Follow-up of serum KL-6 lung fibrosis biomarker levels in 173 patients with systemic sclerosis

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ABSTRACT

Objective. *A single-centre retrospective longitudinal study to investigate the predictive value of KL-6 serum levels for the outcome of interstitial lung fibrosis in a large systemic sclerosis (SSc) patient cohort.*

Methods. *ELISA tests for the mucin like glycoprotein KL-6 were performed in sera of 173 SSc patients. The clinical and laboratory data were evaluated by a standardised protocol of chest x-ray, lung function tests, echocardiography and high-resolution computed tomography. 158 patients were 29±22 months later reinvestigated, 9 patients (2 lcSSc, 7 dcSSc) died from SSc-related causes, and 6 patients were lost to follow-up.*

Results. *Serum titer of KL-6 was negatively correlated with lung function parameters, independent of the time of investigation. There was a significantly higher probability of death among patients with high level of baseline KL-6. There was no statistically significant difference in the deterioration and improvement rates between groups with normal and elevated KL-6 level at study entry, even in patients in early phase of disease (disease duration <3 years). Serum levels of KL-6 significantly decreased in patients receiving cyclophosphamide treatment in spite of the fact that the spirometry results (FVC and DLCO) did not show a significant change.*

Conclusion. *KL-6 can be used as a lung fibrosis severity marker, but its role as a marker for disease activity is questionable. Furthermore, following cyclophosphamide treatment serum KL-6 levels may decrease independently of the lung function parameters.*

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Introduction

Systemic sclerosis (SSc) is a connective tissue disease of unknown origin. Interstitial lung disease (ILD) is one of the most frequent disease manifestation of SSc. ILD plays an important role both in the prognosis and outcome of SSc, therefore an early detection and appropriate follow-up of lung fibrosis is of high priority (1, 2). There is an unmet need for prognostic biomarkers providing additional information to lung function tests and high resonance computed CT (HRCT) during the follow-up of pulmonary fibrosis (PF) (3-6).

Serum KL-6 ("Krebs von den Lungen-6": a mucin-like glycoprotein) is excreted from the lung alveolar and bronchial epithelial cells (3), and its serum level probably indicates the damage in alveolar type II cells (7, 8). KL-6 is a chemotactic factor for most fibroblasts and it has been shown to have pro-fibrotic and anti-apoptotic effects on lung fibroblasts. The increased level of KL-6 in epithelial lining fluid in small airways may cause intra-alveolar fibrosis in fibrosing lung diseases (9). It has been proposed by our group (10) and others (11-14) that KL-6 may be an appropriate marker for monitoring the severity and activity of interstitial lung diseases (4, 15, 16). KL-6 has also been suggested to be a good marker for disease activity in other systemic autoimmune disorders (11-13). It is not clear, however, whether KL-6 is a marker for disease activity (ongoing alveolitis) or rather reflects the tissue damage resulting in lung fibrosis (10, 14, 17, 18). Our previous cross sectional study (10) as well as others (19, 20) indicated that KL-6 levels reflect disease severity. Moreover, we have not enough data about the prognostic value of KL-6 for survival of patients with SSc or monitoring of effect of the treatment (17).

In our follow-up study presented here we assessed serum KL-6 levels in 173 patients with SSc. Our results indicated that KL-6 is a useful severity marker of pulmonary interstitial disease at the time of first investigation, but it is not appropriate for estimating the deterioration of lung disease during the follow-up.

Methods

Patients

In our retrospective longitudinal study, 173 consecutive patients with SSc from a tertiary care centre were included (Table I). Classification into diffuse (49 patients) or limited (124 patients) cutaneous systemic sclerosis (dcSSc or lcSSc) subgroups was performed at the entry (21). With regard to the overlap symptoms, 12 out of the 173 patients also fulfilled the criteria for Sjögren's syndrome (22), 12 for rheumatoid arthritis (23), 4 for secondary antiphospholipid syndrome (24), and 3 for systemic lupus erythematosus (25). Nine patients had polymyositis (26). Early stage of disease was defined if the disease duration was lower than 3 years (Table I).

The clinical and laboratory data of 158 patients was re-analysed 29±22 months later. Nine patients (2 lcSSc, 7 dcSSc) died, and 6 (3 lcSSc, 3 dcSSc) were lost to follow-up.

