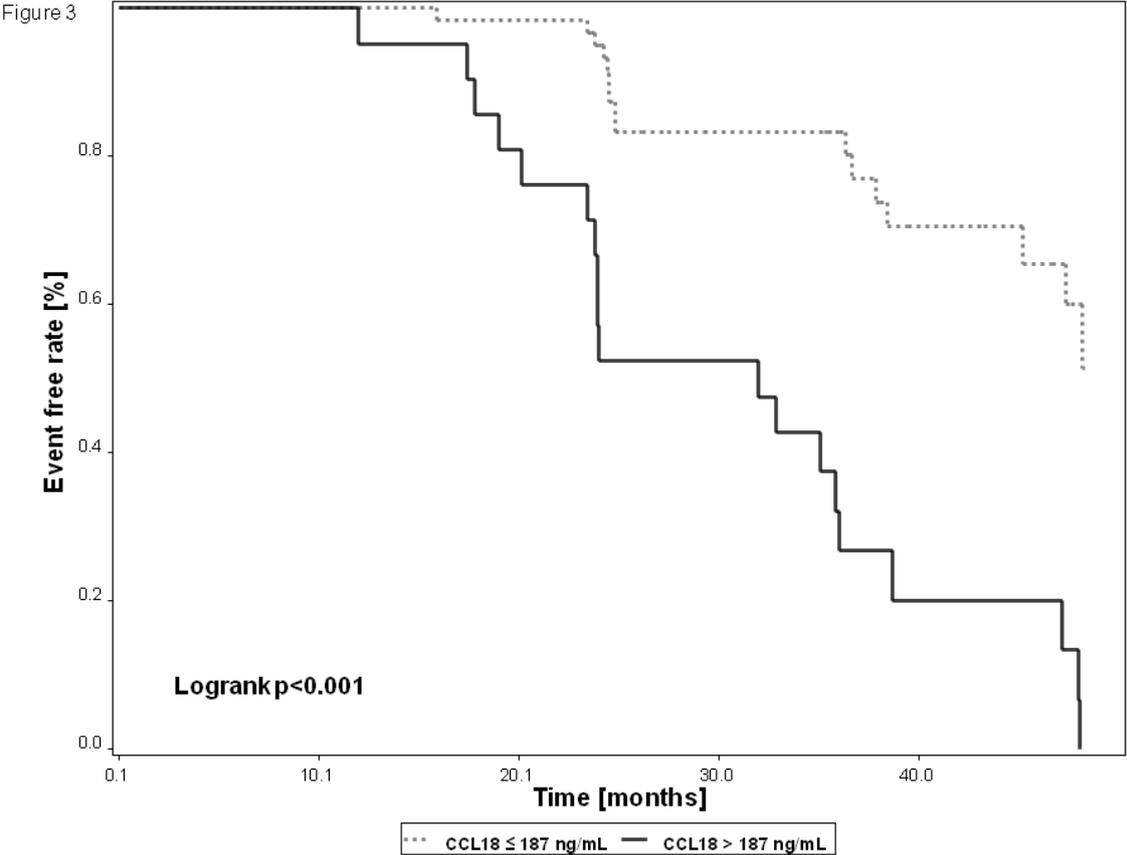


Figure 4: Change in CCL18 levels during the follow-up

Black line — represents SSc patients with baseline serum CCL18 level above the best threshold of CCL18 (187 ng/mL) and grey line — those with baseline serum CCL18 level equal or below the best threshold.

Figure 4

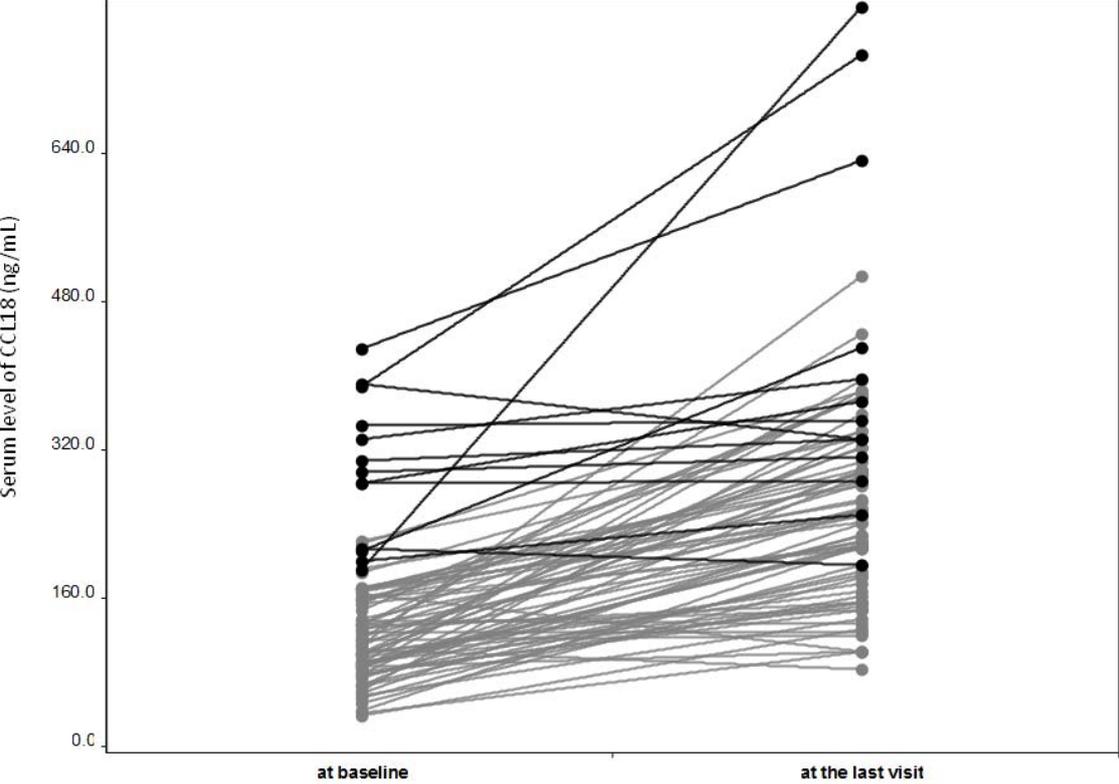


Figure S1 (supplemental data): serum CCL18 levels from systemic sclerosis patients with and without interstitial lung disease at baseline and healthy controls

Systemic sclerosis patients with interstitial lung disease at baseline (○), systemic sclerosis patients without interstitial lung disease at baseline (●) and healthy controls (△).

Figure S1

