RESPIRATORY INVESTIGATION 50 (2012) 3–13

Contents lists available at SciVerse ScienceDirect

Respiratory Investigation

journal homepage: www.elsevier.com/locate/resinv

Review

Utility of KL-6/MUC1 in the clinical management of interstitial lung diseases

Nobuhisa Ishikawa[a], *Noboru Hattori*[a,*], *Akihito Yokoyama*[b], *Nobuoki Kohno*[a]

[a]*Department of Molecular and Internal Medicine, Graduate School of Biomedical Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan*

[b]*Department of Hematology and Respiratory Medicine, Kochi Medical School, Kochi University, Kohasu Oko-cho, Nankoku-City, Kochi 783-8505, Japan*

ARTICLE INFO

Article history:
Received 27 December 2011
Received in revised form
3 February 2012
Accepted 8 February 2012
Available online 8 March 2012

Keywords:
KL-6
MUC1
Serum biomarker
Interstitial lung disease
Ethnic differences

ABSTRACT

Interstitial lung diseases (ILDs) are a diverse group of pulmonary disorders characterized by various patterns of inflammation and fibrosis in the interstitium of the lung. Because injury and/or regeneration of type II pneumocytes are prominent histological features of ILDs, substances derived from type II pneumocytes have been the focus of research investigating potential biomarkers for ILD. One important biomarker for ILD is the high-molecular-weight glycoprotein, Krebs von den Lungen-6 (KL-6). KL-6 is now classified as a human MUC1 mucin protein, and regenerating type II pneumocytes are the primary cellular source of KL-6/MUC1 in the affected lungs of patients with ILD. KL-6/MUC1 is detectable in the serum of patients with ILD, and extensive investigations performed primarily in Japan have revealed that serum KL-6/MUC1 is elevated in 70–100% of patients with various ILDs, including idiopathic interstitial pneumonias, collagen vascular disease-associated interstitial pneumonia, hypersensitivity pneumonia, radiation pneumonitis, drug-induced ILDs, acute respiratory distress syndrome, pulmonary sarcoidosis, and pulmonary alveolar proteinosis. The results from these various studies have supported the utility of KL-6/MUC1 as a serum biomarker for detecting these various ILDs. Moreover, KL-6/MUC1 serum levels have been demonstrated to be useful for evaluating disease activity and predicting the clinical outcomes of various ILD types. Based on these observations, we believe that KL-6/MUC1 is currently one of the best and most reliable serum biomarkers available for ILD management.

Abbreviations: ILDs, interstitial lung diseases; IIPs, idiopathic interstitial pneumonias; CVD-IP, collagen vascular disease-associated interstitial pneumonia; HP, hypersensitivity pneumonia; RP, radiation pneumonitis; D-ILDs, drug-induced ILDs; ARDS, acute respiratory distress syndrome; IPF, idiopathic pulmonary fibrosis; UIP, usual interstitial pneumonia; NSIP, nonspecific interstitial pneumonia; HRCT, high-resolution computed tomography; SLB, surgical lung biopsy; mAb, monoclonal antibody; KL-6, Krebs von den Lungen-6; CEA, carcinoembryonic antigen; ELISA, enzyme-linked immunosorbent assay; CLIEA, chemiluminescent enzyme immunoassay; VNTR, variable number tandem repeat; TACE, TNF-α converting enzyme; ADAM17, disintegrin and metalloproteinase 17; ELF, epithelial lining fluid; ECM, extracellular matrix; PAP, pulmonary alveolar proteinosis; ROC, receiver operating characteristic; SSc, systemic sclerosis; PM/DM, polymyositis/dermatomyositis; EAA, extrinsic allergic alveolitis; FLD, farmer's lung disease; NSCLC, non-small cell lung cancer; EGFR-TKIs, epidermal growth factor receptor tyrosine kinase inhibitors; SBRT, stereotactic body radiotherapy; DAD, diffuse alveolar damage; CIP, chronic interstitial pneumonia; BALF, bronchoalveolar lavage fluid; ALI, acute lung injury; DIC, disseminated intravascular coagulation; AUC, area under the curve

*Corresponding author. Tel.: +81 82 257 5196; fax: +81 82 255 7360.
E-mail address: nhattori@hiroshima-u.ac.jp (N. Hattori).

2212-5345/$ - see front matter © 2012 The Japanese Respiratory Society. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.resinv.2012.02.001

Contents

1. Introduction

Interstitial lung diseases (ILDs) are a diverse group of pulmonary disorders characterized by various patterns of inflammation and fibrosis in the interstitium of the lung, including idiopathic interstitial pneumonias (IIPs), collagen vascular disease-associated interstitial pneumonia (CVD-IP), hypersensitivity pneumonia (HP), radiation pneumonitis (RP), drug-induced ILDs (D-ILDs), acute respiratory distress syndrome (ARDS), and sarcoidosis [1–4]. Moreover, based on histological features, IIPs have been further classified into several types, including idiopathic pulmonary fibrosis (IPF) with the histopathology of usual interstitial pneumonia (UIP) and nonspecific interstitial pneumonia (NSIP).

High-resolution computed tomography (HRCT), bronchoscopic examination, and/or surgical lung biopsy (SLB) are fundamental steps required to make a definite diagnosis of various ILDs, including IIPs [4–6]. Furthermore, serial lung function testing is generally used to monitor disease activity and/or predict the prognosis in patients with ILDs [7]. However, these examinations require specific medical facilities and may result in considerable discomfort to patients. Thus, the identification of serum biomarkers for ILDs would greatly improve current diagnostic methods. Serum biomarkers offer several advantages over other methods, including being generally easy to perform, inexpensive, reproducible, and less invasive. To date, various serum biomarkers have been tested for their use in ILDs [8–13]. Among these, biomarkers derived from type II pneumocytes have been of particular interest, because ILDs show a common pathophysiological development, i.e., type II pneumocyte injury or remodeling [8]. The most widely used biomarkers for ILDs derived from type II pneumocytes are KL-6 and 2 surfactant proteins, SP-A and SP-D. These 3 biomarkers have been studied independently by 2 Japanese research groups (Hiroshima University and Sapporo Medical University) and are currently in wide clinical use in Japan. Lactate dehydrogenase (LDH) has also been used as a biomarker for ILDs in Japan; however, LDH serum levels are not specific for lung damage and have been superseded by KL-6, SP-A, and SP-D [8]. This review, from the research group that discovered KL-6, discusses the clinical application of KL-6 as one of the most promising serum biomarkers for patients with various types of ILDs.

2. From discovery to clinical application: the novel glycoprotein, KL-6

A murine IgG1 monoclonal antibody (mAb) was developed to recognize a sialylated sugar chain, designated as Krebs von den Lungen-6 (KL-6), by immunizing a mouse with the human lung adenocarcinoma cell line VMRC-LCR [14]. KL-6 was first suggested as a serum tumor biomarker for pulmonary, breast, and pancreatic cancers. However, the diagnostic accuracy of KL-6 as a tumor marker was found to be inferior to that of carcinoembryonic antigen (CEA) based on the high rate of false positive cases in patients with pulmonary fibrosis. Further investigations in our laboratory revealed the possibility of KL-6 as a biomarker for ILDs, because patients with benign noninterstitial lung disease did not show a significant elevation in the serum levels of KL-6 [15]. A cooperative study on KL-6 as a serum biomarker was initiated with the diagnostic division of Eidia Co., Ltd. (Tokyo, Japan) in 1992. The findings of this study led to the development of an enzyme-linked immunosorbent assay (ELISA) that enabled the determination of the absolute amount of KL-6 in samples collected in clinical practice. KL-6 has been approved by Japan's Health Insurance Program as a diagnostic marker for ILDs since 1999, and KL-6 levels are examined in more than 2,000,000 samples per year in Japan. A chemiluminescent enzyme immunoassay (CLEIA) system has now been developed that can measure serum KL-6 levels within 1 h in ordinary Japanese clinical settings. However, the measurement of KL-6 is currently not possible for clinical practices in

most countries. For instance, the KL-6 ELISA kit, available from SCETI Bioscience Export Co., Ltd. (Tokyo, Japan), under contract with Edia Co., Ltd., is available for research purposes only.

