Use of Serum Clara Cell 16-kDa (CC16) Levels as a Potential Indicator of Active Pulmonary Fibrosis in Systemic Sclerosis

MINORU HASEGAWA, MANABU FUJIMOTO, YASUHITO HAMAGUCHI, TAKASHI MATSUSHITA, KATSUMI INOUE, SHINICHI SATO, and KAZUHIKO TAKEHARA

ABSTRACT. ***Objective.*** To clarify the clinical significance of concentrations of serum Clara cell 16-kDa protein (CC16; previously denoted CC10) in the diagnosis and monitoring of pulmonary fibrosis (PF) in patients with systemic sclerosis (SSc); and to compare CC16 levels with levels of the current most reliable serum markers for PF, such as Krebs von den Lungen-6 (KL-6) antigen and surfactant protein-D (SP-D).

Methods. Serum levels of CC16, KL-6, and SP-D were determined by ELISA in 92 patients with SSc, 20 patients with systemic lupus erythematosus (SLE), and 20 healthy controls. In a retrospective longitudinal study, correlation of serum CC16 levels with the activity of PF was assessed in 16 SSc patients with PF.

Results. Although CC16 levels were higher in patients with SSc than in SLE patients or healthy controls, the difference was not significant. Increased serum CC16 levels were associated with involvement of PF, especially active PF, as well as KL-6 and SP-D. Receiver operating characteristic curve analysis revealed that the utility of CC16 is slightly inferior to KL-6, but was comparable with that of SP-D for detecting PF in patients with SSc. In the longitudinal study, serum levels of CC16, KL-6, and SP-D were significantly decreased in the inactive disease phase compared to the active disease phase.

Conclusion. CC16 levels can be used as a potential serum biomarker for PF in addition to KL-6 and SP-D in patients with SSc. (First Release Jan 15 2011; J Rheumatol 2011;38:877–84; doi:10.3899/jrheum.100591)

Key Indexing Terms:
SERUM CLARA CELL 16-kDa (CC16) PULMONARY FIBROSIS
SYSTEMIC SCLEROSIS BIOMARKER

Systemic sclerosis (SSc) is a connective tissue disease characterized by tissue fibrosis in the skin and internal organs. Pulmonary fibrosis (PF) develops in more than half of patients with SSc and is one of the major SSc-related causes of death[1,2]. To assess fibrotic activity, high-resolution computed tomography and pulmonary function tests are performed. However, lung-specific serological markers may provide an easier and less invasive approach for closely monitoring the activity of PF in patients with SSc.

Krebs von den Lungen-6 (KL-6) and surfactant protein-D (SP-D) are currently the most reliable serum markers for PF. KL-6 antigen is expressed mainly by alveolar type II pneumocytes and respiratory bronchiolar epithelial cells including Clara cells[3], whereas SP-D is produced and secreted by alveolar type II pneumocytes and Clara cells[4]. Recent studies revealed that levels of KL-6 and SP-D are elevated in serum from patients with PF, including SSc-related PF[3,5,6]. These studies suggested that serum levels of KL-6 and SP-D are serologic markers of the severity and activity of PF in SSc[7,8,9]. However, some SSc patients with active PF showed discrepancies in the serum levels of these markers. Serum KL-6 levels can be increased in patients with adenocarcinoma of the lung, breast, or pancreas[10]. Elevated serum levels of SP-D can be observed in patients with bacterial pneumonia and pulmonary tuberculosis[11,12,13]. Further, KL-6 or SP-D does not necessarily reflect the activity of PF in some patients. Therefore, an

From the Department of Dermatology, Kanazawa University Graduate School of Medical Science, Kanazawa; Division of Health Sciences, Kanazawa University Graduate School of Medical Science; and Department of Dermatology, Faculty of Medicine, University of Tokyo, Tokyo, Japan.

Supported by funds for research on intractable diseases from the Ministry of Health, Labor and Welfare of Japan.

M. Hasegawa, MD, PhD, Assistant Professor; M. Fujimoto, MD, PhD, Associate Professor; Y. Hamaguchi, MD, PhD, Assistant Professor; T. Matsushita, MD, PhD, Instructor, Department of Dermatology, Kanazawa University Graduate School of Medical Science; K. Inoue, MSc, Associate Professor, Division of Health Sciences, Kanazawa University Graduate School of Medical Science; S. Sato, MD, PhD, Professor, Chair, Department of Dermatology, Faculty of Medicine, University of Tokyo; K. Takehara, MD, PhD, Professor, Chair, Department of Dermatology, Kanazawa University Graduate School of Medical Science.

Address correspondence to Dr. M. Hasegawa, Department of Dermatology, Kanazawa University Graduate School of Medical Science, 13-1 Takaramachi, Kanazawa, Ishikawa 920-8641, Japan.
E-mail: minoruha@derma.m.kanazawa-u.ac.jp

Accepted for publication December 1, 2010.

additional serum marker may be helpful for reliable monitoring of PF.

Clara cell 16-kDa protein (CC16) is a 15.8-kDa homodimeric protein secreted throughout the tracheobronchial tree, especially in the terminal bronchioles where Clara cells are localized. This protein was previously referred to as the 10-kDa CC10 because of an underestimation of its molecular weight[4]. Although the exact function of CC16 remains to be clarified, studies suggest that CC16 plays a protective role in the respiratory tract during oxidative stress and inflammatory responses[14]. CC16 has been shown to modulate the production and activity of various mediators of the inflammatory response, including phospholipase A_2, interferon-γ, and tumor necrosis factor-α *in vitro*[15]. In addition, CC16 has been considered a peripheral biomarker for assessing the integrity of the lung epithelium. Increased CC16 concentrations have been found in serum from patients with lung injury such as sarcoidosis and idiopathic PF[4,16]. In contrast, serum CC16 levels are decreased in patients with asthma[17].