Demographic, clinical, and laboratory items were recorded by a standard protocol as previously described (2). Patients underwent echocardiography, spirometry and high-resolution computed tomography (HRCT) if clinically indicated.

Pulmonary arterial hypertension (PAH) was defined by right heart catheterisation (27). Laboratory tests included blood cell counts, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), haemoglobin, haematocrit, serum creatine kinase (CK), lactate dehydrogenase (LDH), total protein, albumin, complement (C3, C4), which were evaluated in all cases.

Informed consent was obtained from all patients. The project was approved by the local Research Ethics Committee (no. 2720/2006).

Treatment

Fifty-three patients were treated with cytostatic agents including cyclophosphamide (parenteral), oral azathioprine, leflunomide, methotrexate and cyclosporine. 46 cases out of the 56 patients also received a simultaneous methylprednisolone therapy (mean (SD) dosage: 7.1mg±1.9mg). The total number of patients with cyclophosphamide therapy was 37 (Table I). The decision of cyclophosphamide treatment was based on the individual judgment of the treating physician: in 34 patients with lung involvement (based on either HRCT or decreased FVC values) was cyclophosphamide started. In 3 early diffuse SSc patients with extensive skin involvement cyclophosphamide was also initiated.

Twelve patients received exclusively intravenous cyclophosphamide therapy without other cytostatic agent(s) during the follow-up with an average dose of 942 mg (±219 mg)/month, for 9.0 months (±4.6 months). In another 20 patients cyclophosphamide treatment (1020±196 mg, for 9.7±7.1months) was followed by azathioprine (106.3±33.3 mg/day) as maintenance therapy. Another 4 patients with scleroderma-rheumatoid arthritis overlap syndrome received leflunomide (20 mg/day) after the pulse cyclophosphamide therapy (1100±622 mg; 9.3±10.2 months). One patient with scleroderma-lupus overlap syndrome received cyclosporine-A therapy (100 mg/day) after the cyclophosphamide therapy.

Table I. Baseline clinical and laboratory characteristics in patients with SSc.

	All (n=173)	Patients with overlap (n=40)	Early (n=32)	Cyclophosphamide treated (n=37)
Age	57.6 (11.3)	54.3 (12.0)	54.3 (12.9)	51.1 (14.0)
Female/male	154/19	32/8	29/3	30/7
Disease duration in year	8.2 (6.9)	8.7 (6.6)	1.4 (0.6)	7.1 (6.7)
ANA	141	34	31	29
Scl70/ACA	69/30	17/5	14/15	22/1
dsDNS/CCP/Jo-1/SSA-B	26/11/2/14	9/5/1/4	3/3/0/3	4/4/1/5
DcSSc/LcSSc	49/124	10/30	7/25	19/18
mRSS (SD)	5.8 (5.5)	5.9 (5.7)	5.8 (6.8)	8.4 (6.9)
FVC% (SD)	95.5 (21.0)	90.1 (23.5)	99.4 (16.9)	80.3 (16.7)
DLCO% (SD)	66.3 (18.8)	56.2 (19.1)	64.2 (16.0)	55.8 (16.7)
KL6 (U/ml) at first investigation (SD)	1465 (1530)	2162 (2050)	1108 (1030)	2440 (2183)
No. of patients with cyclophosphamide therapy (%)	37 (21.3)	11 (27.5)	8 (25.0)	37 (100)

Investigation of the lung involvement

Lung involvement was investigated as previously described (28). Briefly, cases with signs of lung fibrosis on chest x-ray, and/or restrictive ventilatory impairment, and/or decreased diffusing capacity were investigated. Forced vital capacity (FVC), total lung capacity (TLC), diffusing capacity for carbon monoxide (DLCO), and carbon monoxide diffusing capacity adjusted for alveolar volume (DLCO/VA) were investigated. Cases with either signs of lung fibrosis on chest x-ray or with decreased lung function parameters underwent a HRCT. Pulmonary fibrosis (PF) was defined as pulmonary fibrosis on chest x-ray or on HRCT with decreased DLCO% or FVC% (<80%). Deterioration, improvement or constant stage of pulmonary fibrosis was defined based on the changes in forced vital capacity. Improvement was defined as an increase >10%, and disease deterioration as a decrease <10% in the FVC values (29).

