

SERUM KL-6 AS A NOVEL DISEASE MARKER IN ADOLESCENT AND ADULT CYSTIC FIBROSIS

S. Ohshimo¹, F. Bonella¹, N. Grammann², K. Starke², A. Cui¹, P.C. Bauer¹, H. Teschler², N. Kohno³, J. Guzman⁴, U. Costabel¹

¹Medical Faculty, University of Duisburg-Essen, and Department of Pneumology/Allergy, Ruhrlandklinik, Essen; ²Medical Faculty, University of Duisburg-Essen, and Department of Pneumology, Ruhrlandklinik, Essen; ³Department of Molecular and Internal Medicine, Division of Clinical Medical Science, Programs for Applied Biomedicine, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan; ⁴General and Experimental Pathology, Ruhr-University, Bochum

ABSTRACT. *Background:* Cystic fibrosis (CF) is a chronic progressive disease leading to obstructive pulmonary impairment, fibrosis and shortened life expectancy. Serum levels of KL-6, high molecular weight human MUC1 mucin, are increased in the majority of patients with various interstitial lung disorders. Whether they are also elevated in CF has not been investigated before. *Objective:* To evaluate whether serum KL-6 levels are elevated and correlate with pulmonary function variables in CF. *Design:* Serum KL-6, lactate dehydrogenase (LDH) and C-reactive protein (CRP) levels were measured in 72 consecutive CF and 80 age- and sex-matched healthy control subjects. The relationship between serum KL-6 levels and pulmonary function variables was analyzed. *Results:* Serum KL-6 levels in CF patients were significantly increased compared to healthy subjects. Receiver operating characteristic curve analysis revealed that the diagnostic accuracy of KL-6 was better than that of LDH and CRP. Serum KL-6 levels showed an inverse relationship with vital capacity (VC) % predicted and forced expiratory volume in one second (FEV₁) % predicted. *Conclusions:* Serum KL-6 levels are elevated and appear to be correlated with pulmonary function variables in CF. These results suggest that KL-6 may be a useful non-invasive marker to monitor disease severity. (*Sarcoidosis Vasc Diffuse Lung Dis* 2009; 26: 47-53)

KEY WORDS: KL-6, cystic fibrosis, biological marker

Abbreviations

α_1 -PI	α_1 -proteinase inhibitor	COPD	Chronic obstructive pulmonary disease
AUC	Area under the curve	CRP	C-reactive protein
BALF	Bronchoalveolar lavage fluid	ELISA	Enzyme-linked immunosorbent assay
BCF	Cystic fibrosis	FEV ₁	Forced expiratory volume in one second
		IL	Interleukin
		IPF	Idiopathic pulmonary fibrosis
		LDH	Lactate dehydrogenase
		NE	Neutrophil elastase
		ROC	Receiver operating characteristic
		TNF	Tumor necrosis factor
		VC	Vital capacity
		VEGF	Vascular endothelial growth factor

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Correspondence: Ulrich Costabel, MD

Department of Pneumology/Allergy, Ruhrlandklinik

Tueschener Weg 40, 45239 Essen, Germany

Tel. +49 (0) 201 433 4021

Fax: +49 (0) 201 433 4029

E-mail: ulrich.costabel@ruhrlandklinik.de

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INTRODUCTION

Cystic fibrosis (CF) is the most common life-shortening genetic disorder among Caucasian individuals, with an estimated frequency of 1:3,400 live births (1). The main pathogenetic factor of CF is a mutation in the cystic fibrosis transmembrane regulator gene (2). Despite dramatic therapeutic advances, CF continues to be progressive, resulting in chronic pulmonary infection and shortened life expectancy. Previous studies have shown that impaired pulmonary function is the most sensitive prognostic factor for CF patients (3-7). Sensitive serum markers for predicting impaired pulmonary function and prognosis may be therefore useful to monitor disease progression. Although the specificity of a blood test is, in general, limited for evaluating the local pathology in the small airways of CF patients, it may still be beneficial because blood sampling is minimally invasive and can easily be repeated.

