

Advances in the study of biomarkers of idiopathic pulmonary fibrosis in Japan

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Summary

Idiopathic pulmonary fibrosis is an intractable disease with a median survival time of 2 to 3 years. Serum levels of Krebs von den Lungen-6 (KL-6), surfactant protein A (SP-A), and surfactant protein D (SP-D) are useful biomarkers for idiopathic pulmonary fibrosis and they are widely used in Japan. Based on clinical use in Japan, a combination of KL-6, SP-A, and SP-D is useful at diagnosing interstitial lung diseases and predicting the prognoses for patients with these diseases. However, the differential diagnosis of idiopathic pulmonary fibrosis from other interstitial lung diseases is still challenging. Several other biomarkers have been identified and are being studied in Japan.

Keywords: Interstitial lung disease, biomarker, KL-6, SP-A, SP-D

1. Introduction

Idiopathic pulmonary fibrosis (IPF) is a specific form of chronic, progressive, fibrosing interstitial pneumonia of unknown etiology (1). Although its exact epidemiology is still unknown, IPF is reported to have no distinct geographical distribution, a greater prevalence in males, and mostly affect individuals who smoke (2). IPF has the characteristic appearance of usual interstitial pneumonia (UIP), which is usually limited to the lungs. It is the most common and severe form of idiopathic interstitial pneumonia (IIP). It is progressive, irreversible, and associated with an extremely poor prognosis. No effective pharmacological therapies have been identified to date. The median survival time for patients with IPF is 2 to 3 years from the time of diagnosis (3).

The prevalence of IPF in Japan is reported to be 11.8 cases per 100,000 population. IPF has been designated as an intractable disease by the Ministry of Health, Labor, and Welfare of Japan, and patients diagnosed with the condition receive full insurance coverage (4). In 1989, Kohno first reported Krebs von den Lungen-6 (KL-6) as a new serum indicator for

IPF (5). Since then, several types of biomarkers were identified and have been studied extensively mostly in Japanese. Several types of biomarkers for IPF have been used clinically in Japan. The current article briefly reviews advances in the study of biomarkers for IPF in Japan.

2. Search strategy

A search of the Medline database was done using a combination of the keywords "idiopathic pulmonary fibrosis" and "biomarker" or a combination of the keywords "idiopathic pulmonary fibrosis" and "marker". All articles related to Japan were reviewed. Searches of the databases of the Japan Science and Technology Agency (J-GLOBAL) and Japan Medical Abstracts Society (JMAS) were also done using a combination of the keyword "idiopathic pulmonary fibrosis" or "idiopathic interstitial pneumonia" and "biomarker." All abstracts were reviewed and the full text of relevant articles was reviewed.

3. Biomarkers used in clinic in Japan

The diagnosis of IPF was once a major challenge to clinicians. According to the American Thoracic Society's (ATS) 2000 guidelines on diagnosis and treatment of IPF (6), the golden standard for diagnosing IPF is by pathological findings. However, a lung biopsy

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is not feasible for many patients suspected of having IPF, so the identification of diagnostic biomarkers for IPF would help both clinicians and patients, particularly in cases where a lung biopsy cannot be obtained. With the development of high-resolution computed tomography (HRCT), the ATS had revised its criteria so that patients with UIP patterns according to HRCT can be diagnosed as having IPF without the need for a lung biopsy (1). This sounds as though the value of biomarkers for IPF has decreased. Because of the potential side effects of many types of therapies for IPF, however, IPF should be carefully be diagnosed, and biomarkers may help in this regard. Biomarkers also have important value in predicting the progression and activity of IPF as well as patient response and prognosis.

3.1. KL-6

KL-6 is the earliest and most intensively studied biomarker of interstitial lung diseases (ILDs) in Japan. KL-6 is a high-molecular-weight glycoprotein classified as a polymorphic epithelial mucin. It is expressed on the surface of various epithelial cells and highly expressed by regenerating type II pneumocytes. It was first investigated as a serum tumor biomarker, but it resulted in a very high rate of false positives in patients with pulmonary fibrosis (7). A later study (5) identified it as a biomarker for ILDs.