Measurements

Chest radiographs and respiratory functions tests were performed within 3 months from blood sample collection. Evaluation of KL-6 was performed using an ELISA kit (Sanko-Junyako, Tokyo, Japan), according to the manufacturer's protocol. Normal value is lower than 500 U/ml.

Data analysis

Normality of the data was assessed by using the Kolmogorov-Smirnov test. As the investigated parameters showed non-Gaussian distribution, non-parametric tests were used for comparison. Data are presented as mean ± SEM, median (upper and lower quartile values) or percentages, as appropriate. The Mann-Whitney U-test was used for comparisons between two groups of patients. Data of the same group of patients at the study entry and at the time of second investigation were compared employing the Wilcoxon signed

rank test. Correlations were analysed using Spearman's rank correlation. Dichotomous data were compared by using Fisher's exact test. Survival data were compared with Kaplan-Meier test. GraphPad Prism 6 (GraphPad Software, Inc. La Jolla, CA USA) was used for all statistical analyses.

Results

KL-6 and activity markers at baseline and the end of the follow-up investigation

In accordance with our previous findings (10) no correlation was found between KL-6 and ESR, CRP or complement values (data not shown). A weak positive correlation was observed between serum levels of KL-6 and modified Rodnan skin score (mRSS), LDH, FVC and DLCO (Table II). We found similar results during the second investigation, except that there was no correlation between serum KL-6 levels and mRSS (Table II).

Table II. Baseline serum KL-6 levels showed significant correlation with lung function parameters both at baseline and reinvestigation.

Correlations between KL-6 and		mRSS	LDH	FVC%	DLCO%
All patients	Baseline (n=173)	r=0.231 p=0.005	r=0.365 p<0.001	r=-0.249 p=0.002	r=-0.395 p<0.001
	Reinvestigation (n=158)	r=0.107 p=0.247	r=0.225 p=0.006	r=-0.319 p<0.001	r=-0.400 p<0.001
Patients without overlap	Baseline (n=133)	r=0.197 p=0.038	r=0.312 p<0.001	r=-0.251 p=0.004	r=-0.331 p<0.001
	Reinvestigation (n=122)	r=0.092 p=0.385	r=0.261 p=0.005	r=-0.228 p=0.011	r=-0.330 p< .001
Patients with overlap	Baseline (n=40)	r=0.341 p=0.045	r=0.297 p=0.084	r=-0.465 p=0.003	r=-0.576 p<0.001
	Reinvestigation (n=36)	r=0.322 p=0.082	r=0.317 p=0.056	r=-0.384 p=0.021	r=-0.336 p=0.045

After first investigation, clinical and laboratory data of 158 patients were re-analysed 29±22 months later.

Table III. Baseline KL-6 levels could not predict any change in FVC during the follow-up.

		Steady-state FVC%	Deterioration in FVC%	Improvement in FVC%
All patients (n=158)	Normal level of KL-6 at baseline	22	3	6
	Elevated level of KL-6 at baseline	87	22	18
Early patients (n= 32)	Normal level of KL-6 at baseline	6	2	0
	Elevated level of KL-6 at baseline	17	4	3

Change in pulmonary fibrosis during the follow-up assessed by changes in forced vital capacity

If we defined improvement as >10% increase in FVC at follow-up, deterioration as >10% decrease in FVC at follow-up and steady-state as change in FVC less than 10% in any direction, we have found similar number of patients with improvement (n=24) and deterioration (n=25) in their FVC. There were 109 patients with no significant change in FVC. Furthermore, there was no significant difference in the median KL-6 levels at study entry between patients with improving, worsening and steady-state FVC (795 (530–2255) U/ml, 1151 (683-1749) U/ml, 813 (529–1425) U/ml, respectively). KL-6 levels were similarly not significantly different if we compared these data in patients with worsening FVC and KL-6 levels in patients without deterioration (*i.e.* patients with steady-state and improving FVC) (data not shown).

Predictive value of baseline KL-6 levels

There was no significant difference in deterioration and improvement rate between groups with normal and elevated KL-6 level at study entry (Table III). The same results were found in early SSc patients (Table III). The baseline KL-6 levels did not differentiate between patients with and without progression by receiver operating characteristic (ROC) analysis (area= 0.559, p=0.344).