KL-6 is a mucin-like glycoprotein with a molecular weight of 200 kd and has been classified as human MUC1 mucin (8). KL-6 has been reported to be a sensitive marker for various interstitial lung diseases, including idiopathic pulmonary fibrosis (IPF), radiation pneumonitis, drug-induced pneumonitis, collagen vascular disease-associated interstitial pneumonitis, extrinsic allergic alveolitis, pulmonary sarcoidosis and pulmonary alveolar proteinosis (9-12). In addition, prognostic significance of KL-6 has been also demonstrated in patients with IPF and drug-induced pneumonitis with diffuse alveolar damage or chronic interstitial pneumonia pattern (10, 13). However, the significance of serum KL-6 in CF patients has not been investigated.

The aim of the present cross-sectional study was to evaluate whether serum KL-6 levels are elevated and correlate with pulmonary function variables in CF.

METHODS

Study Subjects

Consecutive patients with CF admitted to Ruhrlandklinik (Essen, Germany) for routine assessments between February and November 2007 were studied. The diagnosis was based on compatible

clinical findings, genetic mutation or sweat test. At the enrollment, patients with obvious interstitial lung diseases of known etiology, malignancy or an acute infection were excluded. Age- and sex-matched healthy subjects were selected from our serum bank as controls for serum KL-6 levels. All subjects were Caucasians. There were 72 patients with CF (all non-current smokers) 40 healthy non-smokers, and 40 healthy current smokers. No significant differences were observed in sex and age distribution between the groups (Table 1). The CF patients had a mean height of 170.0 ± 9.7 cm, a mean body weight of 59.5 ± 12.4 kg and a mean body mass index (BMI) of 20.5 ± 3.4 . Serum samples were obtained from all subjects when taking routine blood tests and stored at -80°C until analysis of KL-6. Serum lactate dehydrogenase (LDH) and C-reactive protein (CRP) levels were measured at the routine clinical examinations. The pancreatic insufficiency of the patients was evaluated by the 72-hour fecal fat excretion. Documented informed consent was obtained from all subjects. The study was approved by the Institutional Review Board.

KL-6 Assay

Serum KL-6 level was measured by a sandwich enzyme-linked immunosorbent assay (ELISA) using the Eitest KL-6 ELISA kit (Eisai, Tokyo, Japan) according to the manufacturer's protocol. All samples were measured in duplicate and mean values were used for subsequent analysis.

Pulmonary Function Tests

Vital capacity (VC) and forced expiratory volume in one second (FEV_1) were analyzed by using spirometry (ZAN 400 Sniff, ZAN Messgeraete

Table 1. Characteristics of the 72 subjects enrolled

Factors	CF	Healthy (Non-smokers)	Healthy (Current smokers)
Number, n	72	40	40
Gender, Male/Female, n	32/40	18/22	14/26
Age (years), mean \pm SD (range)	30.6 ± 9.9 (15-63)	32.6 ± 10.0 (16-58)	35.5 ± 12.4 (19-64)

GmbH, Germany). Values were expressed as percentages of predicted normal values.

Statistical Methods

Data are expressed as mean ± standard deviation. Comparison of non-normally distributed variables between groups was done with the Mann-Whitney *U* test. Correlations between serum KL-6 levels and pulmonary function valuables were analyzed with linear regression analysis. Fisher's PLSD procedure was used for multiple comparison analysis. Paired *t* test was used to compare the areas under the ROC curve. All statistical analyses were done using SPSS version 13.0 for Windows (SPSS Inc., Chicago, IL). Differences were considered statistically significant when the *p* value was < 0.05.

RESULTS

Serum KL-6, LDH, CRP Levels in Healthy Subjects

The distribution of serum KL-6 levels in 80 healthy subjects is shown in Figure 1. The mean serum KL-6 level was significantly higher in male subjects than in females (mean ± SD, male 262 ± 79

U/mL, female 225 ± 81 U/mL; *p* < 0.03). The mean LDH and CRP levels were not different between male and female subjects (LDH, mean ± SD, male 180 ± 30 U/mL, female 179 ± 26 U/mL; CRP, mean ± SD, male 0.1 ± 0.3 mg/dL, female 0.1 ± 0.4 mg/dL). Serum levels of KL-6 and LDH, but not of CRP, correlated with age (*r* = 0.51, *p* <0.0001; *r* = 0.42, *p* = 0.0001, respectively).