KL-6's value in diagnosing patients with ILDs compared to healthy controls has been demonstrated by several studies (8,9). The clinical cut-off value for distinguishing patients with ILDs from healthy subjects and patients with lung diseases other than ILDs had been set at the level of 500 U/mL (10). That said, serum levels of KL-6 were also significantly higher in patients with active pulmonary tuberculosis, with a false positive rate of 28% (11). A study (12) found that serum levels of KL-6 were elevated in 70-100% of patients with ILDs and therefore cannot be used to differentiate IPF from other interstitial pneumonias. Data from a study (13) with a large Japanese sample were used to create Figure 1 to show the different rates at which KL-6 tests positive based on a cut-off value of 500 U/mL; data are from 225 patients with various lung diseases and 200 healthy individuals.

Serum levels of KL-6 are also related to therapeutic efficacy. A study treated 14 Japanese patients with rapidly progressive IPF (14) with high-dose corticosteroid pulse therapy and it followed all of the patients for at least 3 weeks. Patients whose serum levels of KL-6 significantly decreased had a better response and better long-time survival. Several studies (15,16) found that serum levels of KL-6 were related to the extent and activity of IPF.

Serum levels of KL-6 are also related to the prognosis for a patient with IPF. Elevated serum levels

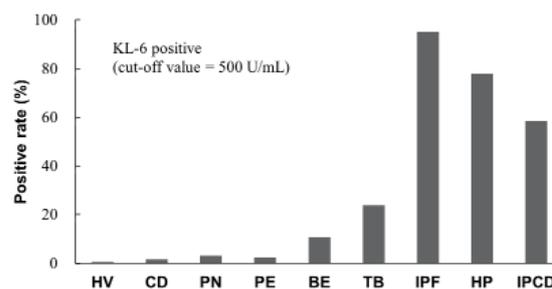


Figure 1. Different rates at which KL-6 tests positive in different lung diseases (Ref. 13). Abbreviations: HV, health volunteer; CD, collagen disease; PN, ordinary pneumonia; PE, pulmonary emphysema; BE, bronchiectasis; TB, pulmonary tuberculosis; HP, hypersensitivity pneumonia; IPCD, interstitial pneumonia with collagen disease.

of KL-6, and especially levels over 1,000 U/mL, upon initial measurement are reported to be related to increased mortality (17). The progression of IPF is significantly faster in patients with KL-6 levels of 1,000 U/mL or higher upon initial measurement than in patients with KL-6 levels below 1,000 U/mL (18).

3.2. Surfactant proteins

Surfactant protein A (SP-A) and surfactant protein D (SP-D) are both water-soluble lung-specific proteins. They are also collectins, a subgroup of the C-type lectin family. They are mainly produced by alveolar epithelial type II pneumocytes and Clara cells within the lungs. Elevated serum levels of SP-A in patients with IPF were first noted in 1993 (19). Serum levels of SP-A in patients with IPF and pulmonary alveolar proteinosis (PAP) were found to be significantly higher than those in healthy volunteers and patients with other non-interstitial lung diseases. A study found that serum levels of SP-A indicate the activity of IPF (20). Another study (12) found that SP-A was better at distinguishing IPF from other ILDs. That study found that serum levels of SP-A in patients with UIP were significantly higher than in patients with non-specific interstitial pneumonia (NSIP).

Elevated serum levels of SP-D were first found to be a possible marker for IPF in 1995 (21). In that study, serum levels of SP-D were significantly higher in patients with IPF, IPCD, and PAP. Serum levels of SP-D were significantly related to the activity of IPF and IPCD. They also closely indicated the severity of PAP.

Serum levels of SP-A and SP-D were also reported to indicate the prognosis for patients with IPF (22). In that study, serum levels of SP-A and SP-D were significantly correlated with the extent of alveolitis. Serum levels of SP-D were also related to the extent of parenchymal collapse on CT and deterioration in pulmonary function. Patients with higher levels of SP-A

Table 1. Rates at which SP-A and SP-D test positive in different lung diseases (Ref. 23)

Rate of testing positive (%)	SP-A	SP-D
70-100	IPF	IPF, IPCD, PAP
50-70	PAP	
30-50	IPCD	
10-30	SAR, PN, DPB, TB, PC	SAR, PN, DPB, TB, PE
0-10	BA, PE	BA

Abbreviations: IPF, idiopathic pulmonary fibrosis; IPCD, interstitial pneumonia with collagen disease; PAP, pulmonary alveolar proteinosis; SAR, pulmonary sarcoidosis; PN, ordinary pneumonia; DPB, diffuse panbronchiolitis; TB, pulmonary tuberculosis; PE, pulmonary emphysema; BA, bronchial asthma.

and SP-D had a worse 3-year survival rate.