These findings suggest that CC16 is a candidate serum biomarker for PF in SSc. To test this hypothesis, we evaluated serum levels of CC16 and examined the correlation with clinical features in patients with SSc.

MATERIALS AND METHODS

Patients. Serum samples were obtained from 92 Japanese patients with SSc (77 women, 15 men). All patients fulfilled the criteria proposed by the American College of Rheumatology (ACR; formerly, the American Rheumatism Association)[18]. Patients were grouped according to the degree of skin involvement, based on the classification system proposed by LeRoy, *et al*[19]. Thirty-five patients (31 women, 4 men) had limited cutaneous SSc (lcSSc) and 57 patients (49 women, 8 men) had diffuse cutaneous SSc (dcSSc). The average age of the SSc patients was 52.3 ± 13.5 years (mean ± SD). Disease duration was 5.5 ± 6.6 years in patients with lcSSc and 3.3 ± 7.3 years in patients with dcSSc. Fifty-six patients (61% of total SSc patients, 8 lcSSc and 48 dcSSc) had PF. None of the SSc patients received any treatment, including corticosteroids, D-penicillamine, or other immunosuppressive therapy, at their first visit.

Antinuclear antibodies were determined by indirect immunofluorescence using HEp-2 cells as a substrate. Autoantibody specificities were further assessed by ELISA and immunoprecipitation. Anticentromere antibodies were present in 29 patients, antitopoisomerase I antibodies were present in 39 patients, anti-U1 RNP antibodies were present in 2 patients, anti-U3 RNP antibodies were present in 2 patients, anti-RNA polymerase I and III antibodies were present in 6 patients, anti-Th/To antibodies were present in 2 patients, and antinuclear antibodies of unknown specificity were present in 6 patients. Six patients tested negative for autoantibodies. Twenty patients with systemic lupus erythematosus (SLE; 17 women, 3 men; mean age 51.7 ± 15.9 yrs) who fulfilled the ACR criteria[20] and did not have PF were also evaluated as disease controls. No patients with SLE were treated with corticosteroid or immunosuppressive agents at this timepoint. In addition, 20 healthy age- and sex-matched Japanese volunteers (17 women, 3 men; mean age 54.2 ± 16.1 yrs) served as controls. All SSc and SLE patients and healthy controls involved in this study were nonsmokers and had no other respiratory diseases, including asthma and sarcoidosis, or impaired renal function.

To determine whether the change in serum pneumoprotein levels correlated with the activity of PF, we analyzed serum samples obtained at the time of active and inactive phase of PF in 16 SSc patients (11 women, 5 men). Five patients had lcSSc and 11 had dcSSc. The age of these patients was 53.7 ± 11.6 years and the disease duration was 3.1 ± 1.7 years. Nine patients with antitopoisomerase I antibodies and no patients with anticentromere antibody were included. These patients initially exhibited active PF. Twelve patients received oral corticosteroid therapy (prednisolone ~20 mg/day) and intravenous cyclophosphamide pulse therapy (500~1000 mg, once per month × 6) for treatment of active PF. The other 4 patients were treated with oral corticosteroid therapy (prednisolone ~20 mg/day). Activity of PF was stabilized by the treatment during the followup period in all patients (3.2 ± 2.0 yrs).

Samples of venous blood were drawn and allowed to clot, and centrifuged shortly after clot formation. Sera were removed, and all samples were stored at –70°C prior to use.

Clinical assessments. Complete medical histories, physical examinations, and laboratory tests were conducted on all patients. The degree of skin involvement was determined according to the modified Rodnan skin thickness score, as described[21]. Organ system involvement was defined as described[22] with some modifications: pulmonary fibrosis = bibasilar interstitial fibrosis on high-resolution computed tomogram (HRCT); pulmonary hypertension = clinical evidence of pulmonary hypertension and increased mean pulmonary arterial pressure (> 40 mm Hg) documented by echocardiography, in the absence of severe pulmonary interstitial fibrosis; esophagus = hypomotility shown by barium radiography; heart = pericarditis, congestive heart failure, or arrhythmias requiring treatment; kidney = malignant hypertension and rapidly progressive renal failure with no other explanation; joint = inflammatory polyarthralgias or arthritis; and muscle = proximal muscle weakness and elevated serum creatine kinase. Pulmonary function, including vital capacity (VC) and diffusing capacity for carbon monoxide (DLCO), was also tested. Erythrocyte sedimentation rates (ESR) and C-reactive protein (CRP) were considered elevated when each value was higher than 20 mm/h and 0.5 mg/dl, respectively.

PF activity was initially determined by HRCT of the chest and pulmonary function testing. Specifically, PF was considered to be active when the following 2 criteria were met: (1) a ground-glass appearance or reticular pattern on HRCT of the chest[23]; and (2) > 10% change in VC or > 15% change in DLCO within 1 year[24]. PF activity was monitored by serial HRCT scans of the chest and by pulmonary function testing, as described[25,26,27].

The study protocol was approved by the Kanazawa University Graduate School of Medical Science. Informed consent was obtained from all study participants.

Measurement of serum CC16 concentrations. Serum levels of CC16 were measured in duplicate with a specific competitive ELISA kit (APC Biomaterials, Rockville, MD, USA), according to the manufacturer's protocol. Briefly, horseradish peroxidase (HRP) conjugated to recombinant human CC16 is captured by the anti-CC16 antibody coating the wells, generating a signal (A_{450}) proportional to the amount of CC16-HRP conjugate bound. The CC16-HRP conjugate is premixed with the sample to be assayed. The assay thus measures a decrease in signal as CC16 in the sample competes with the CC16-HRP conjugate for binding sites. The detection limit of this assay is 10 ng/ml.