Serum KL-6, LDH, CRP Levels in CF Patients

The mean serum KL-6 level was significantly elevated in CF patients compared with healthy subjects (mean ± SD, CF 410 ± 200 U/mL, healthy non-smokers 228 ± 73 U/mL, healthy current smokers 252 ± 90 U/mL, *p* <0.0001, *p* <0.0001, respectively) (Figure 2). The comparison of LDH and CRP levels between CF and healthy controls is shown in table 2. There was no significant difference in serum KL-6, LDH and CRP levels between healthy non-smokers and healthy current smokers. Pancreatic insufficiency was observed in 62 (86%) of CF patients. No significant correlation was found between serum KL-6 levels and the presence of pancreas insufficiency. Site of involved organ(s), presence of chronic infection(s), use of inhalation therapy, use of oral medication and frequency of hospitalization had no

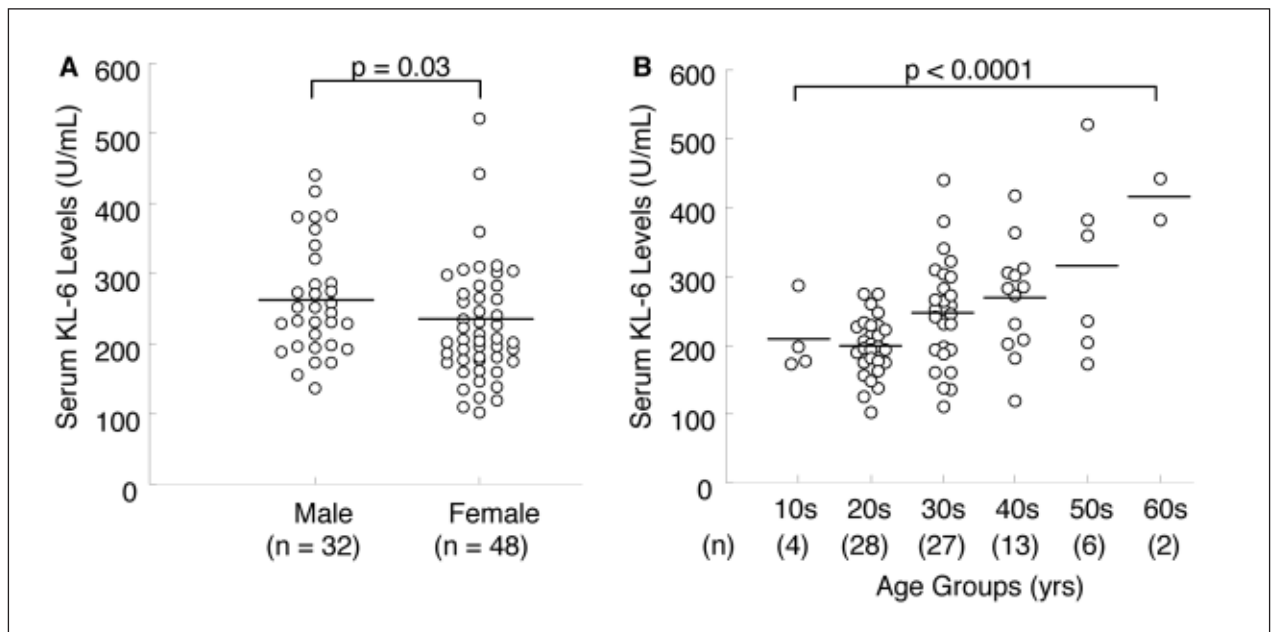


Fig. 1. Scatter plot graph showing sex (A) and age (B) distribution of serum KL-6 levels in 80 healthy subjects. Bars indicate average values.