3. Biochemical and biological properties of KL-6

As described above, we developed an mAb to recognize an undefined high-molecular-weight (200 kDa) glycoprotein designated KL-6. Using this anti-KL-6 mAb, we purified KL-6 from the culture medium of human breast cancer YMB-S cells. Because sialidase digestion or periodate oxidation of KL-6 reduced the binding of anti-KL-6 mAb to KL-6, the epitope on KL-6 was suggested to be a carbohydrate-containing sialic acid [14]. Subsequently, KL-6 was classified as "Cluster 9 (MUC1)" at the Third World International Workshop of the International Association for the Study of Lung Cancer on lung tumor and differentiation antigens according to the results of immunohistochemical and flow cytometry studies, although the precise epitope structure recognized by the anti-KL-6 mAb was unclear [16,17]. A previous study from our laboratory clearly demonstrated that KL-6 was a submolecule of MUC1 based on the results of a carbohydrate composition analysis [18]. In accordance with these different observations, KL-6/MUC1 is commonly used to denote the KL-6 molecule. Recently, the possible carbohydrate epitopes of the anti-KL-6 mAb have been reported to be novel *O*-linked glycans containing 6′sulfo-Gal/GalNAc of MUC1 [19].

MUC1 (episialin, polymorphic epithelial mucin) is a large glycoprotein containing 3 domains: (1) a cytoplasmic tail, (2) a single transmembrane region, and (3) an extracellular domain (Fig. 1a). The extracellular region of MUC1 contains sites of *O*- and *N*-linked glycosylation and a variable number tandem repeat (VNTR) domain with 20–100 repeats of a 20-amino acid sequence [20,21]. MUC1 has an extended, rigid structure protruding 200–500 nm above the plasma membrane and is found on the apical surface of normal glandular epithelial cells. The MUC1 extracellular domain can be shed into the pulmonary epithelial lining fluid (ELF) through the action of TNF-α converting enzyme (TACE; also called a disintegrin and metalloproteinase 17 [ADAM17]) and potentially ADAM9 [21,22]. In addition, some soluble MUC1 may result from alternative splicing. Transfection studies have revealed that MUC1 reduces cell–cell and cell–extracellular matrix (ECM) interactions, decreases cell aggregation, and prevents E-cadherin-mediated cell–cell adhesion and integrin-mediated cell–ECM adhesion. Previous studies have demonstrated that E-cadherin can be functionally suppressed by MUC1 overexpression [23] and that anti-KL-6/MUC1 mAb induces the capping of MUC1 and facilitates E-cadherin-mediated cell–cell interactions [24].

As described below in this review, the clinical importance of KL-6/MUC1 in the management of ILD has been established. However, very little is known about the pathophysiological role of KL-6/MUC1 in patients with ILDs. Previous studies from our laboratory demonstrated that purified KL-6/MUC1 has chemotactic and anti-apoptotic effects on fibroblasts and that the proliferative and anti-apoptotic effects of KL-6/MUC1 are additive to those of transforming growth factor-β [18,25]. These results support the hypothesis that KL-6/MUC1 is one of the key molecules involved in the intra-alveolar fibrotic process and pulmonary fibrosis. Moreover, these results indicate that KL-6/MUC1 may become a promising molecular target for the treatment of pulmonary fibrosis.

4. Expression of KL-6/MUC1 in tissues

Several studies have evaluated the expression of KL-6/MUC1 using immunohistochemistry (Table 1) [14,15,26]. KL-6/MUC1 is moderately expressed in type II pneumocytes and respiratory bronchiolar epithelial cells and only weakly expressed in basal cells of the terminal bronchiolar epithelium of normal lung tissues. On the other hand, type I pneumocytes, goblet cells, and mucous cells of the bronchial glands do not express KL-6/MUC1. Furthermore, KL-6/MUC1 is not expressed by the epithelial cells of the stomach, small intestine, or large intestine, with the exception of the fundic gland cells in the stomach. In addition to strong expression in lung, pancreatic, and breast cancer tissues, KL-6/MUC1 is strongly expressed by atypical and/or regenerating type II pneumocytes in tissue sections obtained from patients with ILDs [14,27,28]. Ohtsuki et al. reported linear and continuous staining for KL-6/MUC1 on the cell surface of regenerating type II pneumocytes in patients with IPF or NSIP, but only discontinuous staining in normal lung tissues (Fig. 1b) [29,30]. KL-6/MUC1 is also strongly expressed in areas of destruction in the pulmonary structures, loose stroma, and endothelial cells of lymph vessels, as well as the contents of these regions [31]. Weak to moderate expression was also observed in several cancer tissues, such as stomach, colon, and hepatocellular tumors [32–34]. KL-6/MUC1 is also expressed in the premature lung during the early weeks of pregnancy, and its expression persists even after lung maturation [35,36].

5. Positive rates of KL-6/MUC1 serum levels in various diseases

A clinical cut-off value of 500 U/mL has been established for distinguishing patients with ILDs from healthy subjects and patients with lung diseases other than ILDs [37]. KL-6/MUC1 serum levels higher than the cut-off value have been observed in more than 70% of patients with ILDs, including IIPs, CVD-IP, HP, RP, D-ILDs, ARDS, pulmonary sarcoidosis, and pulmonary alveolar proteinosis (PAP, Table 2) [15,38–50]. Interestingly, less than 10% of patients with alveolar pneumonia tested positive for KL-6/MUC1. Meanwhile, 28% of patients with active pulmonary tuberculosis and 2.6% of patients with inactive pulmonary tuberculosis test positive for KL-6/MUC1, with most positive patients showing widespread involvement of the lungs [51]. Patients with advanced stages of lung, pancreatic, and breast cancers showed an almost 50% positive rate [14,27,52–54]. However, the positive rate was low for gastric, hepatocellular, colon, and rectal cancers, and for hepatitis, liver cirrhosis, and pancreatitis.