Measurement of serum KL-6 and SP-D levels. Serum levels of KL-6 and SP-D were measured with specific ELISA kits (Eitest KL-6, Eisai, Tokyo, Japan; SP-D kit, Yamasa, Chiba, Japan), according to the manufacturers' protocols. Briefly, 96-well plates were coated with monoclonal antibodies to KL-6 or SP-D and diluted serum samples were added to duplicate wells. After washing, bound antibodies were detected with peroxidase-conjugated monoclonal antibodies against KL-6 or SP-D. The detection limits of KL-6 and SP-D are 50 U/ml and 1.56 ng/ml, respectively.

Statistical analysis. Statistical analyses were performed using the Mann-Whitney U test and Wilcoxon's signed-rank test for comparison of sample means, Fisher's exact probability test for comparison of frequencies, and Bonferroni's test for multiple comparisons. Spearman's rank correlation coefficient was used to examine the relationship between 2 contin-

uous variables. The concentrations of CC16, KL-6, and SP-D were analyzed using receiver-operating characteristic (ROC) curves in order to find cutoff values for optimal discriminative accuracy. Statistical analyses were performed using JMP® 7.01 software (SAS Institute, Cary, NC, USA). P values less than 0.05 were considered statistically significant. All values are reported as the mean ± SD.

RESULTS

Serum concentrations of CC16 in SSc patients at initial presentation. Although SSc patients had higher serum levels of CC16 at the initial presentation (mean 71.7 ± 118.6 ng/ml) compared with the levels of healthy controls (27.7 ± 17.1 ng/ml) and SLE controls (30.6 ± 19.0 ng/ml), the difference was not significant (Figure 1A).

Serum CC16 levels were significantly elevated in SSc patients with PF (n = 56) compared with SSc patients without PF (n = 36, 90.8 ± 110.7 vs 42.1 ± 80.7 ng/ml; $p < 0.01$; Figure 2A). Although several cases of SSc without PF showed increased CC16 levels, these patients did not show any characteristic difference. Serum levels of KL-6 were significantly elevated in SSc patients with PF compared with SSc patients without PF (711.0 ± 535.1 vs 275.2 ± 330.4 U/ml; $p < 0.001$; Figure 2A). Serum levels of SP-D were also significantly elevated in SSc patients with PF compared with SSc patients without PF (146.2 ± 94.6 vs 88.9 ± 75.0 ng/ml; $p < 0.01$; Figure 2A).

Further, SSc patients with active PF (n = 18) showed significantly elevated serum CC16 levels compared to patients with inactive PF (n = 38, 168.8 ± 161.5 vs 53.8 ± 95.0 ng/ml; $p < 0.01$; Figure 3A). SSc patients with active PF also showed significantly elevated serum levels of KL-6 and SP-D compared to patients with inactive PF (1225.1 ± 630.7 vs 467.5 ± 231.2 ng/ml; $p < 0.001$; and 207.7 ± 122.6 vs 117.1 ± 60.8 U/ml; $p < 0.01$, respectively; Figure 3A).

Serum CC16 levels were significantly associated with SP-D ($r = 0.40$, $p < 0.0001$), but not with KL-6 ($r = 0.22$, $p = 0.10$, data not shown).

Thus, serum levels of CC16 as well as KL-6 and SP-D were elevated in SSc patients with PF, especially active PF.

ROC curve analysis. To evaluate the value of CC16 for diagnosis of PF in patients with SSc, ROC curve analysis was performed and the result was compared with that of KL-6 and SP-D. For this analysis, serum CC16 levels in SSc patients with PF were compared to serum CC16 levels observed in SSc patients without PF. KL-6 had excellent diagnostic capacity as demonstrated by an area under the curve (AUC) of 0.89 (Figure 2B). KL-6 level of 302 U/ml or higher was diagnostic of PF with a sensitivity of 85.5% and specificity of 85.3%. AUC of SP-D was 0.72 and SP-D level of 91.0 ng/ml or higher was diagnostic of PF with a sensitivity of 71.4% and specificity of 77.2%. AUC of CC16 was 0.76 and the value was inferior to that of KL-6 but was comparable with that of SP-D (Figure 2B). CC16 level of 46.0 ng/ml or higher was diagnostic of PF with a sensitivity of 51.8% and specificity of 88.8% (Figure 2B).

Similar analysis was also assessed for diagnosis of active PF in SSc patients with PF. For this analysis, serum pneumoprotein levels in SSc patients with active PF were used and compared to levels observed in SSc patients with inactive PF. The AUC of KL-6 (0.94) was higher compared with that of CC16 (0.82) and SP-D (0.75, Figure 3B). Cutoff levels set as the closest point to 100% sensitivity and 100% specificity were 46.0 ng/ml for CC16 (sensitivity 94.4%, specificity 68.4%), 729.0 U/ml for KL-6 (sensitivity 88.9%, specificity 89.2%), and 147.0 ng/ml for SP-D (sensitivity 72.5%, specificity 82.9%).

Although KL-6 seems to be the best marker of PF, this is still not perfect. While 2 patients without PF showed markedly elevated KL-6 (Figure 2A), these patients were