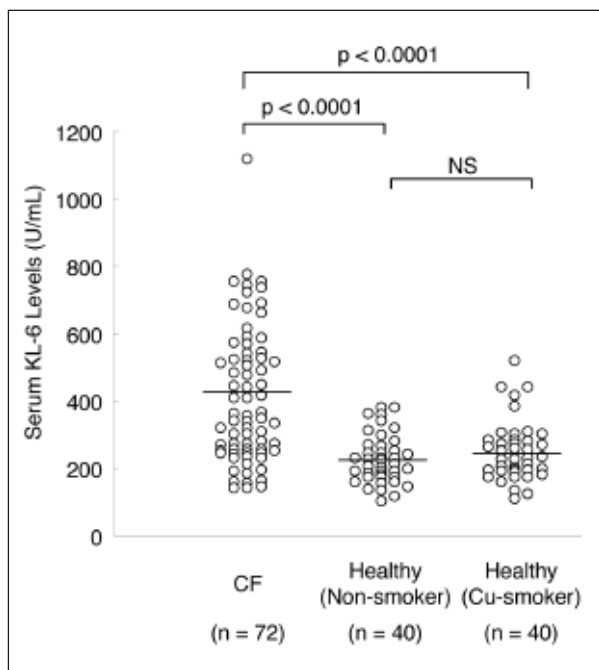


Fig. 2. Scatter plot graph showing the distribution of serum KL-6 levels in patients with CF, healthy non-smoker subjects and healthy current smoker (Cu-smoker) subjects. Bars indicate average values. NS: Not statistically significant.

Table 2. Comparison of serum level of LDH and CRP between CF group and healthy control

Marker	CF	Healthy (Non-smokers)	Healthy (Current smokers)
LDH (IU/L)	195±37	176±29 *	183±26
CRP (mg/dL)	1.9±3.2	0.1±0.4 *	0.1±0.3 *

* Fisher's PLSD test, $p < 0.05$ compared with CF group

significant effect on serum KL-6 levels (*data not shown*).

Receiver Operating Characteristic (ROC) Curve Analysis

ROC curve analysis was used to evaluate the sensitivity, specificity and diagnostic accuracy of serum KL-6, LDH and CRP levels (Figure 3). The largest area under the curve (AUC) was found for KL-6: KL-6, 0.79 (95% confidence interval (CI), 0.71 to 0.86); LDH, 0.62 (95% CI, 0.53 to 0.71); CRP, 0.71 (95% CI, 0.62 to 0.81). When the cut-off levels were set as the closest point to 100% sensitiv-

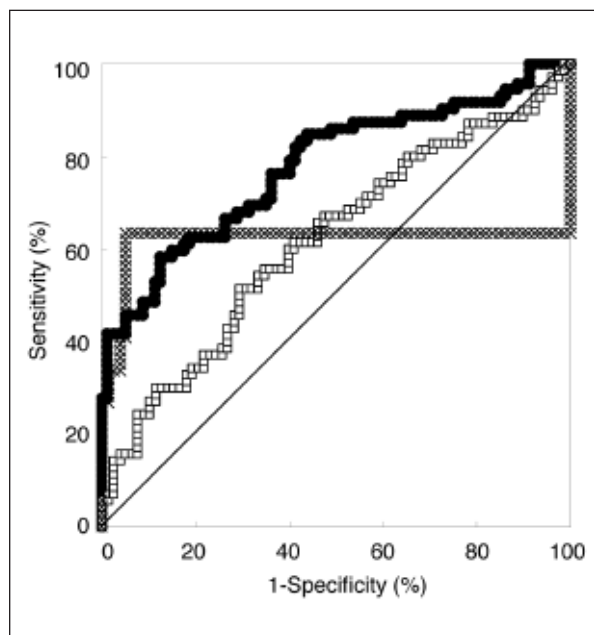


Fig. 3. Receiver operating characteristic curves for KL-6, LDH and CRP for the detection of cystic fibrosis. Closed circles indicate KL-6, opened squares indicate LDH and crosses indicate CRP.

ity and 100% specificity, the levels were 310 U/mL for KL-6 (sensitivity, 63%; specificity, 85%; and diagnostic accuracy, 74%, respectively), 195 IU/L for LDH (51%; 71%; and 61%) and 0.6 mg/dl for CRP (58%; 95%; and 77%, respectively). The differences in the AUC between KL-6, LDH and CRP was significant (KL-6 and LDH, $p = 0.0005$; KL-6 and CRP, $p = 0.0002$, LDH and CRP, $p = 0.001$).