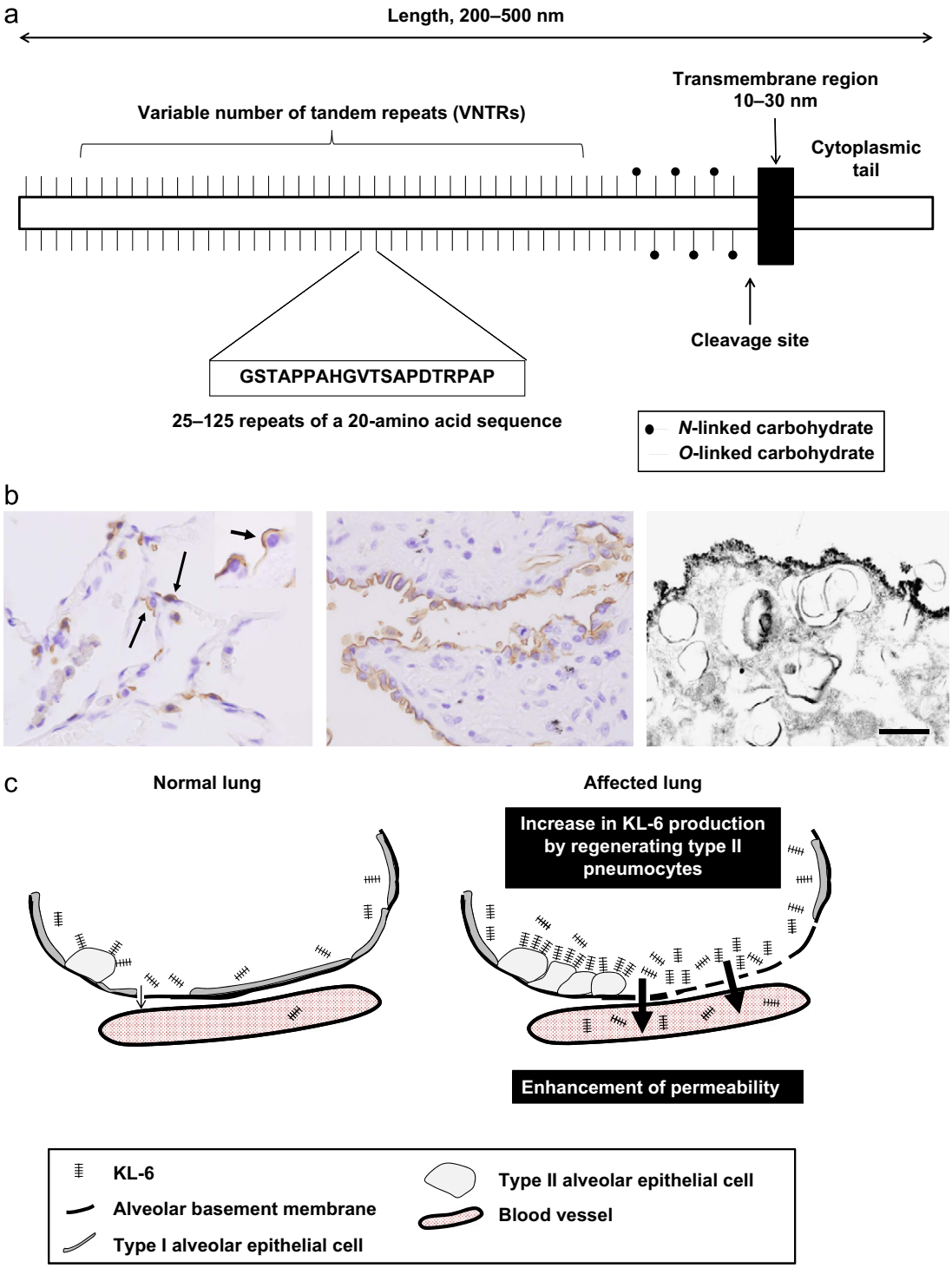

Fig. 1 – (a) Structure of MUC1. MUC1 is a large glycoprotein that contains 3 domains: (1) a cytoplasmic tail, (2) a single transmembrane region, and (3) an extracellular domain. The extracellular region contains sites of O- and N-linked glycosylation and a variable number tandem repeat (VNTR) domain of 20–100 repeats of a 20-amino acid sequence, (b) KL-6/MUC1 expression on the surface of type II pneumocytes. A discontinuous positive reaction (arrows) with anti-KL-6 antibody was observed in presumably normal lung tissue from a case of pneumothorax (left panel; magnification, ×400). Note the distinct dome-shaped positivity of the type II alveolar cells on staining with KL-6 antibody (inset at left panel; magnification, ×800). Linear and continuous staining for KL-6/MUC1 was observed on the cell surface of regenerating type II pneumocytes in patients with IPF (middle panel; magnification, ×400). Immunoelectron microscopic findings revealed that the reaction with anti-KL-6 antibody exhibits a linear pattern on the cell surface of type II pneumocytes in a patient with NSIP (right panel; magnification, ×400). Note that positive surface granular structures are approximately 100–200 nm in diameter. Scale bar = 0.5 μm. Modified from [29], with permission from the publisher. (c) Mechanism for the blood uptake of KL-6/MUC1. The increased serum levels of KL-6 in patients with ILDs may be due to an increase in KL-6 production by regenerating alveolar type II pneumocytes and/or enhanced permeability following the destruction of alveolar capillaries in the affected lung.

Table 1 – KL-6 expression in various tissues. Modified from [26]

	Negative	Positive		
		Weak	Moderate	Strong
Normal				
Lung	Type I pneumocytes	Basal cells of terminal bronchi	Type II pneumocytes	
	Ciliated bronchial cells		Respiratory bronchi epithelial cells	
	Goblet cells Mucous cells of the bronchial gland		Serous cells of the bronchial glands	
Others	Surface mucous cells of the stomach Pyloric cells of the stomach		Fundic gland cells of the stomach Ductal epithelial cells of the mammary gland	
	Epithelial cells of the duodenum Epithelial cells of the rectum Epithelial cells of the colon Acinar cells of the pancreas Leukocytes Red blood cells		Ductal epithelial cells of the pancreas	
Interstitial lung disease	Granuloma Giant cells			Regenerating type II pneumocytes
Malignant cells	Some malignant cells	Most malignant cells		Lung cancer Pancreatic cancer Breast cancer

Table 2 – Positive rate of KL-6 in various diseases. Modified from [26]

	Positive rate			
	0–10%	10–30%	30–70%	70–100%
Benign disease				
Lung	Alveolar pneumonia Bronchial asthma	Pulmonary tuberculosis (total) Pneumoconiosis	Diffuse panbronchitis Sarcoidosis	Idiopathic interstitial pneumonias Collagen vascular disease-associated interstitial pneumonitis
	COPD		Pulmonary tuberculosis with wide-spread involvement of the lung field	Hypersensitivity pneumonitis
	Bronchiectasis Pneumoconiosis			Radiation pneumonitis Drug-induced pneumonitis Acute respiratory distress syndrome Pulmonary sarcoidosis pulmonary alveolar proteinosis
Others	Hepatitis Liver cirrhosis Pancreatitis Cholecystitis			
Malignancies				
	Gastric cancer Colon cancer Rectal cancer Hepatic cancer		Lung cancer Pancreatic cancer Breast cancer	

6. Clinical evaluation of serum KL-6/MUC1 levels

More than 50 papers investigating the clinical significance of KL-6 in various types of ILDs have been published from our research groups, and more than 350 papers on KL-6/MUC1 can be found in PubMed, with an increasing number of reports from international groups of researchers [8]. The data from these reports suggest that KL-6/MUC1 serum levels are useful for (1) detecting the presence of disease, (2) evaluating disease activity, and (3) predicting clinical outcomes in various types of ILDs. The clinical utility of KL-6/MUC1 in various types of ILDs is summarized in Table 3.