KL-6 levels and pulmonary hypertension

We found 13 patients with elevated mean pulmonary arterial pressure based on right heart catheterisation, however only 5 patients could be diagnosed with isolated PAH (*i.e.* with normal FVC % and chest x-ray). There was no significant difference in serum levels of KL-6 between patients with established pulmonary arterial hypertension and patients with no pulmonary hypertension (781 (356–1243) U/ml, and 818 (613–1696) U/ml, respectively, p=0.249). Patients with isolated PAH showed significantly lower serum levels of KL-6 than patients with simultaneous PAH and PF (333 (285–787) U/ml, 1060 (546–1426) U/ml, respectively, p=0.045).

KL-6 and mortality

KL-6 levels of the fatal cases were significantly higher compared to patients who survived (2341 (1793-4314) U/ml; 818 (589-1528) U/ml, respectively, p<0.001) (Fig. 1). ROC analysis showed the best sensitivity/specificity ratio (77%/76%, area =0.789, p=0.003) with a baseline KL-6 value of 1592 U/ml. Furthermore, patients with very high serum level of KL-6 (*i.e.* higher than three times the normal value (>1500 U/ml)) showed significantly higher probability of death than without it (RR=1.144, p=0.0026) (Fig. 2).

Predictive value of changes of KL-6 level

Significant decrease in KL-6 values (decrease of more than 10%) was seen equally frequent in the regressive and steady-state PF groups (14/24 patients, 53/109 patients, respectively, p=0.499). Significant increase in KL-6 values (increase of more than 10%) had also similar prevalence in the progressive and steady-state pulmonary fibrosis groups (9/25 patients, 42/109 patients, respectively, p=1.000), thus the change in KL-6 levels could not predict disease deterioration or improvement. Larger changes in KL-6 values (25%–90%) could also not predict the change in pulmonary fibrosis (data not shown). The FVC% progression rate (4/22 *vs.* 21/136) did not indicate any difference when cases (n=22) with an increasing

KL-6 level (>500 U/m (30)) were compared to the rest of the patients (n=136) (p= 0.754).

Treatment

Fifty-three patients (30.6%) were treated with immunomodulatory drugs (see *Methods*). Data of forty-nine patients who participated at the re-investigation were available for reassessment of KL-6 levels and stage of pulmonary fibrosis. One patient was lost for follow-up, and 3 patients died from SSc-related causes. Out of the 120 patients, who were not treated with immunosuppressive drugs during the study, 109 participated in the second investigation (5 patients were lost for follow-up, and 6 patients died). All six patients who died in this latter group had moderate to severe pulmonary fibrosis.

Cyclophosphamide therapy

Thirty-seven patients were treated with cyclophosphamide during the follow-up (see *Methods*). Baseline FVC % of treated patients with pulmonary fibrosis (n=34) was significantly lower than in non-treated group (81% (70-90%) and 102% (92–116%), respectively, p<0.001). Serum levels of KL-6 were significantly higher in the cyclophosphamide treated group at baseline compared to patients with no current immunomodulatory treatment (1970 (856–4444) U/ml, and 747 (506–1192) U/ml, respectively, p<0.001).

Thirty-one patients with available follow-up data were treated with cyclophosphamide due to their pulmonary disease.

There was no change in FVC during the follow-up either in cyclophosphamide treated ((baseline: 81% (70–90%); at re-investigation: 79% (66–93%), p=0.996)) or in non-treated group (baseline: 102% (92–116%); at re-investigation: 102% (90–113%), p=0.381) (Fig. 3).

Eight patients show increasing FVC (improvement) and 5 patients decreasing FVC (deterioration). The other 18 patients remained in the same range of FVC. The number of patient with improvement and deterioration was not significantly different between cyclophosphamide treated and not treated patients (data not shown).