Correlation of Serum KL-6 Levels with Pulmonary Function Test Variables in CF patients

In CF patients, the mean \pm SD values of VC % predicted, FEV₁ % predicted and FEV₁/VC were 65 \pm 20%, 45 \pm 21% and 56 \pm 13%, respectively. Serum KL-6 levels showed an inverse relationship with VC % predicted ($r = -0.37$, $p = 0.001$) (Figure 4A), with FEV₁ % predicted ($r = -0.35$, $p = 0.003$) (Figure 4B) and with FEV₁/VC ($r = -0.27$, $p = 0.02$) (Figure 4C). When the 72 CF patients were categorized into two groups based on the values of VC % predicted (above or equal to 40%, $n = 59$; under 40%, $n = 13$) or FEV₁ % predicted (above or equal to 40%, $n = 40$; under

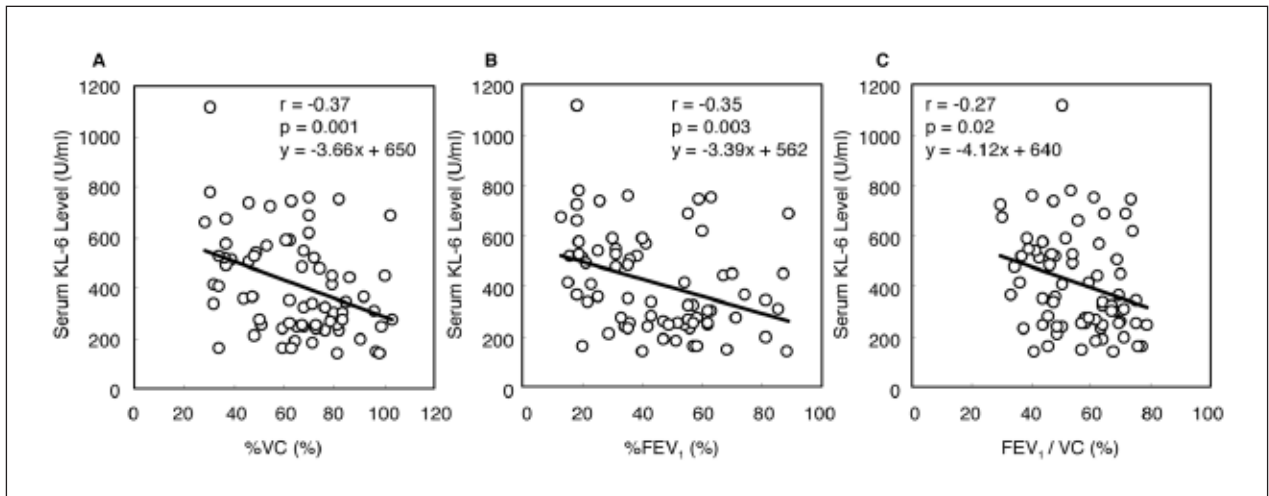


Fig. 4. Scatter diagrams showing the correlation of serum KL-6 levels in CF patients with VC % predicted (A), FEV₁ % predicted (B) and FEV₁/VC (C). The r value indicates a correlation coefficient. The equation indicates a regression equation. Lines indicate regression lines

40%, n = 32), patients with the lower VC or FEV₁ showed significantly higher serum KL-6 levels (p = 0.01, p = 0.0009).

DISCUSSION

This is the first study to explore the utility of KL-6 as a serum marker in patients with CF. Serum KL-6 levels in CF patients were significantly increased independent of age and sex compared to healthy control subjects. Increased serum KL-6 levels correlated with lower values of FEV₁ and VC. Serum KL-6 levels in CF patients were not influenced by extrapulmonary lesions, presence of chronic infection or mode of therapy. Interestingly, compared with traditional markers of cell injury or inflammation such as LDH and CRP, serum KL-6 discriminated best between CF patients and healthy controls.