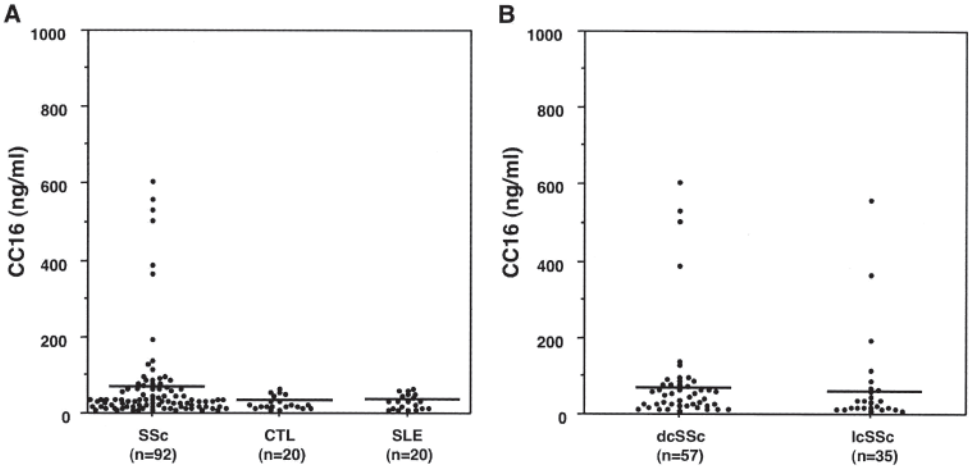

Figure 1. A. Levels of CC16 in serum samples from patients with systemic sclerosis (SSc), and systemic lupus erythematosus (SLE) as well as healthy controls (CTL). B. Serum CC16 levels in patients with diffuse cutaneous SSc (dcSSc) and limited cutaneous SSc (lcSSc). Serum CC16 levels were determined by competitive ELISA. Bars show group means.

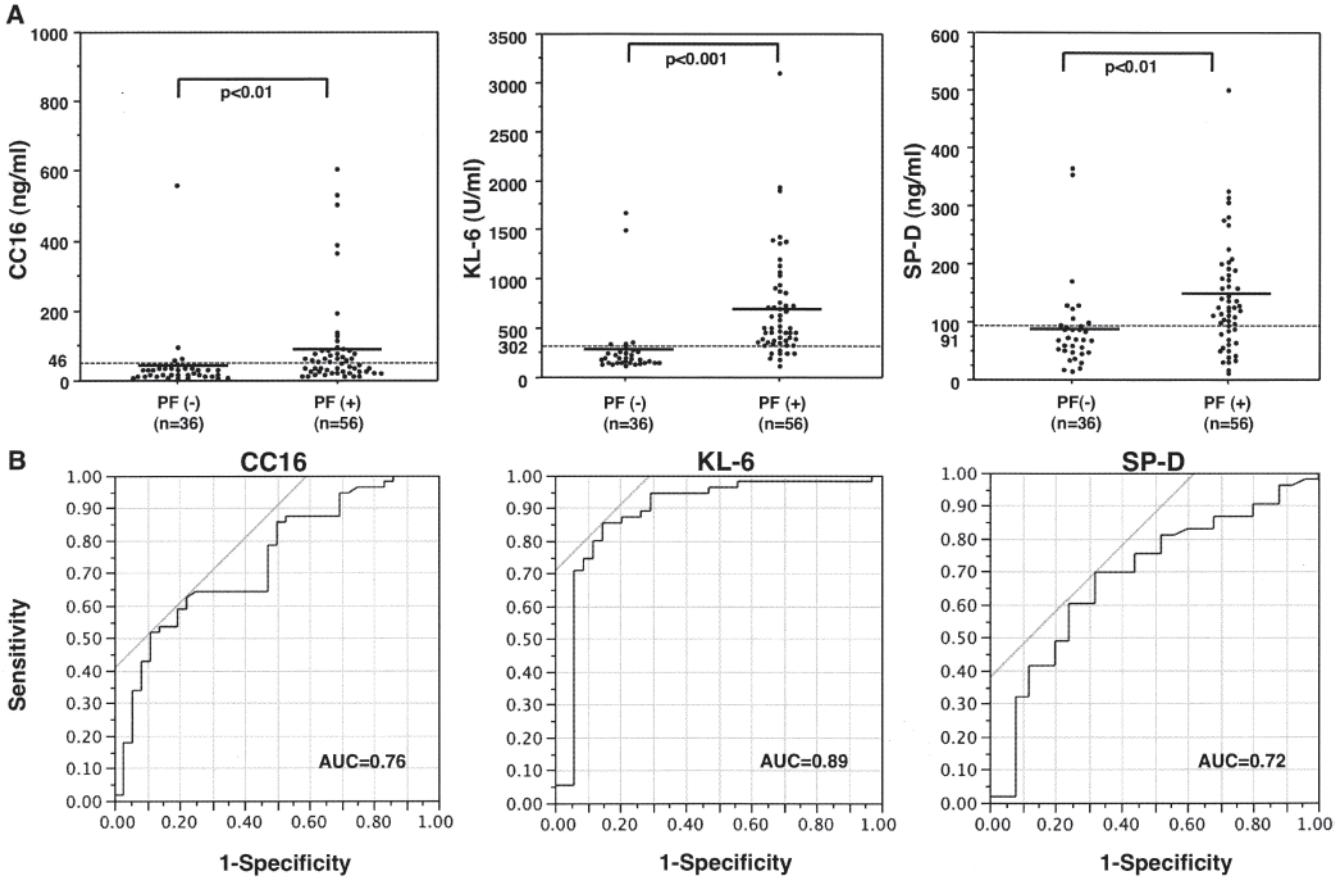

Figure 2. A. Serum levels of CC16, KL-6, and SP-D from SSc patients with pulmonary fibrosis (PF) and without PF. Bars show group means. B. ROC curves show the sensitivity and 1 – specificity of CC16, KL-6, and SP-D for detection of PF in patients with SSc. AUC: area under the ROC curve.

negative for CC16 and positive for SP-D (data not shown). Among 8 patients with PF that showed negative KL-6 (Figure 2A), 2 cases were positive for both CC16 and SP-D, and 3 cases were positive only for CC16, and 3 cases were positive only for SP-D (data not shown). These findings suggest that use of CC16 and SP-D in combination with KL-6 is valuable as an indicator of PF. Thus, ROC curve analysis suggests that CC16 is as useful as SP-D for diagnosis of PF or active PF, although it may not be as diagnostic as KL-6.

