Serum marker KL-6/MUC1 for the diagnosis and management of interstitial pneumonitis

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Abstract: Interstitial pneumonitis includes more than a hundred diseases in which alveolitis is the main manifestation of the affected lung. Symptoms such as dry cough and exertional dyspnea, fine crackles on chest auscultation, interstitial infiltrates on chest X-ray films and CT scans, respiratory function tests, and Ga-67 scintigraphy have been used for the diagnosis and the evaluation of disease activity. However, the poor prognosis of some types of interstitial pneumonitis has not been improved . We discovered a high molecular weight mucin-like antigen, designated KL-6, which is also known as MUC1. The serum level of KL-6/MUC1 was elevated in 70-100% of patients with interstitial pneumonitis, such as pulmonary fibrosis (either idiopathic or related to collagen-vascular disorders), hypersensitivity pneumonitis, sarcoidosis, and radiation pneumonitis. The levels were significantly higher in patients with active disease than in those with inactive disease. In contrast, patients with noninterstitial lung disease did not show a significant elevation of KL-6/MUC1. Furthermore, the serum KL-6/MUC1 level was found to be an early predictive marker of the therapeutic effect of high-dose corticosteroids in patients with rapidly progressing idiopathic pulmonary fibrosis. These results indicate that KL-6/MUC1 may be a useful serum marker for the diagnosis and monitoring of patients with interstitial pneumonitis. J. Med. Invest. 46 : 151-158, 1999

Key words **:** *KL-6, KL-6/MUC1, interstitial pneumonitis, serum marker*

INTRODUCTION

Interstitial lung disease includes a variety of pulmonary diseases, which can be classified into the following five groups ; collagen vascular disease-associated interstitial pneumonitis (IP), drug and treatment-induced IP, primary or unclassified disease-related IP, occupational and environmental IP, and idiopathic fibrotic disease. In all these diseases, disruption of the distal lung parenchyma is the leading morphological change (1). Most of the diseases feature progressive alveolitis that results in fibrotic remodeling of alveolar spaces. In idiopathic pulmonary fibrosis (IPF), which is a representative type of IP, no improvement of survival has been achieved over the last two decades. Almost half of the patients die within 5 years of the onset of dyspnea, and only 20% respond to therapy. These poor outcome statistics have engendered a certain negative attitude to the management of IP. Most patients receive little intensive therapy early in the disease and obtain little benefit from more intensive therapy when their disease is advanced. As Wells and du Bois stated, something needs to be done about this situation (2).

We should place a high value on both the use of video-assisted thoracoscopic surgery (VATS) as a less invasive approach for lung biopsy (3) and on a recently advocated histological classification that describes' nonspecific interstitial pneumonia (NSIP) ' as one of the histological manifestations of IPF (4). The former will increase the willingness of practitioners to utilize invasive diagnostic procedures. The latter provides notice of the pres-

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ence of a pathological entity that might respond to therapy more readily than usual interstitial pneumonia (UIP). However, only a small proportion of patients with UIP respond to therapy. Such responders are believed to have relatively more reversible changes than non-responders. The reversible changes are mainly inflammatory, while the irreversible changes are fibrotic. A prominent ground-glass pattern on CT (5) and lymphocytosis in bronchoalveolar lavage fluid are indicative of potential reversibility (6). Most IPF patients, however, have irreversible manifestations and a predominant reticular pattern on CT. Also, the rate of progression of IPF is highly variable, so practitioners need markers of progressive disease. The carbon monoxide diffusing capacity (DL_{CO}) (7), oxygen desaturation on exercise (7), and clearance of inhaled Tc-99 m diethylenetriamine pentacetate (DTPA) (8) are currently the most reliable predictors of disease progression, although a large number of other functional and clinical indices are not reliable. However, these examinations require specific facilities and can cause considerable discomfort to the patient.

We found a circulating high molecular weight mucin-like glycoprotein, KL-6 (9), which is classified in human MUC1 mucin (10). KL-6/MUC1 has been evaluated as a marker of disease activity for IP and is common to most IP (11-16). As the result of such clinical studies, KL-6 was approved as a serum diagnostic marker for IP in Japan on June 1, 1999. This review summarizes the clinical role of KL-6/MUC1 for IP.