6.1. *IIPs*

The first report from our laboratory describing KL-6/MUC1 serum levels demonstrated that KL-6/MUC1 serum levels in patients with various types of ILDs were significantly higher than those of healthy control subjects [15]. KL-6/MUC1 serum levels were found to be particularly high in IIP patients with a positive uptake of ^{67}Ga-citrate in their diseased lung. Furthermore, a significant positive correlation was observed between changes in KL-6/MUC1 serum levels and the subjective and objective signs of disease activity in patients whose clinical courses were followed. In another study, the KL-6/MUC1 serum levels in 33 patients with ILDs (21 patients with IPF and 12 with CVD-IP) were compared to 82 control subjects (70 healthy controls and 12 patients with bacterial pneumonia) [46]. A receiver operating characteristic (ROC) curve drawn in this study revealed the superiority of KL-6/MUC1 to SP-A, SP-D, and MCP-1 as a diagnostic marker of ILDs, as revealed by the diagnostic accuracy, sensitivity, specificity, and likelihood ratios. Based on these data, KL-6/MUC1 is thought to be useful for distinguishing most ILDs from other benign lung diseases, such as alveolar pneumonia. However, KL-6/MUC1 serum levels are elevated in 70–100% of patients with ILDs and therefore cannot be used to differentiate patients with IPF from those with NSIPs [55]. A detailed analysis of the relationship between KL-6/MUC1 serum levels and disease extent on HRCT in patients with NSIP revealed that KL-6/MUC1 serum levels are significantly correlated with the extent of interstitial disease [25,56,57]. Follow-up CT and changes in KL-6/MUC1 serum levels after treatment showed that the percent change in disease extent is reflected in the levels of KL-6/MUC1. Together, these observations indicate that KL-6/MUC1 serum levels may reflect the presence of fibrotic lung lesions accompanied by regenerating epithelial cells.

Acute exacerbation is a critical prognostic factor that shortens the survival period of patients with IPF. A small study followed 14 patients with rapidly progressive IPF who received weekly high-dose corticosteroid pulse therapy for at least 3 weeks [43]. KL-6/MUC1 serum levels significantly decreased in survivors, but tended to increase in nonsurvivors, suggesting that changes in KL-6/MUC1 serum levels can predict the efficacy of high-dose corticosteroid pulse therapy. However, this study was conducted prior to the 2002 ATS/ERS classification of ILDs; therefore, the diagnosis of some of these patients may be different from what is now accepted.

Elevated serum KL-6/MUC1 (KL-6/MUC1 levels $\geq$1000 U/mL) in IPF patients at the initial visit were associated with increased mortality [48]. Satoh et al. also reported that the progression of the disease was significantly faster in patients with ILDs whose KL-6/MUC1 levels were 1000 U/mL or more at the initial measurement than in patients whose KL-6/MUC1 levels were less than 1000 U/mL [58].

6.2. *CVD-IP*

ILDs are common manifestations in patients with collagen vascular disease (CVD), with an overall incidence estimated at 15% [59]. In a study conducted in our laboratory, KL-6/MUC1 serum levels were shown to be elevated in patients with CVD-IP compared to those of control subjects (healthy subjects and patients with bacterial pneumonia) [46]. In another study from our laboratory, the serum levels of KL-6/MUC1 were measured in 177 patients with rheumatoid arthritis. The results showed that an increase in KL-6/MUC1 serum levels was correlated with the presence of active CVD-IP [60]. Nakajima et al. evaluated the serum levels of KL-6/MUC1 in patients with CVD with or without ILDs and demonstrated that KL-6/MUC1 serum levels are useful markers in the diagnosis and evaluation of CVD-IP disease activity [61]. The utility of KL-6/MUC1 as a serum biomarker to detect ILDs and evaluate disease activity in patients with systemic sclerosis

Table 3 – Clinical utility of KL-6 in various types of ILDs.

Disease	Detection of disease		Evaluation of disease activity		Prediction of the prognosis	
	Utility	References	Utility	References	Utility	References
IIPs	++	[15,41,46,55]	++	[41,43]	++	[48,58]
CVD-IP	++	[41,46,60–64,66–69]	++	[41,61–69]	NE	
HP	++	[15,41,78,80,81]	++	[41,78,79]	NE	
RP	+	[15,38,82,83]	++	[38,39,82,83]	+	[84]
D-ILDs	+	[47,86]	++	[47,86]	++	[47,86]
ARDS	++	[49,87,92]	++	[49,87,92]	++	[50,87,88]

IIPs: idiopathic interstitial pneumonias; CVD-IP: collagen vascular disease-associated interstitial pneumonia; HP: hypersensitivity pneumonia; RP: radiation pneumonitis; D-ILDs: drug-induced interstitial lung diseases; ARDS: acute respiratory distress syndrome; ++: high utility, +: moderate utility NE: not evaluated.

(SSc) and polymyositis/dermatomyositis (PM/DM) has also been reported in several publications [62–69]. Furthermore, the serum levels of KL-6/MUC1 in patients with SSc are correlated with functional lung impairment, as expressed by diffusing capacity for carbon monoxide (DL_{CO}) reduction [69].

Glucocorticosteroids, immunosuppressants, and biological agents are widely used for the treatment of CVD, particularly in rheumatoid arthritis [70,71]. However, these therapies sometimes cause adverse effects, such as the occurrence of opportunistic infection. One such infection is *Pneumocystis jiroveci* pneumonia (PCP). This infection results in the appearance of ground-glass opacity on HRCT imaging and resembles acute exacerbation of ILD. Several reports demonstrate that KL-6/MUC1 serum levels are elevated in patients with PCP [72–75]. However, β-D-glucan may be more reliable as a serum diagnostic marker for PCP than KL-6/MUC1, since KL-6/MUC1 is too sensitive for underlying ILDs. Therefore, KL-6/MUC1 can be used to determine the extent of damaged alveolar epithelium and alveolar–capillary permeability, whereas β-D-glucan is a marker for fungal volume.

6.3. HP

HP, also known as extrinsic allergic alveolitis (EAA), is an immunologically mediated lung disease induced by the inhalation of antigens present in various systemic organs [76]. The clinical manifestation of HP can be divided into acute, subacute, or chronic types [77]. In patients with summer-type acute HP, KL-6/MUC1 serum levels are elevated [15,41]. Takahashi et al. evaluated KL-6/MUC1 serum levels in 272 farmers in a daily farming community and showed that KL-6/MUC1 serum levels were significantly higher in patients with farmer's lung disease (FLD) compared to farmers without FLD. In patients with FLD, KL-6/MUC1 serum levels were correlated with the activity of the disease [78]. Several reports demonstrate that KL-6/MUC1 serum levels are useful for evaluating disease activity in patients with HP caused by spores of the Japanese mushroom [79,80]. Inase et al. reported that KL-6/MUC1 serum levels were also elevated in cases of chronic HP with the potential to develop end-stage lung fibrosis similar to IPF [81].

6.4. RP

RP is a common complication that restricts the use of radiotherapy against lung cancer and sometimes leads to progressive respiratory failure or even death. The utility of KL-6/MUC1 in distinguishing RP from lung cancer is limited. However, the serum levels of KL-6/MUC1 are useful for the early diagnosis of RP in patients with lung cancer who receive radiation therapy [38,39]. Goto et al. monitored KL-6/MUC1 serum levels in patients with lung cancer at multiple time points after the start of radiation therapy and showed a correlation between the changes in serum KL-6/MUC1 levels and the clinical course of RP [82]. Furthermore, patients whose KL-6/MUC1 serum levels rose more than 1.5 times higher than baseline levels showed a trend toward the development of severe life-threatening RP [83,84]. Yamashita et al. retrospectively evaluated the incidence rate and risk factors of severe RP after stereotactic body radiotherapy (SBRT) for 117 patients with lung cancers. Grade 4–5 RP was observed in 9 patients (7.7%), and a correlation was found between the incidence of grade 4–5 RP and higher serum KL-6/MUC1 levels [84].