Serum CC16 levels and correlation with clinical features in SSc. Serum levels of CC16 were comparable in dcSSc and lcSSc patients (78.3 ± 124.3 vs 60.8 ± 109.5 ng/ml, Figure 1B). When the cutoff value was determined as 46 ng/ml as described above, elevated serum CC16 levels were observed in 35.9% of the SSc patients (33 of 92; Table 1). SSc patients with elevated CC16 levels had PF more frequently than patients with normal CC16 levels (88% vs 46%, respectively; $p < 0.001$; Table 1). Patients with elevated serum CC16 levels showed significantly decreased VC and DLCO values (predicted percentages) relative to those in SSc patients with normal CC16 levels ($p < 0.01$ for both comparisons). Antitopoisomerase I antibodies were present more frequently in SSc patients with elevated levels of CC16 than in those with normal levels, whereas anticentromere antibodies were found less frequently ($p < 0.05$, both comparisons). It is well known that patients with antitopoisomerase I antibodies frequently develop severe PF, whereas patients with anticentromere antibodies usually do not develop PF. Serum CC16 levels and the type of autoantibody present do not likely have a direct association, since serum CC16 levels were also found to be closely correlated with PF among patients with antitopoisomerase I antibodies or patients without anticentromere antibodies (data not shown).

Serum levels of CC16 were significantly inversely correlated with percentage VC ($r = –0.28$, $p < 0.05$) or percentage DLCO ($r = –0.20$, $p < 0.05$) values in patients with SSc (Table 2). The direct correlation of CC16 with percentage VC and percentage DLCO was modest compared with that of KL-6 ($r = –0.33$, $p < 0.01$, and $r = –0.46$, $p < 0.0001$, respectively), but was comparable with that of SP-D ($r = –0.21$, $p < 0.05$, and $r = –0.36$, $p < 0.01$, respectively). No other significant correlations were detected between serum CC16 levels and clinical or laboratory findings, as shown in Table 1. Thus, serum CC16 levels were specifically associated with the involvement of PF.

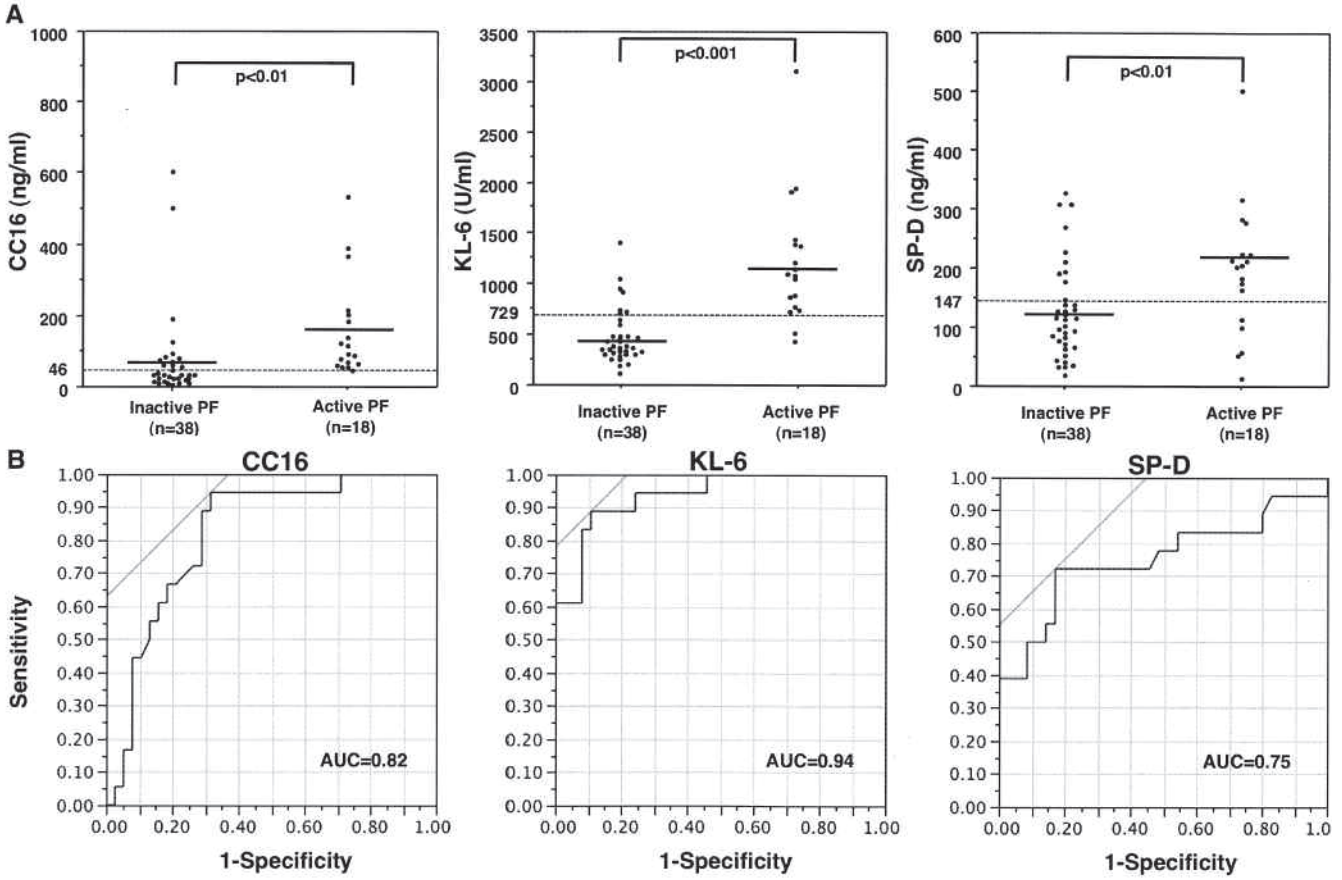

Figure 3. A. Serum levels of CC16, KL-6, and SP-D from SSc patients with active pulmonary fibrosis (PF) and inactive PF. Bars show group means. B. ROC curves show the sensitivity and 1 – specificity of CC16, KL-6, and SP-D for the detection of active PF in SSc patients with PF. AUC: area under the ROC curve.