BIOCHEMICAL AND BIOLOGIC PROPERTIES OF KL-6/MUC1

KL-6 was originally found using a murine monoclonal antibody, KL-6 antibody, which was obtained from a hybridoma established from the splenocytes of a BALB/c mouse immunized with a human pulmonary adenocarcinoma cell line, VMRC-LCR (9). The hybridoma was selected because it produced a monoclonal antibody which reacted with pulmonary adenocarcinoma cell lines and pulmonary epithelial cells, but not with fibroblast cell lines, endothelial cells, or pulmonary interstitium. KL-6 antibody recognized an undefined sialylated carbohydrate chain on a high molecular weight mucin-like glycoprotein (9). The molecule bearing the sialylated carbohydrate was later defined as MUC1 (10). MUC1 (episialin, polymorphic epithelial mucin) is a large glycoprotein (>200 kDa) that has three domains : (i) a cytoplasmic tail ; (ii) a single transmembrane region, and (iii) an extracellular domain. The extracellular domain contains repeating sequences of 20 amino acids. MUC1 has an extended and rigid structure protruding 200-500 nm above the plasma membrane, and is found at the apical surface of normal glandular epithelial cells. However, it often covers the entire cell surface in carcinomas and its level of expression is more than 10 times higher in carcinoma cells than in normal cells. Transfection studies have revealed that MUC1 reduces cell-cell and cell-extracellular matrix interactions, decreases cell aggregation, and prevents E-cadherin-mediated cell-cell adhesion and integrin-mediated cell-extracellular matrix adhesion (17-19). We have also shown that decreased MUC1 expression induces E-cadherin-mediated cell adhesion of breast cancer cell lines in suspension culture (20). These results indicate that membrane MUC1 regulates cell adhesion properties. In several malignancies, MUC1 expression has been shown to be correlated with the metastatic potential of the primary tumor and with a poor prognosis (21-26).

Soluble MUC1 molecules bear sialyl Le^a and sialyl Le^x carbohydrate epitopes that regulate cell-cell interactions (27). KL-6/MUC1 is also chemotactic for human fibroblasts (28). Since this chemotactic activity is completely inhibited by anti-KL- 6 monoclonal antibody, the epitope recognized by this antibody may be crucial for such activity.

TISSUE DISTRIBUTION OF KL-6/MUC1

The distribution of KL-6/MUC1 was examined using an indirect immunostaining method (9, 11). As shown in Table 1, KL-6/MUC1 was expressed by atypical and/or regenerating type II pneumocytes in tissue sections from patients with interstitial pneumonitis as strongly as in adenocarcinoma of the lung, pancreas and breast. However, cells in granulomas were not positive. In the normal lung tissue, type II pneumocytes, respiratory bronchiolar epithelial cells, and serous cells of the bronchial glands showed positive expression of KL-6/MUC 1. On the other hand, type I pneumocytes, goblet cells, and mucous cells of the bronchial glands did not. In the gastro-intestinal tract, KL-6/MUC1 was not expressed by epithelial cells of the stomach, small intestine and large intestine a part from

Table 1. Expression of KL-6 in tissues

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

fundic gland cells. Weak to moderate expression was observed in most malignant cells.

SERUM LEVELS OF KL-6/MUC1 IN VARIOUS DISEASES

The KL-6/MUC1 level was measured by a sandwich enzyme immunoassay using KL-6 antibody as both the capture and tracer antibody (9). From the results in healthy individuals, the cut-off value of serum KL-6/MUC1 was set at 500 or 520 U/ml (9, 15). In benign lung diseases, abnormally high serum levels of KL-6/MUC1 were observed in more than 70% of patients with interstitial pneumonitis, such as IPF, hypersensitivity pneumonitis, radiation pneumonitis, pulmonary sarcoidosis, collagen vascular disease-associated interstitial pneumonitis (CVD-IP), and pulmonary alveolar proteinosis (29, 30), as shown in Table 2 (11-13). Interestingly, KL-6/MUC1 was positive in less than 10% of patients with alveolar pneumonia, including mycoplasma pneumonia. These results indicated that elevation of the serum KL-6/MUC1 level was not influenced by the intensity of inflammation in the peripheral lung tussue. Pulmonary tuberculosis showed a 10-30% positive rate and most positive patients had widespread involvement of the lungs. The KL-6/MUC1 level might be influenced by the severity of pulmonary fibrotic lesions, because there was a significant negative correlation between KL-6/MUC1 and % vital capacity (31). In granulomatous lung diseases, such as pulmonary tuberculosis and sarcoidosis, the increased level of serum KL-6 might originate from the enhanced production of the antigen from proliferated regenerating type II pneumocytes, not from cells in the granuloma.

