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# Impact of serum SP-A and SP-D levels on comparison and prognosis of idiopathic pulmonary fibrosis

## A systematic review and meta-analysis

Kai Wang, MD<sup>b</sup>, Qing Ju, MD<sup>a</sup>, Jing Cao, MD<sup>a</sup>, Wenze Tang, MPH<sup>c</sup>, Jian Zhang, MD<sup>a,\*</sup>

### Abstract

**Background and objective:** Idiopathic pulmonary fibrosis (IPF) has a poor prognosis in general; however, it is heterogeneous to detect relative biomarkers for predicting the disease progression. Serum biomarkers can be conveniently collected to detect and help to differentially diagnose IPF and predict IPF prognosis. This meta-analysis aimed to evaluate the use of serum surfactant proteins A and D (SP-A and SP-D) for differential diagnosis and prognosis of IPF.

**Methods:** Relevant articles were searched in *PubMed*, *Embase*, and *Chinese National Knowledge Infrastructure* databases and reviewed by 2 independent readers. Standard mean difference (SMD) and 95% confidence interval (CI) were calculated to assess the difference in serum levels of SP-A/D among patients with IPF, when compared to patients with non-IPF interstitial lung disease (ILD), pulmonary infection, and healthy control. Hazard ratio (HR) and 95% CI were used to compare the relative risk of mortality.

**Results:** Twenty-one articles (totalling 1289 IPF patients) were included in final meta-analysis. Serum SP-A levels were significantly higher in patients with IPF than in patients with non-IPF ILD (SMD: 1.108 [0.584, 1.632],  $P < .001$ ), or pulmonary infection (SMD: 1.320 [0.999, 1.640],  $P < .001$ ) and healthy controls (SMD: 2.802 [1.901, 3.702],  $P < .001$ ). There was no significant difference in serum SP-D levels between patients with IPF and those with non-IPF ILD patients (SMD: 0.459 [-0.000, 0.919],  $P = .050$ ). Serum SP-D levels were significantly higher in patients with IPF than in patients with pulmonary infection (SMD: 1.308 [0.813, 1.803],  $P < .001$ ) and healthy controls (SMD: 2.235 [1.739, 2.731],  $P < .001$ ). Risk of death in patients with IPF and elevated serum SP-A was increased 39% compared to patients with low SP-A groups. Elevated SP-D increased risk by 111% when compared to low SP-D. In acute exacerbation of IPF, serum SP-A/D were higher than those in stable stage. The comparisons and prognosis might be different in Asian and Caucasian patients.

**Conclusions:** Serum SP-A/D detection might be useful for differential diagnosis and prediction of survival in patients with IPF.

**Abbreviations:** 95% CI = 95% confidence interval, AE = acute exacerbation, AHP = acute hypersensitivity pneumonitis, ARDS = acute respiratory distress syndrome, ATS = American Thoracic Society, CBD = chronic beryllium disease, CCL18 = CC-chemokine ligand 18, CHP = chronic hypersensitivity pneumonitis, CNKI = Chinese National Knowledge Infrastructure, COPD = chronic obstructive pulmonary disease, CPI = composite physiologic index, CRP = clinical, radiological, and physiological, CTD-IP = connective tissue disease-associated interstitial pneumonia, CVD-IP = collagen vascular disease-associated interstitial pneumonia, Dlco = diffusing capacity of the lung for carbon monoxide, FEV<sub>1</sub> = forced expiratory volume in 1 second, FVC = forced vital capacity, GAP = gender, age, and two lung physiology variables (FVC and Dlco), HR = hazard ratio, HRCT = high-resolution computed tomography, ILD = interstitial lung disease, I-NSIP = idiopathic non-specific interstitial pneumonia, IPF = idiopathic pulmonary fibrosis, KL-6 = Krebs von den Lungen-6, MMP-7 = matrix metalloproteinase-7, PAP = pulmonary alveolar proteinosis, PSS = progressive systemic sclerosis, SMD = standard mean difference, SP-A = surfactant protein-A, SP-D = surfactant protein-D, SSC-ILD = scleroderma-associated interstitial lung disease, UIP = usual interstitial pneumonia.