6.5. D-ILDs

Various agents can cause pulmonary toxicity, including ILDs, which often results in a fatal outcome. Moreover, a high incidence of ILDs is reported in patients with advanced non-small cell lung cancer (NSCLC) treated with epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs), particularly in Japanese populations [85]. The serum levels of KL-6/MUC1 were examined in 30 patients with D-ILDs who were classified into 4 different HRCT patterns [47]. The absolute KL-6/MUC1 serum levels at the onset of D-ILDs increased only in life-threatening disease types, such as those ILDs displaying diffuse alveolar damage (DAD) and chronic interstitial pneumonia (CIP) patterns. Serum KL-6/MUC1 levels increased or decreased in accordance with the clinical outcome of the disease in patients with the DAD and CIP patterns. Recently, the clinical records and radiographs of 341 patients with advanced NSCLCs who were treated with EGFR-TKIs were retrospectively reviewed, and changes in serum KL-6/MUC1 levels were also monitored in patients who developed D-ILDs [86]. Although absolute KL-6/MUC1 serum levels could not discriminate between life-threatening and non-life-threatening D-ILDs at either the baseline reading or the onset of D-ILDs, the ratio of the serum KL-6/MUC1 level at the onset of D-ILDs to that at the baseline could clearly discriminate between the 2 outcomes.

6.6. ARDS

ARDS is characterized by the influx of protein-rich edema fluid into air spaces, with the influx resulting from the increased permeability of the alveolar–capillary barrier. A previous study examined KL-6/MUC1 levels in the serum and pulmonary ELF or bronchoalveolar lavage fluid (BALF) of patients with ARDS or acute lung injury (ALI) [87,88]. These studies reported that the KL-6/MUC1 levels in these samples were significantly higher in nonsurvivors than in survivors. A recent study from our laboratory evaluated the levels of KL-6/MUC1 in ELF and serum obtained at multiple time points from patients with ARDS [50]. A comparison of the kinetics of KL-6/MUC1 levels in ELF and serum between survivors and nonsurvivors revealed that only the KL-6/MUC1 levels in ELF on days 0–3 after the diagnosis of ARDS were significantly higher in nonsurvivors than in survivors. In another study from our laboratory, KL-6/MUC1 serum levels, serially measured in patients with ARDS, along with the indices of respiratory failure, inflammation, coagulation, fibrinolysis, and multiple organ dysfunctions were shown to be associated with the development of disseminated intravascular coagulation (DIC) [49].

The expression of MUC1-associated sialyl Lewis^a has been demonstrated in previous studies [89–91], and studies from our laboratory have also found the presence of sialyl Lewis^a on KL-6/MUC1 [54,92]. This KL-6/MUC1 molecule containing sialyl Lewis^a was designated as SLAK, and an ELISA system using both anti-sialyl Lewis^a and anti-KL-6 antibodies was developed to measure SLAK levels in samples. The serum

levels of SLAK in patients with ARDS were found to be useful in predicting future development of DIC [92].

6.7. *Pulmonary sarcoidosis*

Sarcoidosis is a chronic systemic disorder characterized by noncaseating epithelioid cell granulomas and the accumulation of T-lymphocytes and macrophages in multiple organs [93]. The serum levels of KL-6/MUC1 in patients with sarcoidosis are increased and significantly influenced by the severity of lung involvement and the positive uptake of ^{67}Ga-citrate in the diseased lung [42]. Janssen et al. evaluated the ability of serum KL-6/MUC1, SP-D, and Clara cell 16 (CC16) levels to discriminate between patients with sarcoidosis and control subjects and concluded that KL-6/MUC1 was the best discriminative biomarker [94]. The investigators also observed a trend in which the serum KL-6/MUC1 levels were associated with pulmonary disease outcomes in the patients with sarcoidosis. In another study evaluating the significance of various biomarkers in patients with pulmonary sarcoidosis, Miyoshi et al. measured the serum levels of KL-6/MUC1, serum amyloid A, soluble interleukin 2 receptor, lysozyme, and angiotensin-converting enzyme [95]. These researchers demonstrated that KL-6/MUC1 serum levels were significantly correlated with the number of the total cells, lymphocytes, and $CD4^+$ T lymphocytes in BALF and were the single indicator of increased parenchymal infiltration in chest radiographs.

7. Mechanism for blood uptake of KL-6/MUC1

The primary cellular source of KL-6/MUC1 in the affected lungs of patients with ILDs is regenerating type II pneumocytes [15,29], and KL-6/MUC1 is present at high concentrations in BALF [40]. KL-6/MUC1 levels in BALF were significantly correlated with the total cell number, lymphocytes, neutrophils, and albumin concentrations in BALF and with serum KL-6/MUC1 levels in patients with ILDs. A correlation between KL-6/MUC1 serum levels and albumin levels in BALF was also found in patients with chronic beryllium disease, suggesting the utility of serum KL-6/MUC1 levels as a marker for the permeability of the air–blood barrier [96]. Both the destruction of the alveolar–capillary barrier and the enhancement of alveolar-capillary permeability are thought to be necessary for the leakage of KL-6/MUC1 into systemic circulation, since KL-6/MUC1 is a high-molecular-weight glycoprotein. As shown in Fig. 1c, the increase in serum KL-6/MUC1 levels in patients with ILDs results from an increase in KL-6/MUC1 production by regeneration of alveolar type II pneumocytes and/or enhancement of permeability following destruction of the alveolar–capillary barrier in the affected lung.

Simultaneous measurement of the serum levels of KL-6/MUC1, SP-A, and SP-D in patients with ILDs sometimes reveals a discrepancy between these serum markers. For instance, a transient increase in the serum levels of SP-A and SP-D following mild lung injury is frequently observed, while serum KL-6/MUC1 levels remain unchanged [8]. This discrepancy suggests that increases in serum KL-6/MUC1 levels do not reflect the intensity of inflammation, but rather indicate the extent of damaged alveolar epithelium and alveolar-capillary permeability (Table 4).

8. Association between serum KL-6/MUC1 levels and genetic variants in the MUC1 gene

Measurements of serum KL-6/MUC1 levels have been performed primarily in Japanese populations, and therefore, the data for non-Japanese populations are rather limited. We recently found that the levels of circulating KL-6/MUC1 were higher in European populations than in Japanese populations [97–100]. Another recent study evaluating the relationship between the functional A-to-G polymorphism at nucleotide position 568 (exon 2; rs4072037) in the MUC1 gene and serum KL-6/MUC1 levels in Caucasian subjects revealed that the genotype of this polymorphism affected serum KL-6/MUC1 levels. KL-6/MUC1 levels were highest for the GG genotype, lowest for the AA genotype, and in an intermediate range for the AG genotype [100]. The HapMap data (http://hapmap.ncbi.nlm.nih.gov/) indicate that the distributions of the AA, AG, and GG genotypes differ between European subjects (30.1%, 55.8%, and 14.2%, respectively) and Japanese subjects (69.8%, 25.6%, and 4.7%, respectively). These observations suggest an ethnic difference in the serum levels of KL-6/MUC1 and imply that a different cut-off level for KL-6/MUC1 is needed in Caucasians to discriminate between patients with ILDs and healthy subjects.

Table 4 – Acute phase reactant and serum KL-6 levels as serum indicators for the activity of interstitial lung diseases.