Adenocarcinoma of the lung, pancreas and breast showed an almost 50% positive rate. However, the positive rate was low for gastric, colon and rectal cancer. Furthermore, the false-positive rate was low for hepatitis, liver cirrhosis, and pancreatitis. These characteristics were different from those of other serum markers, such as CA19-9 and carcinoembrionic antigen (CEA).

CLINICAL USEFULNESS OF KL 6/MUC1 IN INTERSTITIAL PNEUMONITIS

There had been only a small number of studies about the serological evaluation of interstitial pneumonitis

Table 2. Abnormal serum KL-6 level in various diseases

		Positive rate			
		0-10%	10-30%	30-70%	70-100%
Benign diseases	Lung	Alveolar pneumonia Bronchial asthma Chronic bronchitis Emphysema Bronchiectasis Pneumoconiosis	Pulmonary tuberculosis	Diffuse panbronchiolitis Sarcoidosis Pulmonary tuberculosis with widespread involvement of the lungs	Idiopathic pulmonary fibrosis Hypersensitivity pneumonitis Radiation pneumonitis Collagen vascular disease-associated interstitial pneumonitis Pulmonary sarcoidosis Drug-induced pneumonitis Pulmonary alveolar proteinosis
	Others	Hepatitis Liver cirrhosis Pancreatitis Cholecystitis			
Malignant diseases		Gastric cancer Colon cancer Rectal cancer Hepatic cell cancer	Squamous cell carcinoma of the lung	Pulmonary adenocarcinoma Pancreatic adenocarcinoma Breast adenocarcinoma	

until we reported on KL-6 in 1989 (11). DeRemee first reported that serum lactate dehydrogenase (LDH) activity might give useful clinical information for the management of patients with diffuse IP in 1968 (32). However, LDH activity has no specificity for IP, i.e., hepatic damage caused by the treatment of IP and collagen vascular disease in the absence of IP frequently leads to increased LDH activity. Furthermore, the sensitivity is not high. Practitioners see many patients with IPF whose fibrosis progresses slowly and surely, although LDH is always in the normal range. In the assessment of the clinical usefulness of serum markers, we should evaluate their effectiveness for the following ; (i) differential diagnosis, (ii) evaluation of disease activity, (iii) prediction of the prognosis, and (iv) monitoring.

KL-6/MUC1 is useful for distinguishing most IP from other benign lung diseases, such as alveolar pneumonia, and from benign diseases of other organs (11, 15). KL-6/MUC1 can not be used to distinguish a specific IP from the others or to differentiate IP from adenocarcinoma of the lung, pancreas, and breast. However, serum KL-6/MUC1 is useful for the early diagnosis of radiation pneumonitis in patients with lung cancer who are receiving radiation therapy (12, 13).

The levels are significantly higher in patients with active interstitial pneumonitis, such as IPF, hypersensitivity pneumonitis, and CVD-IP, than in those with inactive disease (11, 15). In sarcoidosis, the KL-6/MUC1 level is increased and is significantly influenced by the severity of lung involvement (16). In chronic pulmonary berylliosis, the increase of serum KL-6/MUC1 is correlated with functional and radiologic abnormalities (33).

The progression of IPF in patients who showed KL-6/MUC1 levels $\geq$ 415 U/ml at first diagnosis was significantly faster than that of patients with KL-6/MUC1 levels < 415 U/ml (34).

In patients with rapidly progressing IPF who received high-dose corticosteroid therapy, the decrease of KL-6/MUC1 but not LDH levels was significantly related to a favorable outcome and an increase was related to a poor outcome in the first cycle of treatment when a clinical effect might not be evident (35). These characteristics mean that KL-6/MUC1 is a useful serum indicator for monitoring IP patients.