**Keywords:** idiopathic pulmonary fibrosis, meta-analysis, surfactant protein-A, surfactant protein-D

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## 1. Introduction

Idiopathic pulmonary fibrosis (IPF) is a chronic and progressive fibrotic interstitial lung disease (ILD) of unknown etiology.<sup>[1]</sup> Several studies have indicated that the worldwide incidence of IPF seems to be increasing, especially in Europe and North America.<sup>[2]</sup> IPF is histologically characterized by the usual interstitial pneumonia (UIP) pattern. True UIP patterns are also found in rheumatoid lung, asbestosis, and, rarely, in sarcoidosis.<sup>[3]</sup> The primary diagnostic method of IPF in current clinical practice is the high-resolution computed tomography (HRCT), lung biopsy.<sup>[4]</sup> Incorporating with noninvasive biomarkers (eg, matrix metalloproteinase-7 [MMP7], surfactant protein-D [SP-D]) would provide additional evidence for HRCT and clinical history when distinguish IPF from other ILDs, and they may have the ability to predict risk for acute exacerbation (AE) of IPF patients.<sup>[5]</sup>

The median survival of patients with IPF is 3 years and the 5-year survival rate is 20% to 40%.<sup>[6,7]</sup> Pathologist features,<sup>[8]</sup> clinical parameters,<sup>[9,10]</sup> and prediction models,<sup>[11,12]</sup> including lung function test, the 6-minute walk test, and the clinical, radiological, and physiological (CRP) scoring system, have been used as prognostic tools. However, since the clinical course of individual patients is highly variable and unpredictable, these physiologic parameters have limitations.<sup>[13]</sup> Alternatively, non-invasive biomarkers may be helpful in identifying patients with IPF and predicting long-term outcome.

Type II counterproductive molecules, Krebs von den Lungen-6 (KL-6), surfactant protein-A (SP-A), SP-D, MMP-7, and CC-chemokine Gilligan 18 (CCL18) have emerged as potential diagnostic and prognostic biomarkers of IPF.<sup>[14]</sup> SP-A and SP-D are hydrophobic, collagen-containing calcium-dependent lectins, with a range of nonspecific immune functions at pulmonary and cardiopulmonary sites.<sup>[15]</sup> SP-A and SP-D play crucial roles in the pulmonary immune response, and are secreted by type II pneumocytes, nonciliated bronchiolar cells, submucosal glands, and epithelial cells of other respiratory tissues, including the trachea and bronchi. SP-D is important in maintaining pulmonary surface tension, and is involved in the organization, stability, and metabolism of lung parenchyma.<sup>[16]</sup>

Serum SP-A and SP-D have been identified as biomarkers for pulmonary diseases, including acute respiratory distress syndrome (ARDS),<sup>[17]</sup> chronic obstructive pulmonary disease (COPD),<sup>[18]</sup> and progressive systemic sclerosis (PSS).<sup>[19]</sup> Previous studies had reported that serum SP-A and/or SP-D were significantly different from IPF and some non-IPF ILD patients (eg, rheumatoid arthritis-associated ILD).<sup>[5]</sup> Meanwhile, SP-A and SP-D levels in BALF may also play a role in differential diagnosis from IPF and other ILDs (eg, sarcoidosis)<sup>[20]</sup> and predicting survival in IPF patients.<sup>[21]</sup> In IPF patients, serum SP-A or SP-D also plays helpful roles in differential diagnosis<sup>[22]</sup> as well as predicting prognosis.<sup>[14]</sup> Before this study, the potential role of serum SP-A and SP-D as diagnostic and prognostic biomarkers in patients with IPF had not been studied by meta-analysis. The goal of this study was to evaluate the role of SP-A and SP-D in the diagnosis and prognosis of IPF.

## 2. Materials and methods

### 2.1. Search strategy and ethics permission

In March 2017, we performed systematic searches in *PubMed*, *EMBASE*, and the *Chinese National Knowledge Infrastructure* (CNKI). We used the following search terms: “Surfactant

protein” or “SP” and “Idiopathic pulmonary fibrosis” or “IPF” or “Interstitial lung disease” or “Pulmonary fibrosis.” We also identified relevant publications by reviewing the reference of the search results. The meta-analysis was approved by the Ethics Committee of the Xijing Hospital, the First Affiliated Hospital of the Fourth Military Medical University.