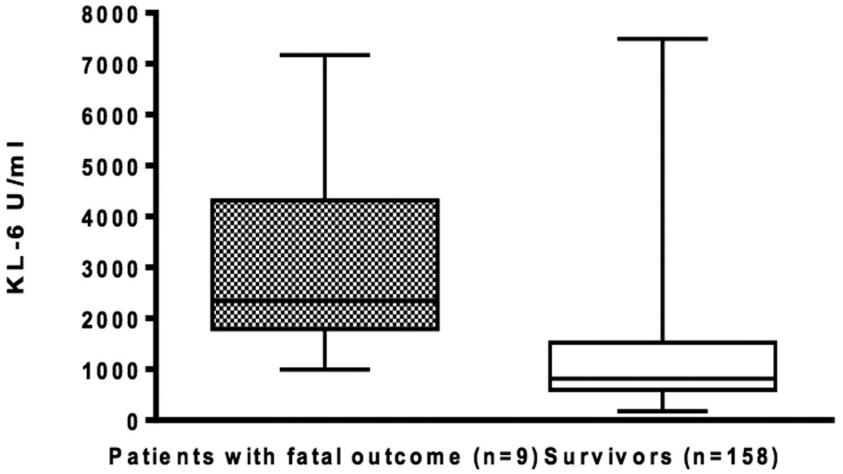

Fig. 1. KL-6 serum levels in patient with fatal outcome were significantly higher than in survivors (p<0.001).

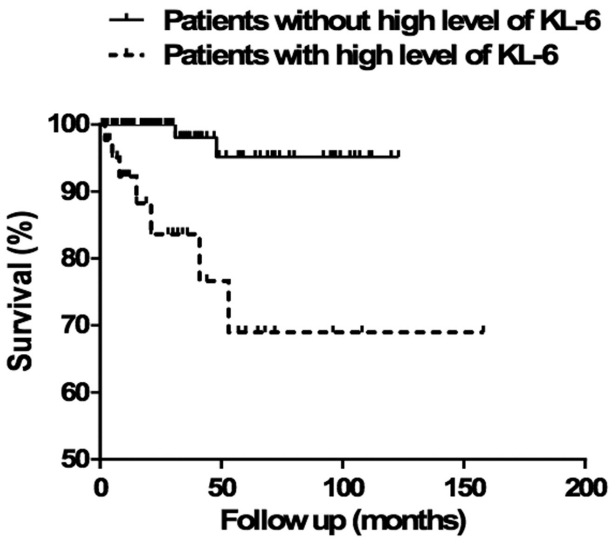

Fig. 2. Patients with high level of KL-6 show significantly higher probability for fatal outcome than patients with low level of KL-6. Kaplan-Meier analysis to compare survival rates between patients with very high level of KL-6 (higher than 3 times of normal, n=50) and rest of the patients (KL-6 levels lower or equal than 3 times of normal, n=117). Number of patients with fatal outcome is 7/50 (high KL-6) and 2/117 (not high KL-6), respectively, p<0.001.

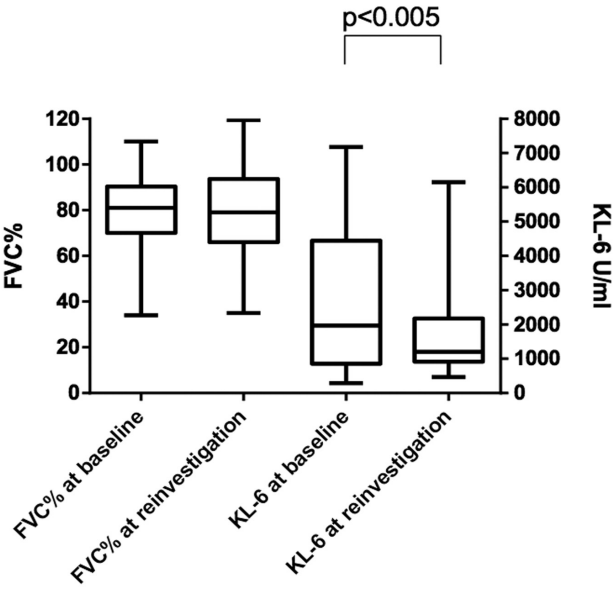

Fig. 3. Serum levels of KL-6 significantly decreased following a cyclophosphamide treatment in spite of the fact that the spirometry results (FVC and DLCO) did not show significant change (p=0.976). Cyclophosphamide treated patients with pulmonary fibrosis (n=31).

Serum levels of KL-6 significantly decreased in patients with cyclophosphamide treatment compared to serum levels before therapy (before: 1970 (857–4444), after: 1205 (918–2176), n=31, $p<0.005$) (Fig. 3). Group of patients with deterioration (n=5) and improvement (n=8) did not show any significant change in serum levels of KL-6 after therapy (patients with deterioration 2197 (801-7123) U/ml and 1028 (918-5826) U/ml, $p=0.188$; patients with improvement: 2007 (796–2530) U/ml and 989 (471–2658) U/ml, $p=0.148$). Patients with steady-state disease (n=18) show significant decrease in serum levels of KL-6 after therapy compared to the values at study entry (1818 (523–4882) U/ml, 1315 (469-3103) U/ml, respectively, $p<0.05$).