		KL-6	
		Normal range	Increased
Acute phase reactant	Normal range	Inactive	Inflammation (–) Insufficient repair of damaged alveoli • Progressing alveolar remodeling
	Increased	Inflammation (± ~ +) Epithelial barrier damage (–) • Alveolar remodeling (–) • Mild to severe respiratory dysfunction	Inflammation (± ~ +) Presence of alveolar damage • Rapid progression of alveolar remodeling • Severe clinical manifestation

9. Conclusions and future directions

In this review, we summarized the utility of KL-6/MUC1 in the clinical management of patients with various types of ILDs. Based on the results from a number of reports investigating KL-6/MUC1, the serum levels of KL-6/MUC1 are thought to be useful for (1) detecting the presence of disease, (2) evaluating disease activity, and (3) predicting outcomes in various types of ILDs. Because the measurement of serum KL-6/MUC1 levels is rapid, inexpensive, reproducible, less invasive, and easier to perform than SLB, HRCT, BAL, and pulmonary function tests, we believe that this biomarker would provide a significant benefit to the clinical management of patients with ILDs.

In Japan, KL-6/MUC1 has been used in clinical practice for more than 10 years; however, evidence from clinical trials validating the clinical efficacy of KL-6/MUC1 remains limited. In addition, we are aware of ethnic differences in the prevalence of pulmonary diseases such as D-ILDs and cystic fibrosis [85,101,102] and in the serum levels of KL-6/MUC1 [97–100]. In order to establish KL-6/MUC1 as an internationally useful serum biomarker, further prospective and international studies to determine the clinical efficacy of KL-6/MUC1 in the management of patients with ILDs are necessary.

Conflict of interest

Nobuoki Kohno received patent royalties/licensing fees from Eisai Co., Ltd.

Nobuhisa Ishikawa, Noboru Hattori, Akihito Yokoyama, they have no potential conflict of interest.

Acknowledgments

This work was supported by the Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan. The authors appreciate insightful suggestions from Y. Ohtsuki (Matsuyama-Shimin Hospital, Matsuyama, Japan) regarding the figures.

REFERENCES

[1] American Thoracic Society. Idiopathic pulmonary fibrosis: diagnosis and treatment. International consensus statement. American Thoracic Society (ATS), and the European Respiratory Society (ERS). Am J Respir Crit Care Med 2000;161:646–64.

[2] American Thoracic Society. European Respiratory Society. American Thoracic Society/European Respiratory Society International Multidisciplinary Consensus Classification of the Idiopathic Interstitial Pneumonias. This joint statement of the American Thoracic Society (ATS), and the European Respiratory Society (ERS) was adopted by the ATS board of directors, June 2001 and by the ERS Executive Committee, June 2001. Am J Respir Crit Care Med 2002;165:277–304.

[3] Raghu G, Collard HR, Egan JJ, et al. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. Am J Respir Crit Care Med 2011;183:788–824.

[4] Kinnula VL, Ishikawa N, Bergmann U, et al. Proteomic approaches for studying human parenchymal lung diseases. Expert Rev Proteomics 2009;6:619–29.

[5] King Jr. TE. Clinical advances in the diagnosis and therapy of the interstitial lung diseases. Am J Respir Crit Care Med 2005;172:268–79.

[6] Kim R, Meyer KC. Therapies for interstitial lung disease: past, present and future. Ther Adv Respir Dis 2008;2:319–38.

[7] British Thoracic Society recommendation. The diagnosis, assessment and treatment of diffuse parenchymal lung disease in adults. Thorax 1999;54(Suppl 1):S1–14.

[8] Nukiwa T. The role of biomarkers in management of interstitial lung disease: implication of biomarkers derived from type II pneumocytes. In: Du Bois RM, Richeldi L, editors. The European Respiratory Monograph 46 Interstitial lung disease. Leeds: Maney Publishing; 2009. p. 47–66.

[9] Tzouvelekis A, Kouliatsis G, Anevlavis S, et al. Serum biomarkers in interstitial lung diseases. Respir Res 2005;6:78.

[10] Prasse A, Müller-Quernheim J. Non-invasive biomarkers in pulmonary fibrosis. Respirology 2009;14:788–95.

[11] Thomeer M, Grutters JC, Wuyts WA, et al. Clinical use of biomarkers of survival in pulmonary fibrosis. Respir Res 2010;11:89.

[12] van den Blink B, Wijsenbeek MS, Hoogsteden HC. Serum biomarkers in idiopathic pulmonary fibrosis. Pulm Pharmacol Ther 2010;23:515–20.

[13] Barlo NP, van Moorsel CH, van den Bosch JM, et al. Predicting prognosis in idiopathic pulmonary fibrosis. Sarcoidosis Vasc Diffuse Lung Dis 2010;27:85–95.

[14] Kohno N, Akiyama M, Kyoizumi S, et al. 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.

[15] Kohno N, Kyoizumi S, Awaya Y, et al. New serum indicator of interstitial pneumonitis activity. Sialylated carbohydrate antigen KL-6. Chest 1989;96:68–73.

[16] Kohno N, Inoue Y, Hamada H, et al. Difference in serodiagnostic values among KL-6-associated mucins classified as cluster 9. Int J Cancer Suppl 1994;8:81–3.

[17] Stahel RA, Gilks WR, Lehmann HP, et al. Third International Workshop on Lung Tumor and Differentiation Antigens: overview of the results of the central data analysis. Int J Cancer 1994;8:6–26.

[18] Hirasawa Y, Kohno N, Yokoyama A, et al. KL-6, a human MUC1 mucin, is chemotactic for human fibroblasts. Am J Respir Cell Mol Biol 1997;17:501–7.

[19] Seko A, Ohkura T, Ideo H, et al. Novel O-linked glycans containing 6'sulfo-Gal/GalNAc of MUC1 secreted from human breast cancer YMBS cells: possible carbohydrate epitopes of KL-6(MUC1) monoclonal antibody. Glycobiology 2012;22:181–95.

[20] Singh PK, Hollingsworth MA. Cell surface-associated mucins in signal transduction. Trends Cell Biol 2006;16:467–76.

[21] Hattrup CL, Gendler SJ. Structure and function of the cell surface (tethered) mucins. Annu Rev Physiol 2008;70:431–57.

[22] Thathiah A, Blobel CP, Carson DD. Tumor necrosis factor-alpha converting enzyme/ADAM 17 mediates MUC1 shedding. J Biol Chem 2003;278:3386–94.

[23] Kondo K, Kohno N, Yokoyama A, et al. Decreased MUC1 expression induces E-cadherin-mediated cell adhesion of breast cancer cell lines. Cancer Res 1998;58:2014–19.

[24] Doi M, Yokoyama A, Kondo K, et al. Anti-tumor effect of the anti-KL-6/MUC1 monoclonal antibody through exposure of surface molecules by MUC1 capping. Cancer Sci 2006;97:420–9.

[25] Ohshimo S, Yokoyama A, Hattori N, et al. KL-6, a human MUC1 mucin, promotes proliferation and survival of lung fibroblasts. Biochem Biophys Res Commun 2005;338:1845–52.