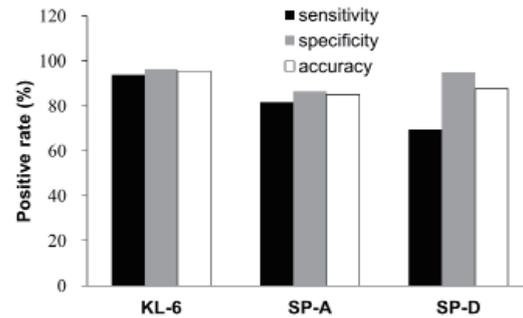
A study (23) of serum levels of SP-A and SP-D in a large Japanese sample set the cut-off value of serum SP-A and SP-D at 48.3 ng/mL and 109 ng/mL, respectively. The study described serum levels of SP-A and SP-D in 87 patients with IPF, 303 patients with HV, and 215 patients with other lung diseases (Table 1).

Many studies have investigated the levels of KL-6, SP-A, and SP-D in bronchoalveolar lavage fluid (BALF). Levels of KL-6 in BALF were reported to be consistent with those in serum (5,8,9), whereas the levels of SP-A and SP-D in BALF were reported to be inversely correlated with levels in serum (19-21). Although the levels of KL-6, SP-A and SP-D in BALF are potential biomarkers for IPF, the inconvenience of obtaining BALF has resulted in their limited use clinically.

KL-6, SP-A, and SP-D have been recognized by Japan's National Health Insurance System as diagnostic markers for ILDs in 1999. KL-6 has been used in clinical practice for more than 10 years in Japan, and KL-6 levels are examined in more than 2,000,000 samples per year in Japan (24). Kits to measure KL-6, SP-A, and SP-D have been successfully developed by reagent manufacturers (Picolumi KL-6: Sanko Junyaku Co., Tokyo, Japan; SP-A test-F: Kokusai Shiyaku Co., Hyogo, Japan; SP-D kit Yamasa; YamasaShoyu Co., Tokyo, Japan). KL-6, SP-A, and SP-D have been included a fourth edition of clinical diagnostic criteria for IPF from a Study Group on IPF of the Ministry of Health, Labor, and Welfare, and all three markers are widely used in many hospitals in Japan.

3.3. The comparative diagnostic value of KL-6, SP-A, and SP-D

A study (25) compared the usefulness of KL-6, SP-A, and SP-D in clinically diagnosing ILDs (Figure 2). KL-6 had the highest specificity and sensitivity for ILDs, but SP-A had the best ability to differentiate IPF from other ILDs (12). To date, KL-6, SP-A, and SP-D have proven value at diagnosing ILDs, but the

**Figure 2. Different diagnostic values of KL-6, SP-A, and SP-D for ILDs (Ref. 25).**

differential diagnosis of ILDs is still difficult. Thus, a combination of KL-6, SP-A, and SP-D is needed to diagnose IPF.

4. Potential biomarkers being studied in Japan

4.1. Vascular endothelial growth factor (VEGF)

Since many interstitial lung diseases are associated with aberrant angiogenesis, the key angiogenesis regulator VEGF has been investigated as a potential biomarker for IPF. A study in the UK (26) found that serum levels of VEGF were not related to IPF. A study in Japan (27) grouped patients depending on the alveolar-arterial difference in oxygen (AaDO₂) and the study found that there were significant differences in the serum levels of VEGF in different groups with a certain AaDO₂ and healthy volunteers. IPF patients with serum levels of VEGF above the median tended to have a worse survival than those with serum levels of VEGF below the median.

4.2. YKL-40

YKL-40, also known as human cartilage glycoprotein 39, is a chitinase-like protein. Its roles in tissue remodeling and fibrosis have been investigated. A study in Japan (28) found that YKL-40 levels in serum and BALF were significantly higher in patients with IPF than in healthy controls. Serum levels of YKL-40 are significantly related to serum levels of KL-6. This means that YKL-40 may be a potential biomarker for IPF.