We have previously reported that serum KL-6 levels are increased in various interstitial lung diseases (9-12). Because of this feature of KL-6, the value of KL-6 in diagnosing CF is limited. The significant correlation of serum KL-6 level with pulmonary function tests, however, indicates that serum KL-6 may be a non-invasive marker for evaluating the degree of lung destruction in CF patients.

Patients with chronic obstructive pulmonary disease (COPD) or asthma also show impaired pul-

monary function tests with low FEV₁ values. It is, therefore, important to know whether serum KL-6 levels are also elevated in COPD or asthma especially during acute exacerbations. Our previous study in a Japanese adult population has demonstrated no significant differences between healthy controls and patients with COPD, asthma, bacterial pneumonia or bronchiectasis (9). Increased serum levels are also rare in diffuse panbronchiolitis (14). Another study of a pediatric population has shown significantly increased serum KL-6 levels in patients with pneumonia, including measles pneumonia, and slightly increased levels in asthmatic patients, more frequently in those with acute exacerbations. The magnitude of the increase KL-6 in asthma was very modest, compared to the 2 fold increase in our CF population (15). It is likely that severe infection or inflammation can evoke alveolar epithelial damage in the immature lungs of these subjects. To date, however, no study has directly evaluated serum KL-6 levels in a CF group in comparison with COPD/asthma patients in a Caucasian adult population. Further studies are needed.

FEV₁ is an important prognostic factor in CF patients (7, 16). Kerem et al. have demonstrated that FEV₁ <30% predicted was associated with a mortality rate of 50% within 2 years (7). Milla et al. have shown that the yearly decline in FEV₁ was predictive of mortality in CF (17). In addition, CF patients with a low FEV₁ are more likely to experience pul-

monary exacerbations (18). The inverse correlation of serum KL-6 with FEV₁ may indicate the potential ability of KL-6 to assess the severity of airway remodeling due to fibrosis in CF. Further studies are needed to assess whether the local concentrations of KL-6 in BALF, sputum or exhaled breath condensate are even better as a biomarker for CF than serum levels.

Only few studies have shown that a serum marker may be useful for predicting decline in lung function in CF patients. McColley et al. have demonstrated that the serum level of vascular endothelial growth factor (VEGF) was increased compared to healthy subjects and predicted the decrease in FEV₁ (19). Carlsson et al. have shown that an increased serum level of autoantibodies against bactericidal permeability increasing protein predicted a decrease in FEV₁ and a poor prognosis (20). Other investigators studied local concentrations of potential biomarkers in CF patients. In this regard, the concentrations of neutrophil elastase (NE), myeloperoxidase (21), α_1 -proteinase inhibitor (α_1 -PI) (22), tumor necrosis factor (TNF)- α , interleukin (IL)-1, IL-6 and IL-8 in BALF or sputum (23-25) could potentially be useful for evaluating pulmonary impairment in CF patients. Wolter et al., however, have demonstrated a divergence between the local and systemic inflammatory response (26). IL-8 and TNF- α were generally undetectable in serum. No or only weak correlations were found between serum and sputum levels of proinflammatory markers, including IL-8, TNF- α , α_1 -PI and NE- α_1 -PI complex (26).

The predominant histological feature of the lung in CF is an inflammation of the small airways with resultant obstruction and surrounding fibrosis. Serum KL-6 levels are known to be increased in various fibrotic lung diseases (9-12). The increase of serum KL-6 levels in interstitial pneumonitis is thought to be due to an enhanced production of KL-6 by regenerating alveolar type II pneumocytes, and/or due to an increased permeability following destruction of the air-blood barrier in the affected lungs (27, 28). The concentration of KL-6 in BALF corresponds well with serum KL-6 in patients with interstitial lung diseases (27). We have previously demonstrated that the purified KL-6 molecule has chemotactic, proliferative and anti-apoptotic effects on fibroblasts *in vitro*, and that the proliferative and

anti-apoptotic effects of KL-6 are additive to those of transforming growth factor- β (29, 30). The increase of serum KL-6 in CF patients is likely to favor accelerated fibrosis.