[26] Kohno N. Serum marker KL-6/MUC1 for the diagnosis and management of interstitial pneumonitis. J Med Invest 1999;46:151–8.
[27] Tanaka S, Hattori N, Ishikawa N, et al. Krebs von den Lungen-6 (KL-6) is a prognostic biomarker in patients with surgically resected nonsmall cell lung cancer. Int J Cancer 2012;130:377–87.
[28] Yamasaki H, Ikeda S, Okajima M, et al. Expression and localization of MUC1, MUC2, MUC5AC and small intestinal mucin antigen in pancreatic tumors. Int J Oncol 2004;24:107–13.
[29] Ohtsuki Y, Fujita J, Hachisuka Y, et al. Immunohistochemical and immunoelectron microscopic studies of the localization of KL-6 and epithelial membrane antigen (EMA) in presumably normal pulmonary tissue and in interstitial pneumonia. Med Mol Morphol 2007;40:198–202.
[30] Ohtsuki Y, Nakanishi N, Fujita J, et al. Immunohistochemical distribution of SP-D, compared with that of SP-A and KL-6, in interstitial pneumonias. Med Mol Morphol 2007;40:163–7.
[31] Hisata S, Kimura Y, Shibata N, et al. A normal range of KL-6/MUC1 independent of elevated SP-D indicates a better prognosis in the patients with honeycombing on high-resolution computed tomography. Pulm Med 2011;2011:806014.
[32] Tanimoto T, Tanaka S, Haruma K, et al. MUC1 expression in intramucosal colorectal neoplasms. Possible involvement in histogenesis and progression. Oncology 1999;56:223–31.
[33] Hiraga Y, Tanaka S, Haruma K, et al. Immunoreactive MUC1 expression at the deepest invasive portion correlates with prognosis of colorectal cancer. Oncology 1998;55:307–19.
[34] Inagaki Y, Xu H, Nakata M, et al. Clinicopathology of sialomucin: MUC1, particularly KL-6 mucin, in gastrointestinal, hepatic and pancreatic cancers. Biosci Trends 2009;3:220–32.
[35] Sakurai J, Hattori N, Nakajima M, et al. Differential expression of the glycosylated forms of MUC1 during lung development. Eur J Histochem 2007;51:95–102.
[36] Sun AP, Ohtsuki Y, Fujita J, et al. KL-6, a human MUC1 mucin, is expressed early in premature lung. Respir Med 2003;97:964–9.
[37] Kitamura S, Hiwada K, Kobayashi J, et al. [Use of the ED046 kit to analyze serum KL-6 in patients with pneumonitis] [Article in Japanese]. Nihon Kyobu Shikkan Gakkai Zasshi 1996;34:639–45.
[38] Kohno N, Hamada H, Fujioka S, et al. Circulating antigen KL-6 and lactate dehydrogenase for monitoring irradiated patients with lung cancer. Chest 1992;102:117–22.
[39] Hamada H, Kohno N, Akiyama M, et al. Monitoring of serum KL-6 antigen in a patient with radiation pneumonia. Chest 1992;101:858–60.
[40] 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.
[41] Kobayashi J, Kitamura S. KL-6: a serum marker for interstitial pneumonia. Chest 1995;108:311–15.
[42] Kobayashi J, Kitamura S. Serum KL-6 for the evaluation of active pneumonitis in pulmonary sarcoidosis. Chest 1996;109:1276–82.
[43] Yokoyama A, Kohno N, Hamada H, et al. Circulating KL-6 predicts the outcome of rapidly progressive idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 1998;158:1680–4.
[44] Takahashi T, Munakata M, Suzuki I, et al. Serum and bronchoalveolar fluid KL-6 levels in patients with pulmonary alveolar proteinosis. Am J Respir Crit Care Med 1998;158:1294–8.
[45] Ishikawa N, Kondo K, Oguri T, et al. Usefulness of the modified lavage technique of Bingisser and KL-6 monitoring in a patient with pulmonary alveolar proteinosis. Intern Med 2002;41:381–5.
[46] Ohnishi H, Yokoyama A, Kondo 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.
[47] Ohnishi H, Yokoyama A, Yasuhara Y, et al. Circulating KL-6 levels in patients with drug induced pneumonitis. Thorax 2003;58:872–5.
[48] Yokoyama A, Kondo K, Nakajima M, et al. Prognostic value of circulating KL-6 in idiopathic pulmonary fibrosis. Respirology 2006;11:164–8.
[49] Nakashima T, Yokoyama A, Ohnishi H, et al. Circulating KL-6/MUC1 as an independent predictor for disseminated intravascular coagulation in acute respiratory distress syndrome. J Intern Med 2008;263:432–9.
[50] Kondo T, Hattori N, Ishikawa N, et al. KL-6 concentration in pulmonary epithelial lining fluid is a useful prognostic indicator in patients with acute respiratory distress syndrome. Respir Res 2011;12:32.
[51] Inoue Y, Nishimura K, Shiode M, et al. Evaluation of serum KL-6 levels in patients with pulmonary tuberculosis. Tuber Lung Dis 1995;76:230–3.
[52] Ishikawa N, Hattori N, Yokoyama A, et al. Usefulness of monitoring the circulating Krebs von den Lungen-6 levels to predict the clinical outcome of patients with advanced nonsmall cell lung cancer treated with epidermal growth factor receptor tyrosine kinase inhibitors. Int J Cancer 2008;122:2612–20.
[53] Ogawa Y, Ishikawa T, Ikeda K, et al. Evaluation of serum KL-6, a mucin-like glycoprotein, as a tumor marker for breast cancer. Clin Cancer Res 2000;6:4069–72.
[54] Inata J, Hattori N, Yokoyama A, et al. Circulating KL-6/MUC1 mucin carrying sialyl Lewis^{a} oligosaccharide is an independent prognostic factor in patients with lung adenocarcinoma. Int J Cancer 2007;120:2643–9.
[55] 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;58:52–4.
[56] Sakamoto K, Taniguchi H, Kondoh Y, et al. Serum KL-6 in fibrotic NSIP: Correlations with physiologic and radiologic parameters. Respir Med 2010;104:127–33.
[57] Ichiyasu H, Ichikado K, Yamashita A, et al. Pneumocyte biomarkers KL-6 and surfactant protein D reflect the distinct findings of high-resolution computed tomography in nonspecific interstitial pneumonia. Respiration 2012;83:190–7.
[58] Satoh H, Kurishima K, Ishikawa H, et al. Increased levels of KL-6 and subsequent mortality in patients with interstitial lung diseases. J Intern Med 2006;260:429–34.
[59] Antoniou KM, Margaritopoulos G, Economidou F, et al. Pivotal clinical dilemmas in collagen vascular diseases associated with interstitial lung involvement. Eur Respir J 2009;33:882–96.
[60] Oyama T, Kohno N, Yokoyama A, et al. Detection of interstitial pneumonitis in patients with rheumatoid arthritis by measuring circulating levels of KL-6, a human MUC1 mucin. Lung 1997;175:379–85.
[61] Nakajima H, Harigai M, Hara M, et al. KL-6 as a novel serum marker for interstitial pneumonia associated with collagen diseases. J Rheumatol 2000;27:1164–70.
[62] Yamane K, Ihn H, Kubo M, et al. Serum levels of KL-6 as a useful marker for evaluating pulmonary fibrosis in patients with systemic sclerosis. J Rheumatol 2000;27:930–4.
[63] Yanaba K, Hasegawa M, Hamaguchi Y, et al. Longitudinal analysis of serum KL-6 levels in patients with systemic sclerosis: association with the activity of pulmonary fibrosis. Clin Exp Rheumatol 2003;21:429–36.
[64] Yanaba K, Hasegawa M, Takehara K, et al. 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.
[65] Kodera M, Hasegawa M, Komura K, et al. Serum pulmonary and activation-regulated chemokine/CCL18 levels in patients