Correlation between serum CC16 levels and activity of PF. Changes in serum CC16 levels in 16 SSc patients with active PF were also compared with changes in serum KL-6 and SP-D levels (Figure 4). Serum levels of CC16 were decreased significantly in parallel with the suppression of PF activity ($p < 0.05$). Serum levels of KL-6 were also significantly reduced in the inactive phase ($p < 0.05$). Further, serum SP-D levels were significantly decreased in association with the stabilization of PF activity. There were a few cases that showed increased levels of CC16, KL-6, or SP-D after stabilization of PF. In these patients, either 1 or 2 pneumoprotein levels were reduced in association with the disease activity (data not shown). These findings suggest that the utility of each marker for monitoring disease activity can be different in each patient. Thus, serum CC16 levels may indicate the PF activity as well as KL-6 or SP-D.

DISCUSSION

To our knowledge, this study is the first to evaluate serum levels of CC16 in patients with SSc. We found not only that serum CC16 levels were elevated in SSc patients with PF, but also that serum CC16 levels were remarkably higher in patients with active PF compared to patients with inactive PF. Further, CC16 levels were significantly reduced after the stabilization of PF in SSc patients. ROC curve analysis demonstrated that CC16 is as useful as SP-D for the diagnosis of PF or active PF in patients with SSc. Although serum CC16 may be slightly inferior as a marker for diagnosing PF or evaluating the severity of PF compared to KL-6, our data show it can be useful as an additional marker of PF activity in patients with SSc.

Ohnishi, *et al*[28] demonstrated a clear superiority of KL-6 to SP-D, SP-A, and monocyte chemoattractant protein-1 as a diagnostic marker of PF in terms of accuracy, sensitivity, specificity, and likelihood ratio in patients with idiopathic PF and collagen vascular disease-related interstitial pneumonia[28]. In SSc patients, the utility of KL-6 and SP-D has been reported for the evaluation of PF[7,8,9]. In a previous comparative study, we concluded that combined use of these 2 markers would be more effective for diagnosis and monitoring of PF activity in SSc than single use of each marker[9]. Another group's study supports these findings[8]. Our current results suggest that CC16 is another candidate protein for evaluating PF in SSc patients. Although the sensitivity and

Table 1. Clinical and laboratory features of patients with systemic sclerosis according to serum CC16 levels. Except where indicated otherwise, values are percentages.

Characteristic	Elevated CC16, n = 33	Normal CC16, n = 59
Sex, male:female	6:27	9:50
Age, mean ± SD yrs	55.2 ± 10.9	50.3 ± 14.4
Disease duration, mean ± SD yrs	5.4 ± 12.0	3.3 ± 4.0
Clinical features		
MRSS, mean ± SD	12.6 ± 9.7	12.3 ± 10.5
Digital pitting scars/ulcers	37	42
Contracture of phalanges	45	41
Diffuse pigmentation	48	42
Organ involvement		
Pulmonary fibrosis	88**	46
%VC, mean ± SD	93.8 ± 19.3**	105.8 ± 17.5
%DLCO, mean ± SD	57.1 ± 16.9**	70.0 ± 14.5
Pulmonary hypertension	21	10
Esophagus	63	73
Heart	3	2
Kidney	0	0
Joint	19	20
Muscle	7	22
Laboratory findings		
Antitopoisomerase I antibody	61*	32
Anticentromere antibody	3*	32
Elevated ESR	67	39
Elevated CRP	19	12

* $p < 0.05$, ** $p < 0.01$ vs normal serum CC16 levels. MRSS: modified Rodnan skin thickness score; VC: vital capacity; DLCO: diffusing capacity for carbon monoxide; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein.

Table 2. Correlations between serum pneumoprotein levels and values of respiratory function tests in patients with systemic sclerosis.

	VC, %	DLCO, %
CC16	$r = -0.28$, $p < 0.05$	$r = -0.20$, $p < 0.05$
KL-6	$r = -0.33$, $p < 0.01$	$r = -0.46$, $p < 0.0001$
SP-D	$r = -0.21$, $p < 0.05$	$r = -0.36$, $p < 0.01$

specificity were limited compared to KL-6, they were comparable with that of SP-D. Similarly, the sensitivity and specificity for active PF were slightly inferior to KL-6 and were at least comparable to that of SP-D. Further, our retrospective longitudinal study suggested that CC16 is as effective as KL-6 and SP-D to evaluate the activity of PF.

In addition to other important functions, the lung epithelium produces complex secretions, including mucus, surfactant proteins, and several proteins important for host defense[29]. Although CC16 is secreted by Clara cells into bronchoalveolar lavage (BAL) fluid, a significant correlation between CC16 levels in BAL fluid and serum has been reported[30]. Therefore, serum CC16 could potentially provide a less invasive method for assessing airway damage[31]. The exact physiological function of CC16 in the lung is not known, but it is believed to play a role in reducing inflammation in the airways[32] and protecting the respiratory tract from oxidative stress[15,31,32]. Serum CC16 has been shown to be elevated in several conditions related to impairment of the air-blood barrier, including idiopathic PF[16,29]. In our study, the CC16 serum levels as well as SP-D levels were found to be significantly associated with measurements of lung function such as percentage VC, but the correlation factors of CC16 and SP-D were slightly lower than that of KL-6. Serum CC16 levels were significantly associated with SP-D levels but not with KL-6 levels. The molecular weights of CC16 and SP-D (15.8 kDa and 43 kDa, respectively) are lower than that of KL-6 (> 200 kDa). Therefore, CC16 and SP-D may more easily leak into the circulation compared with KL-6 despite considerable lung-blood barrier destruction and subsequent fibrosis[3,15,33]. CC16 and SP-D are secretory proteins, whereas KL-6 is basically a structural component of cell membrane. Additionally, some proteinase to cleave its extracellular domain would be needed for KL-6 to leak into peripheral blood. Further, high molecular weight KL-6 may require more destruction of the lung-blood barrier or regeneration of lymph vessels compared with the other 2 pneumoproteins to leak into the circulation. These differences may explain why KL-6 is a more specific marker for detecting PF and evaluating the severity of PF compared with CC16 and SP-D in patients with SSc. On the other hand, these features may suggest that CC16 and SP-D are more sensitive to monitor the activity of interstitial pneumonia compared with KL-6. Although our study could not clarify this, previous studies demonstrated that SP-D is a more sensitive marker of alveolitis than KL-6 in patients with SSc[8,9].