Stahel et al. have immunohistochemically demonstrated that the location of KL-6 is in the epithelial cells of the pancreatic and mammary ducts, as well as in the type II epithelial cells of the lung (8). Sakuma et al. have shown that serum KL-6 levels decreased by 36% following lobectomy, indicating that circulating KL-6 is predominantly derived from the lung (31). Our study demonstrated no significant correlation between serum KL-6 levels and the presence of pancreatic insufficiency or glucose tolerance, suggesting that KL-6 is a lung-specific marker in CF patients.

Our study is the first to show the distribution of serum KL-6 levels in healthy Caucasian subjects. In these subjects, serum KL-6 levels are higher in males than in females and show a correlation with age, whereas there was no difference between healthy smokers and non-smokers. These findings are compatible with a previous study performed in Japanese subjects (32). The reason for the increased serum KL-6 in male or aged healthy subjects is unclear to date.

The potential limitation of our study is the almost exclusive enrollment of adult patients. Because this was a single center study, a sufficient number of children could not be enrolled. A multicenter longitudinal study including pediatric patients will be important to evaluate the further utility of KL-6 in CF.

In conclusion, we demonstrated increased serum KL-6 levels in CF patients and an inverse correlation with FEV₁ and VC. These findings suggest that serum KL-6 may be a useful marker to assess the disease severity. Further studies are required to validate these investigations and to evaluate the utility of serum KL-6 as a prognostic marker for CF patients.

REFERENCES

1. Kosorok MR, Wei WH, Farrell PM. The incidence of cystic fibrosis. *Stat Med* 1996; 15: 449-62
2. Riordan JR, Rommens JM, Kerem B, et al. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science* 1989; 245: 1066-73