Patients with overlap syndrome

Patients, who have not only SSc but also fulfill the criteria of another systemic autoimmune disease, demonstrated a significantly higher serum level of KL-6 than SSc patients without overlap (1226 (641-3347) U/ml and 813 (589-1425) U/ml, respectively, $p<0,05$) (Table I). These particular overlap cases showed a female predominance, but the other demographic parameters did not differ from patients without overlap phenomena (Table I).

The baseline FVC% and DLCO% of overlap patients were significantly lower than in patients without overlap (FVC: 85 (77–105)%, 100 (82–110)%, respectively, $p<0.05$; DLCO: 56 (44–69)%, 72 (60–82)%, respectively, $p<0.001$). In the overlap group, the correlation coefficients between baseline KL-6 and the baseline/follow-up values of lung function parameters were slightly higher than in the patients group without overlap (Table II). However, either the survival or progression rate did not differ between these two subgroups of patients. We lost 5 patients in the SSc-alone group (n=133) and 4 patients with overlap syndrome (n=40); the progression rate was 21/122 in the SSc-alone group and 4/36 in the overlap group.

Discussion

In our study we demonstrated that KL-6 is a useful negative prognostic marker in SSc-ILD: patients with high titer of KL-6 have higher probability for fatal outcome than patients with low titer (Fig. 1-2). However, there was no statistically significant difference in deterioration and improvement rate between groups with normal and elevated KL-6 level at study entry. Satoh *et al*. reported very similar findings in their work investigating a large number of patients with ILD; however, most of their patients did not have SSc (31). The ROC analysis showed a KL-6 level of 1000 U/ml as the most suitable cut-off value by which to distinguish between patients with fatal outcome and survivors. Our results indicated a slightly higher cut-off value of 1592 U/ml. This difference may be explained by the difference in the proportion of SSc-ILD cases among the studies. We suggest a cut-off of a three times increased KL-6 level (>1500U/ml) for the differentiation between SSc patients with high and low risk for survival (Fig. 2).

In our current study we investigated the deterioration of ILD by assessing FVC values only, as a widely used method in SSc-ILD (29). Change of DLCO is more sensitive (but less specific) than alteration of FVC in SSc-ILD (32). Pulmonary arterial hypertension (PAH) is associated with disproportionately low DLCO, compared to FVC. In our large cohort we also investigated the role of KL-6 in SSc-PAH. Patients with isolated PAH showed significantly lower levels of KL-6 compared to patients with ILD and all patients with isolated PAH showed KL-6 levels in the normal range (<500 UI/ml, data not shown). Serum level of KL-6 was independent from PAH in our study and may be a useful tool in the differentiation between isolated PAH, combined SSc-ILD and SSc-PAH. This is supported by our previous paper (10), where we demonstrated a parallel increase between the extent of lung involvement and KL-6 levels. These results are very similar to the findings of Bonella *et al*., who also demonstrated a significant correlation between KL-6 and HRCT score (16).

We investigated the role of cytostatic therapy on KL-6 values and showed that cytostatic treatment decreases serum levels of KL-6 (Fig. 3). It has previously been suggested that KL-6 may reflect a response to treatment in SSc-ILD (9). In another two previous studies similar results were found (30, 33), however, in the first paper only one, and in the second article only two patients received cyclophosphamide therapy, therefore our article is the first which demonstrated in a large SSc cohort that specifically the cyclophosphamide treatment exerted a KL-6 lowering effect. It is accepted that cyclophosphamide therapy can favourably influence the deterioration of SSc-ILD (29, 34) and there is some evidence for other forms of immunosuppressive therapy (*i.e*. azathioprine, cyclosporine, mycofenolate mofetil, etc.) may also have some beneficial effect on interstitial lung disease in SSc (35-39). The decrease of KL-6 levels after cyclophosphamide therapy was significant only in the subgroup of patients whose FVC did not show any change during our follow-up. The explanation may be that cytostatic therapy influences the serum level of KL-6 only but not the disease deterioration. It is already known that an injury of alveolar type II cells (which secrete KL-6 too) may lead to PF (40). The role of these particular cells in lung fibrosis supported by findings of Serrano-Mollar et al demonstrating that an intratracheal transplantation with alveolar type II cells from healthy animals reversed the bleomycin-induced lung fibrosis in rats (8, 41). Cytostatic therapy may lower the secretion of KL-6 without influencing other functions of these particular cells, or the fibrotic process may be independent from the primary injury. For better understanding this particular phenomenon, KL-6 levels should be investigated following after other therapies including rituximab treatment (8, 42). Previous studies provided some evidence for the role of KL-6 as an activity marker in relation to therapy. Tsukamoto *et al*. has found that KL-6 levels after stem cell transplantation improved significantly, but, only five patients with SSc were enrolled in this particular study (5). Kodera *et al*. showed that improvement in PF activity was associated with significantly decreased KL-6 levels in 21 patients