MECHANISM OF BLOOD UPTAKE FOR KL-6/MUC1

KL-6/MUC1 is present at a high concentration in bronchoalveolar lavage fluid (BALF) (33, 36). The average KL-6/MUC1 level in BALF was reported to be 207$\pm$66 U/ml in nine healthy volun-

teers. In most patients with IP, the levels are increased in BALF, and there is a significant positive correlation between the KL-6/MUC1 levels in serum and BALF. In three healthy volunteers, two patients with IPF, two patients with sarcoidosis, and one patient with hypersensitivity pneumonitis, the KL-6/MUC1 level was estimated in epithelial lining fluid (ELF), which is alveolar fluid from the distal airways and alveoli lavaged by BAL. The average levels in serum, BALF, and ELF were 853 ±1,161,493±457, and 33,683±18,513 U/ml, respectively, and the ELF/serum and ELF/BALF ratios were 231±334 and 104±87, respectively. Since immunohistochemical studies have shown that atypical and/or regenerating pneumocytes in patients with IP express high levels of KL-6/MUC1 (11), the increased KL-6/MUC1 level in ELF may be related to regenerating pneumocytes in the peripheral lung tissue.

In many lung diseases, there is evidence that lung-specific epithelial proteins secreted at high levels into ELF are transferred into the circulation across the air-blood barrier (37, 38). Among patients with chronic beryllium disease, the serum level of KL-6/MUC1 is correlated with albumin in BALF (33). These results suggest that serum KL-6/MUC1 is a useful marker of the permeability of the air-blood barrier, as shown in Fig. 1.

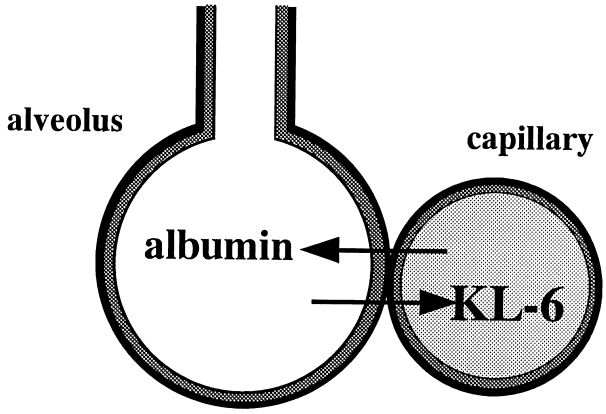

Figure 1. Flux of molecules following an increase in the permeability of the air-blood barrier.

COMPARISON WITH OTHER MARKERS

Sialylated carbohydrate antigens such as CA19-9 (39, 40) and SLX (41, 42), which are sialyl Le^a and sialyl Le^x, respectively, are ligands for E-selectin that have been used as serum markers for IP. However, their diagnostic value was reported to be less than that of KL-6/MUC1 (43).

There are reports that the serum levels of protein markers procollagen N-terminal peptide (44) and type IV collagen 7S (45), indicating collagen synthesis and destruction, respectively, are increased in patients with IP. However, these markers were also of less diagnostic value in the diagnosis of IP than KL-6/MUC1 (46).

IP may be pathophysiologically controlled by cell-cell interactions among immunocytes, fibroblasts, and epithelial and endothelial cells involving cytokines, such as tumor necrosis factor-α (47), basic fibroblast growth factor (48), monocyte chemoattractant protein-1 (MCP-1) (49, 50), and interleukin 8 (51). However, since the serum levels of cytokines may be influenced by the existence of systemic inflammations, the diagnostic value of such cytokines may be lower than that of lung epithelium-specific proteins such as KL-6/MUC1 and surfactant proteins. There are several reports that the serum levels of surfactant proteins A and D (SP-A and SP-D) are useful disease markers for IP (52, 53). As shown in Table 3, it is interesting that KL-6/MUC1 is increased in ELF from a patient with IP (36),

Table 3. Serum markers for interstitial pneumonitis

Marker	Expression	Interstitial pneumonitis		Alveolar pneumonia	Other diseases causing abnormal levels
		ELF	Serum	Serum	
LDH	Cell lysis	↑	↑	→ (~↑)	Any type of tissue damage
KL-6	Constitutive & inducible	↑	↑	→	Pulmonary adenocarcinoma, Pancreatic cancer, breast cancer, primary alveolar proteinosis
SP-A/SP-D	Constitutive & inducible	↓	↑	→	Pulmonary adenocarcinoma, primary alveolar proteinosis
MCP-1	Inducible	↑	↑	→	Inflammatory diseases

while both SP-A and SP-D are decreased (54). The increase of both KL-6/MUC1 and surfactant protein levels in serum is thought to be caused by enhanced permeability of the air-blood barrier in the peripheral lungs. The clinical values of these lung-epithelium specific proteins in the diagnosis and the management of IP should be clarified by comparative prospective studies in the future.

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