There are some limitations to this study. This was not a prospective study and the study groups were not large, especially in the longitudinal study. Further, CC16 is not necessarily a perfectly specific marker for PF as much as KL-6 and SP-D. Reductions in levels of serum CC16 of approximately 30% have been found in smokers[17], and circulating CC16 levels were increased in patients with sarcoidosis and decreased in patients with asthma as compared with healthy controls[17]. Since CC16 is eliminated by glomerular filtration, the concentration of CC16 in serum can be used as a biomarker of lung epithelial injury only when the renal function is normal or moderately decreased[14]. Therefore, smokers and people with asthma, sarcoidosis, or impaired renal function were excluded from this study. The effects of these conditions on CC16 levels need to be considered when evaluating the effectiveness of using CC16 for monitoring PF. In addition, serum CC16 levels were elevated in several SSc patients without PF. Although we could not find any characteristic features in these patients, the kinds of factors other than PF that affect serum CC16 levels should be clarified in future studies.

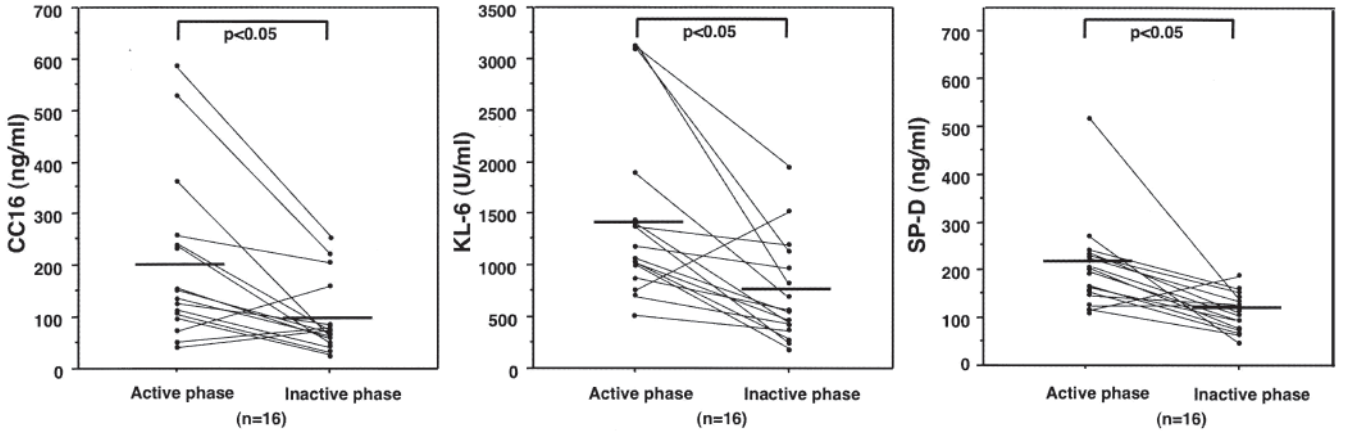

Figure 4. Change in serum levels of CC16, KL-6, and SP-D during active and inactive PF in patients with SSc. Serum samples were obtained during an active phase and an inactive phase of PF in each patient. Bars show group means.

Our data indicate that higher serum CC16 levels reflect increased activity of PF in patients with SSc. KL-6 and SP-D levels are currently the best serum markers of PF, but are still not perfect. Therefore, use of CC16 combined with KL-6 and SP-D may be a more valuable approach to monitor PF in SSc patients. Further prospective and comparative studies in larger populations will be needed to confirm these studies.