with early SSc (disease duration <5 years) (33). In this study 2 patients were treated with cyclophosphamide and both of them showed decrease in KL-6 levels after therapy. One of them showed an increase of KL-6 level seven months after the first visit in parallel with a subacute deterioration of the PF activity. Based on these results, the authors suggested, that an increasing level of KL-6 in a patient with early SSc may indicate an active ILD (33). Doishita *et al*. investigated a large number of patients with connective tissue disease (n=240), however only 22 patients had SSc (10). The authors divided these 240 patients into 3 groups: patients without ILD, patients with inactive and active ILD. Patients with active ILD had a higher KL-6 titer than patients with inactive ILD, and this latter group had elevated KL-6 values compared with patients without ILD. These findings suggest that an elevated level of KL-6 is a marker of the presence of active ILD associated with CTDs (10). Bonella *et al*. found that KL-6 correlated well with Valentini disease activity index (16). Some components of this activity score were also investigated in our recent paper, and similar findings were found. The mRSS and the DLCO% correlated with KL-6 serum level. However, we do not have data about the whole activity index. In our study, KL-6 levels did not show any correlation with hypocomplementemia and ESR, indicating that the evaluation of the disease activity in SSc is difficult. The role of KL-6 as an activity marker for ILD emerged also in other acute pulmonary disorders, such as sarcoidosis or *Pneumocystis carinii* pneumonitis (43, 44). We investigated the predictive value of changes of KL-6 on the deterioration of interstitial lung fibrosis in a large patient cohort (n=158), and found that KL-6 levels at study entry did not exhibit any predictive value for the clinical course of SSc-ILD (Table III).

We are aware of some limitations of our study. Disease duration of patients in our cohort was long (6 years) and probably the nature of ILD differs in early and late SSc. Patients with cyclophosphamide treatment also had a long disease duration (median = 5 years, 3–9 years), their spirometric values were not yet decreased (Fig. 3), suggesting that our cohort had relative mild disease. Thus, re-assessment of these data would be needed in an international multicentre study, in which early diffuse SSc patients with rapid clinical progression were tested: Yanaba *et al*. demonstrated in a longitudinal study that KL-6 levels in 4 early scleroderma patients with Scl70 antibody positivity increased rapidly (more than 500 U/ml in the first 6 months), parallel to the progression of PF (30). In our cohort 6 patients with early disease showed deterioration of FVC% during the follow-up, but only 1 of our 6 patients displayed similar parallel high progression in KL-6. Further investigations with a large cohort of SSc-ILD cases are required. Yanaba *et al*. (30) also demonstrated that patients with almost normal KL-6 levels (n=31) during the follow-up did not exhibit a worsening of lung fibrosis and new onset of PF. Our result are very similar, only 3 out of 22 patients with normal level of KL-6 showed a progression in FVC% indicating that normal KL-6 level is a relatively good marker for good prognosis. It could be further strengthened with measuring of other promising markers of activity, such as CXCL11. CXCL11 concentrations in bronchoalveolar lavage fluid were significantly elevated in the samples taken from SSc patients who did not developed ILD as compared to those who developed ILD (6).

In summary, the KL-6 titers alone cannot be used to monitor the effect of cyclophosphamide treatment or to assess improvement or deterioration of SSc-ILD in late stage disease. However, the baseline investigation of SSc patients is useful to indicate an interstitial pulmonary disease with worse prognosis. Patients with very high titer of KL-6 showed high probability of fatal outcome. Furthermore, KL-6 levels may also be examined in patients with SSc, as patients with KL-6 titers within the normal range at study entry rarely had progressive lung disease.

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