with systemic sclerosis: a sensitive indicator of active pulmonary fibrosis. Arthritis Rheum 2005;52:2889–96.
[66] Hant FN, Ludwicka-Bradley A, Wang HJ, 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.
[67] Bandoh S, Fujita J, Ohtsuki Y, et al. Sequential changes of KL-6 in sera of patients with interstitial pneumonia associated with polymyositis/dermatomyositis. Ann Rheum Dis 2000;59:257–62.
[68] Kubo M, Ihn H, Yamane K, et al. Serum KL-6 in adult patients with polymyositis and dermatomyositis. Rheumatology (Oxford) 2000;39:632–6.
[69] Bonella F, Volpe A, Caramaschi P, et al. Surfactant protein D and KL-6 serum levels in systemic sclerosis: correlation with lung and systemic involvement. Sarcoidosis Vasc Diffuse Lung Dis 2011;28:27–33.
[70] Stuck AE, Minder CE, Frey FJ. Risk of infectious complications in patients taking glucocorticosteroids. Rev Infect Dis 1989;11: 954–63.
[71] Nam JL, Winthrop KL, van Vollenhoven RF, et al. Current evidence for the management of rheumatoid arthritis with biological disease-modifying antirheumatic drugs: a systematic literature review informing the EULAR recommendations for the management of RA. Ann Rheum Dis 2010;69: 976–86.
[72] Hamada H, Kohno N, Yokoyama A, et al. KL-6 as a serologic indicator of *Pneumocystis carinii* pneumonia in immunocompromised hosts. Intern Med 1998;37:307–10.
[73] Tasaka S, Hasegawa N, Kobayashi S, et al. Serum indicators for the diagnosis of *Pneumocystis* pneumonia. Chest 2007;131:1173–80.
[74] Shimizu Y, Sunaga N, Dobashi K, et al. Serum markers in interstitial pneumonia with and without *Pneumocystis jirovecii* colonization: a prospective study. BMC Infect Dis 2009;9:47.
[75] Nakamura H, Tateyama M, Tasato D, et al. Clinical utility of serum beta-D-glucan and KL-6 levels in *Pneumocystis jirovecii* pneumonia. Intern Med 2009;48:195–202.
[76] Bourke SJ, Dalphin JC, Boyd G, et al. Hypersensitivity pneumonitis: current concepts. Eur Respir J Suppl 2001;32: 81s–92s.
[77] Richerson HB, Bernstein IL, Fink JN, et al. Guidelines for the clinical evaluation of hypersensitivity pneumonitis. Report of the Subcommittee on Hypersensitivity Pneumonitis. J Allergy Clin Immunol 1989;84:839–44.
[78] Takahashi T, Munakata M, Ohtsuka Y, et al. Serum KL-6 concentrations in dairy farmers. Chest 2000;118:445–50.
[79] Tsushima K, Furuya S, Yoshikawa S, et al. Therapeutic effects for hypersensitivity pneumonitis induced by Japanese mushroom (Bunashimeji). Am J Ind Med 2006;49:826–35.
[80] Yoshikawa S, Tsushima K, Yasuo M, et al. Hypersensitivity pneumonitis caused by *Penicillium citrinum*, not Enoki spores. Am J Ind Med 2007;50:1010–17.
[81] Inase N, Ohtani Y, Usui Y, et al. Chronic summer-type hypersensitivity pneumonitis: clinical similarities to idiopathic pulmonary fibrosis. Sarcoidosis Vasc Diffuse Lung Dis 2007;24:141–7.
[82] Goto K, Kodama T, Sekine I, et al. Serum levels of KL-6 are useful biomarkers for severe radiation pneumonitis. Lung Cancer 2001;34:141–8.
[83] Hara R, Itami J, Komiyama T, et al. Serum levels of KL-6 for predicting the occurrence of radiation pneumonitis after stereotactic radiotherapy for lung tumors. Chest 2004;125: 340–4.
[84] Yamashita H, Kobayashi-Shibata S, Terahara A, et al. Prescreening based on the presence of CT-scan abnormalities and biomarkers (KL-6 and SP-D) may reduce severe radiation pneumonitis after stereotactic radiotherapy. Radiat Oncol 2010;5:32.
[85] Kudoh S, Kato H, Nishiwaki Y, et al. Interstitial lung disease in Japanese patients with lung cancer: a cohort and nested case–control study. Am J Respir Crit Care Med 2008;177: 1348–57.
[86] Kawase S, Hattori N, Ishikawa N, et al. Change in serum KL-6 level from baseline is useful for predicting life-threatening EGFR-TKIs induced interstitial lung disease. Respir Res 2011;12:97.
[87] Ishizaka A, Matsuda T, Albertine KH, et al. Elevation of KL-6, a lung epithelial cell marker, in plasma and epithelial lining fluid in acute respiratory distress syndrome. Am J Physiol Lung Cell Mol Physiol 2004;286:L1088–94.
[88] Sato H, Callister ME, Mumby S, et al. KL-6 levels are elevated in plasma from patients with acute respiratory distress syndrome. Eur Respir J 2004;23:142–5.
[89] Baeckström D, Karlsson N, Hansson GC. Purification and characterization of sialyl-Le(a)-carrying mucins of human bile; evidence for the presence of MUC1 and MUC3 apoproteins. J Biol Chem 1994;269:14430–7.
[90] Fernandez-Rodriguez J, Dwir O, Alon R, et al. Tumor cell MUC1 and CD43 are glycosylated differently with sialyl-Lewis a and x epitopes and show variable interactions with E-selectin under physiological flow conditions. Glycoconj J 2001;18:925–30.
[91] Burdick MD, Harris A, Reid CJ, et al. Oligosaccharides expressed on MUC1 produced by pancreatic and colon tumor cell lines. J Biol Chem 1997;272:24198–202.
[92] Nakashima T, Yokoyama A, Inata J, et al. Mucins carrying selectin ligands as predictive biomarkers of disseminated intravascular coagulation complication in ARDS. Chest 2010;139:296–304.
[93] Statement on sarcoidosis. Joint Statement of the American Thoracic Society (ATS), the European Respiratory Society (ERS) and the World Association of Sarcoidosis and Other Granulomatous Disorders (WASOG) adopted by the ATS Board of Directors and by the ERS Executive Committee, February 1999. Am J Respir Crit Care Med 1999;160:736–55.
[94] Janssen R, Sato H, Grutters JC, 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.
[95] Miyoshi S, Hamada H, Kadowaki T, et al. Comparative evaluation of serum markers in pulmonary sarcoidosis. Chest 2010;137:1391–7.
[96] Inoue Y, Barker E, Daniloff E, et al. Pulmonary epithelial cell injury and alveolar-capillary permeability in berylliosis. Am J Respir Crit Care Med 1997;156:109–15.
[97] Ohshimo S, Bonella F, Grammann N, et al. Serum KL-6 as a novel disease marker in adolescent and adult cystic fibrosis. Sarcoidosis Vasc Diffuse Lung Dis 2009;26:47–53.
[98] Ohshimo S, Bonella F, Sommerwerck U, et al. Comparison of serum KL-6 versus bronchoalveolar lavage neutrophilia for the diagnosis of bronchiolitis obliterans in lung transplantation. J Heart Lung Transplant 2011;30:1374–80.
[99] Ishikawa N, Mazur W, Toljamo T, et al. Ageing and long-term smoking affects KL-6 levels in the lung, induced sputum and plasma. BMC Pulm Med 2011;11:22.
[100] Janssen R, Kruit A, Grutters JC, et al. The mucin-1 568 adenosine to guanine polymorphism influences serum Krebs von den Lungen-6 levels. Am J Respir Cell Mol Biol 2006;34:496–9.
[101] Wright SW, Morton NE. Genetic studies on cystic fibrosis in Hawaii. Am J Hum Genet 1968;20:157–69.
[102] Yamashiro Y, Shimizu T, Oguchi S, et al. The estimated incidence of cystic fibrosis in Japan. J Pediatr Gastroenterol Nutr 1997;24:544–7.