3. Belkin RA, Henig NR, Singer LG, et al. Risk factors for death of patients with cystic fibrosis awaiting lung transplantation. *Am J Respir Crit Care Med* 2006; 173:659-666
4. Sharma R, Florea VG, Bolger AP, et al. Wasting as an independent predictor of mortality in patients with cystic fibrosis. *Thorax* 2001; 56: 746-50
5. Liou TG, Adler FR, Cahill BC, et al. Survival effect of lung transplantation among patients with cystic fibrosis. *Jama* 2001; 286: 2683-9.
6. Aurora P, Wade A, Whitmore P, Whitehead B. A model for predicting life expectancy of children with cystic fibrosis. *Eur Respir J* 2000; 16: 1056-60.
7. Kerem E, Reisman J, Corey M, Canny GJ, Levison H. Prediction of mortality in patients with cystic fibrosis. *N Engl J Med* 1992; 326: 1187-91.
8. Stahel RA, Gilks WR, Lehmann HP, Schenker T. Third International Workshop on Lung Tumor and Differentiation Antigens: overview of the results of the central data analysis. *Int J Cancer Suppl* 1994; 8: 6-26.
9. 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.
10. Ohnishi H, Yokoyama A, Yasuhara Y, et al. Circulating KL-6 levels in patients with drug induced pneumonitis. *Thorax* 2003; 58: 872-5.
11. Kobayashi J, Kitamura S. Serum KL-6 for the evaluation of active pneumonitis in pulmonary sarcoidosis. *Chest* 1996; 109: 1276-82.
12. Takahashi T, Munakata M, Suzuki I, Kawakami Y. Serum and bronchoalveolar fluid KL-6 levels in patients with pulmonary alveolar proteinosis. *Am J Respir Crit Care Med* 1998; 158: 1294-8.
13. Yokoyama A, Kondo K, Nakajima M, et al. Prognostic value of circulating KL-6 in idiopathic pulmonary fibrosis. *Respirology* 2006; 11: 164-8.
14. Ishii H, Mukae H, Kadota J, et al. High serum concentrations of surfactant protein A in usual interstitial pneumonia compared with non-specific interstitial pneumonia. *Thorax* 2003; 58: 52-7.
15. Imai T, Takase M, Takeda S, Kougo T. Serum KL-6 levels in pediatric patients: reference values for children and levels in pneumonia, asthma, and measles patients. *Pediatr Pulmonol* 2002; 33: 135-41.
16. Moorcroft AJ, Dodd ME, Webb AK. Exercise testing and prognosis in adult cystic fibrosis. *Thorax* 1997; 52: 291-3.
17. Milla CE, Warwick WJ. Risk of death in cystic fibrosis patients with severely compromised lung function. *Chest* 1998; 113: 1230-4.
18. Block JK, Vandemheen KL, Tullis E, et al. Predictors of pulmonary exacerbations in patients with cystic fibrosis infected with multi-resistant bacteria. *Thorax* 2006; 61: 969-74.
19. McColley SA, Stellmach V, Boas SR, Jain M, Crawford SE. Serum vascular endothelial growth factor is elevated in cystic fibrosis and decreases with treatment of acute pulmonary exacerbation. *Am J Respir Crit Care Med* 2000; 161: 1877-80.
20. Carlsson M, Eriksson L, Pressler T, et al. Autoantibody response to BPI predict disease severity and outcome in cystic fibrosis. *J Cyst Fibros* 2007; 6: 228-33.
21. Meyer K C, Zimmerman J. Neutrophil mediators, Pseudomonas, and pulmonary dysfunction in cystic fibrosis. *J Lab Clin Med* 1993; 121: 654-61.
22. O'Connor CM, Gaffney K, Keane J, et al. alpha 1-Proteinase inhibitor, elastase activity, and lung disease severity in cystic fibrosis. *Am Rev Respir Dis* 1993; 148: 1665-70.
23. Bonfield TL, Panuska JR, Konstan MW, et al. Inflammatory cytokines in cystic fibrosis lungs. *Am J Respir Crit Care Med* 1995; 152: 2111-8.
24. Taggart C, Coakley RJ, Grealley P, Canny G, O'Neill SJ, McElvaney NG. Increased elastase release by CF neutrophils is mediated by tumor necrosis factor-alpha and interleukin-8. *Am J Physiol Lung Cell Mol Physiol* 2000; 278: L33-41.
25. Osika E, Cavaillon JM, Chadelat K, et al. Distinct sputum cytokine profiles in cystic fibrosis and other chronic inflammatory airway disease. *Eur Respir J* 1999; 14: 339-46
26. Wolter JM, Rodwell RL, Bowler SD, McCormack JG. Cytokines and inflammatory mediators do not indicate acute infection in cystic fibrosis. *Clin Diagn Lab Immunol* 1999; 6: 260-5.
27. Kohno N, Awaya Y, Oyama T, et al. KL-6, a mucin-like glycoprotein, in bronchoalveolar lavage fluid from patients with interstitial lung disease. *Am Rev Respir Dis* 1993; 148: 637-42.
28. Inoue Y, Barker E, Daniloff E, Kohno N, Hiwada K, Newman LS. Pulmonary epithelial cell injury and alveolar-capillary permeability in berylliosis. *Am J Respir Crit Care Med* 1997; 156: 109-15.
29. Ohshimo S, Yokoyama A, Hattori N, Ishikawa N, Hirasawa Y, Kohno N. KL-6, a human MUC1 mucin, promotes proliferation and survival of lung fibroblasts. *Biochem Biophys Res Commun* 2005; 338: 1845-52.
30. Hirasawa Y, Kohno N, Yokoyama A, Inoue Y, Abe M, Hiwada K. KL-6, a human MUC1 mucin, is chemotactic for human fibroblasts. *Am J Respir Cell Mol Biol* 1997; 17: 501-7.
31. Sakuma T, Takahashi K, Ohya N, Usuda K, Handa M. Serum KL-6, a novel mucin-like glycoprotein, as an indicator of interstitial pneumonitis following lobectomy. *Surg Today* 1999; 29: 121-8.
32. Kobayashi J, Itoh Y, Kitamura S, Kawai T. Establishment of reference intervals and cut-off value by an enzyme immunoassay for KL-6 antigen, a new marker for interstitial pneumonia. *Jpn J Pathol* 1996; 44: 653-658 (in Japanese).