REFERENCES

1. Silver RM. Clinical problems: the lungs. Rheum Dis Clin North Am 1996;22:825-40.
2. Steen VD, Conte C, Owens GR, Medsger TA. Severe restrictive lung disease in systemic sclerosis. Arthritis Rheum 1994;37:1283-9.
3. Kohno N, Kyoizumi S, Awaya Y, Fukuhara H, Yamakido M, Akiyama M. New serum indicator of interstitial pneumonitis activity: sialylated carbohydrate antigen KL-6. Chest 1989; 96:68-73.
4. Hermans C, Bernard A. Lung epithelium-specific proteins: characteristics and potential applications as markers. Am J Respir Crit Care Med 1999;159:646-78.
5. Kobayashi J, Kitamura S. KL-6: a serum marker for interstitial pneumonia. Chest 1995;108:311-5.
6. Hamada H, Kohno N, Akiyama M, Hiwada K. Monitoring of serum KL-6 antigen in a patient with radiation pneumonia. Chest 1992;101:858-60.
7. Asano Y, Ihn H, Yamane K, Yazawa N, Kubo M, Fujimoto M, et al. Clinical significance of surfactant protein D as a serum marker for evaluating pulmonary fibrosis in patients with systemic sclerosis. Arthritis Rheum 2001;44:1363-9.
8. Hant FN, Ludwicka-Bradley A, Wang HJ, Li N, Elashoff R, Tashkin DP, et al. Surfactant protein D and KL-6 as serum biomarkers of interstitial lung disease in patients with scleroderma. J Rheumatol 2009;36:773-80.
9. Yanaba K, Hasegawa M, Takehara K, Sato S. Comparative study of serum surfactant protein-D and KL-6 concentrations in patients with systemic sclerosis as markers for monitoring the activity of pulmonary fibrosis. J Rheumatol 2004;31:1112-20.
10. Kohno N, Akiyama M, Kyoizumi S, Hakoda M, Kobuke K, Yamakido M. Detection of soluble tumor-associated antigens in sera and effusions using novel monoclonal antibodies, KL-3 and KL-6, against lung adenocarcinoma. Jpn J Clin Oncol 1988; 18:203-16.
11. Honda Y, Kuroki Y, Matsuura E, Nagae H, Takahashi H, Akino T, et al. Pulmonary surfactant protein D in sera and bronchoalveolar lavage fluids. Am J Respir Crit Care Med 1995;152:1860-6.
12. Svenssen J, Strandberg K, Tuvemo T, Hamberg M. Thromboxane A2: effects on airway and vascular smooth muscle. Prostaglandins 1977;14:425-36.
13. Takahashi H, Fujishima T, Koba H, Murakami S, Kurokawa K, Shibuya Y, et al. Serum surfactant proteins A and D as prognostic factors in idiopathic pulmonary fibrosis and their relationship to disease extent. Am J Respir Crit Care Med 2000;162:1109-14.
14. Broeckaert F, Clippe A, Knoops B, Hermans C, Bernard A. Clara cell secretory protein (CC16): features as a peripheral lung biomarker. Ann NY Acad Sci 2000;923:68-77.
15. Broeckaert F, Bernard A. Clara cell secretory protein (CC16): characteristics and perspectives as lung peripheral biomarker. Clin Exp Allergy 2000;30:469-75.
16. Lesur O, Bernard A, Arsalane K, Lauwerys R, Begin R, Cantin A, et al. Clara cell protein (CC-16) induces a phospholipase A2-mediated inhibition of fibroblast migration in vitro. Am J Respir Crit Care Med 1995;152:290-7.
17. Shijubo N, Itoh Y, Yamaguchi T, Abe S. Development of an enzyme-linked immunosorbent assay for Clara cell 10-kDa protein: in pursuit of clinical significance of sera in patients with asthma and sarcoidosis. Ann NY Acad Sci 2000;923:268-79.
18. Subcommittee for Scleroderma Criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. Preliminary criteria for the classification of systemic sclerosis (scleroderma). Arthritis Rheum 1980;23:581-90.
19. LeRoy EC, Krieg T, Black C, Medsger TA, Fleischmajer R, Rowell N, et al. Scleroderma (systemic sclerosis): classification, subsets, and pathogenesis. J Rheumatol 1988;15:202-5.
20. Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982;25:1271-7.
21. Clements P, Lachenbrush P, Seibold J, White B, Weiner S, Martin R, et al. Inter and intraobserver variability of total skin thickness score (modified Rodnan TSS) in systemic sclerosis. J Rheumatol 1995;22:1281-5.
22. Steen VD, Powell DL, Medsger TA. Clinical correlations and prognosis based on serum autoantibodies in patients with systemic sclerosis. Arthritis Rheum 1988;31:196-203.
23. Wells AU, Hansell DM, Corrin B, Harrison NK, Goldstraw P, Black CM, et al. High resolution computed tomography as a predictor of lung histology in systemic sclerosis. Thorax 1992;47:738-42.
24. Bouros D, Wells AU, Nicholson AG, Colby TV, Polychronopoulos

V, Pantelidis P, et al. Histopathologic subsets of fibrosing alveolitis in patients with systemic sclerosis and their relationship to outcome. Am J Respir Crit Care Med 2002;165:1581-6.
25. Taylor ML, Noble PW, White B, Wise R, Liu MC, Bochner BS. Extensive surface phenotyping of alveolar macrophages in interstitial lung disease. Clin Immunol 2000;94:33-41.
26. Schnabel A, Reuter M, Gross WL. Intravenous pulse cyclophosphamide in the treatment of interstitial lung disease due to collagen vascular diseases. Arthritis Rheum 1998;41:1215-20.
27. Giacomelli R, Valentini G, Salsano F, Cipriani P, Sambo P, Conforti ML, et al. Cyclophosphamide pulse regimen in the treatment of alveolitis in systemic sclerosis. J Rheumatol 2002;29:731-6.
28. Ohnishi H, Yokoyama A, Kondo K, Hamada H, Abe M, Nishimura K, et al. Comparative study of KL-6, surfactant protein-A, surfactant protein-D, and monocyte chemoattractant protein-1 as serum markers for interstitial lung diseases. Am J Respir Crit Care Med 2002;165:378-81.
29. Janssen R, Sato H, Grutters JC, Bernard A, van Velzen-Blad H, du Bois RM, et al. Study of Clara cell 16, KL-6, and surfactant protein-D in serum as disease markers in pulmonary sarcoidosis. Chest 2003;124:2119-25.
30. Shijubo N, Itoh Y, Yamaguchi T, Shibuya Y, Morita Y, Hirasawa M, et al. Serum and BAL Clara cell 10 kDa protein (CC10) levels and CC10-positive bronchiolar cells are decreased in smokers. Eur Respir J 1997;10:1108-14.
31. Lakind JS, Holgate ST, Ownby DR, Mansur AH, Helms PJ, Pyatt D, et al. A critical review of the use of Clara cell secretory protein (CC16) as a biomarker of acute or chronic pulmonary effects. Biomarkers 2007;12:445-67.
32. Jorens PG, Sibille Y, Goulding NJ, van Overveld FJ, Herman AG, Bossaert L, et al. Potential role of Clara cell protein, an endogenous phospholipase A2 inhibitor, in acute lung injury. Eur Respir J 1995;8:1647-53.
33. Nagae H, Takahashi H, Kuroki Y, Honda Y, Nagata A, Ogasawara Y, et al. Enzyme-linked immunosorbent assay using F(ab')2 fragment for the detection of human pulmonary surfactant protein D in sera. Clin Chim Acta 1997;266:157-71.