4.3. Osteopontin

Osteopontin is a type of glycoprotein involved in immune response and tissue repair. Its role in promoting the migration, adhesion, and proliferation of fibroblasts has been demonstrated in a mouse model of bleomycin-induced pulmonary fibrosis (29). A study in 2005 (30) found that levels of osteopontin in plasma were significantly higher in patients with ILDs than in those

with sarcoidosis or in healthy controls. No significant differences in the plasma concentration of osteopontin were noted in patients with IPF or other ILDs.

4.4. Periostin

Periostin is a type of protein known for its roles in the maintenance and development of bones, teeth, and the heart. A recent study (31) found that serum levels of periostin were significantly higher in patients with IPF than in healthy volunteers and patients with cryptogenic organizing pneumonia (COP). Furthermore, periostin levels were inversely correlated with patients' pulmonary function.

4.5. Napsin A

Napsin A, which is expressed in type II pneumocytes and alveolar macrophages, is a type of aspartic proteinase. A recent study (32) found that serum levels of napsin A were significantly higher in patients with IPF than in healthy controls. Serum levels of napsin A are also correlated with the severity of disease.

4.6. Connective tissue growth factor (CCN2)

CCN2 (also known as CTGF) is a type of secreted peptide that mainly serves to produce extracellular matrix and perform other profibrotic activities. Plasma levels of CCN2 were reported to be significantly higher in patients with IPF than in patients with non-IPF IIPs and healthy controls (33). That study also reported a correlation between plasma CCN2 and changes in the 6-month forced vital capacity (FVC).

4.7. S100A9

S100A9 is a type of calcium-binding protein also known as calgranulin B. It is reported to be a useful marker for non-infectious inflammatory diseases. A study of BALF (34) found that S100A9 levels were significantly higher in patients IPF than in patients with other IPDs and healthy volunteers.

4.8. Heat shock protein 47 (HSP47)

HSP47 is a type of collagen-specific molecular chaperone that mainly functions in biosynthesis and the secretion of collagen. Serum levels of HSP47 in cases of acute exacerbation of IPF (AE-IPF) are reported to be significantly higher than those in cases of stable IPF (35). Details are shown in Table 2.

5. Conclusion

This review briefly presented advances in the study of biomarkers for IPF in Japan. Although no specific

Table 2. Details on other biomarkers being studied in Japan

Biomarker	Location	Difference between ILDs and HV	Difference between IPF and other ILDs	Correlation with disease activity or severity	Correlation with KL-6	Correlation with SP-A or SP-D	Number of HV	Number of IPF	Other IIPs	Ref.
VEGF	serum	Yes*	--	HRCT	--	--	43	41	--	(27)
YKL-40	serum	Yes	--	DLco and PaO ₂	Yes	--	41	63	--	(28)
	BALF	Yes	--	--	--	No	16	18	--	(28)
Osteopontin	plasma	Yes	No	PaO ₂	Yes	--	20	8	8	(29,30)
Periostin	serum	Yes	No	Pulmonary function	--	--	--	51	41	(31)
Napsin A	serum	Yes	--	Forced vital capacity	--	--	20	20	--	(32)
CCN2	plasma	Yes	Yes	Forced vital capacity	--	--	101	33	14	(33)
S100A9	BALF	Yes	Yes	--	--	--	23	28	70	(34)
	serum	No	No	Exacerbation vs. stable	--	--	23	28	70	(34)
HSP47	serum	--	--	--	--	--	--	53	--	(35)

Abbreviations: DLco, diffusing capacity for carbon monoxide; PaO₂, partial pressure of oxygen. *Differences were significant for the subgroup with a certain alveolar-arterial difference in oxygen.

biomarkers for IPF had been identified to date, KL-6, SP-A, and SP-D have shown good specificity and sensitivity at diagnosing ILDs. Of the three, KL-6 has the best specificity and sensitivity at diagnosing ILDs while SP-A is specifically able to differentiate IPF from other ILDs. Based on clinical use in Japan, the combination of KL-6, SP-A, and SP-D provides certain value at diagnosing IPF and predicting the prognosis for patients with the condition.

That said, the differentiation of IPF from other ILDs is still challenging. Biomarkers should help to overcome this challenge. Several other biomarkers have also been identified, but further research and evaluation are still needed.

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