### 2.2. Inclusion and exclusion criteria

Included studies met the following criteria: SP-A and/or SP-D were evaluated in human subjects for the differential diagnosis and prognosis of IPF. In comparison part, patients with IPF were compared to at least one reference group (non-IPF patients or healthy subjects). Studies were written in English or Chinese. A definitive diagnosis of IPF was evident by clinical features, chest HRCT, laboratory findings, and/or surgical lung biopsy. Data, for example, serum levels of SP-A and SP-D, and hazard ratios (HRs), were available from the reviewed articles. In addition, for inclusion in the prognostic evaluation of SP-A/SP-D in patients with IPF, studies had to have a defined patient follow-up schedule, and include death as an endpoint. Studies were excluded if the data were unavailable after attempts to contact author.

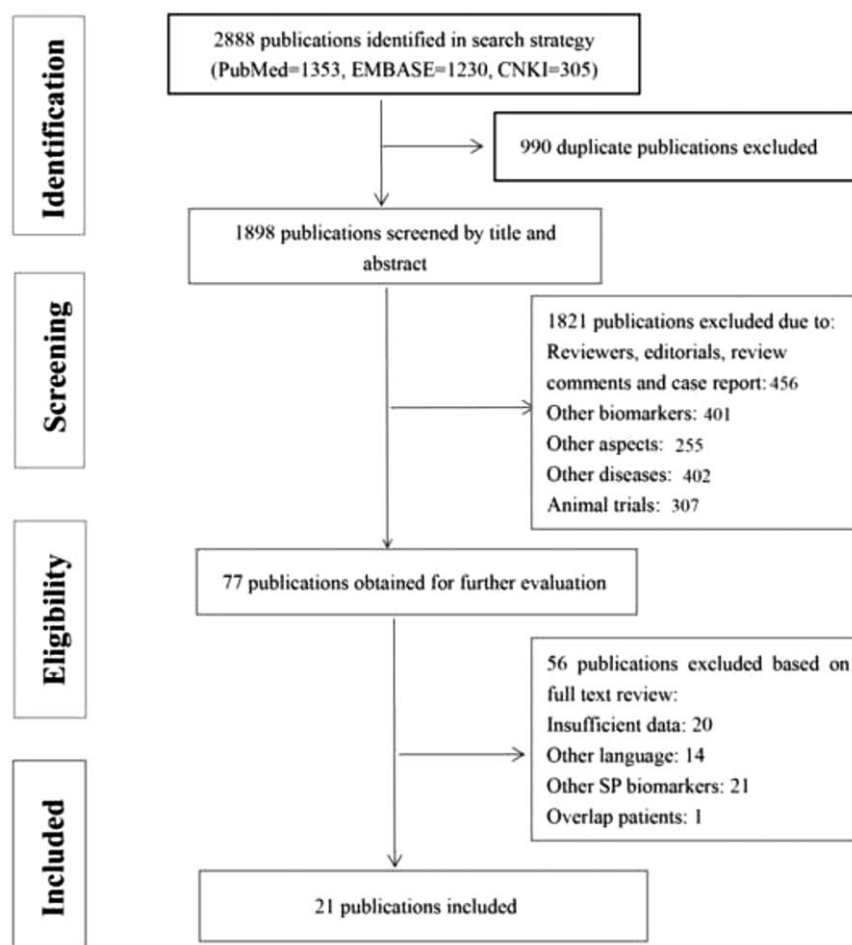
### 2.3. Data extraction and quality assessment

Two reviewers independently screened titles and abstracts for potentially eligible articles. Full texts of articles were obtained, and 2 reviewers independently determined eligibility. Discrepancies were resolved by a discussion or with a third reviewer. For every eligible study, we extracted the following information: first author, year of publication, number and sex of patients with IPF, patient smoking history, study endpoints, study methods, and epidemiological study methods. When the association between SP-A/SP-D and IPF mortality was studied, cutoff values for SP-A and SP-D were also collected. When study data for meta-analysis were not available in the article, we attempted to contact author (s) to obtain original data. Some original data were not available. In this case, we derived mean and standard deviation using median and range from graphs and curve diagrams, according to the method recommended by Hozo et al.<sup>[23]</sup> In one case, the relative risk of mortality data was presented as Kaplan–Meier curves, not HR and 95% confidence interval (CI).<sup>[24]</sup> In this case, we calculated the HR and 95% CI according to the method described by Parmar et al.<sup>[25]</sup> In the analysis of the association of SP-A/SP-D with death of patients with IPF, there was an overlap of subjects in the studies of Greene et al.<sup>[26]</sup> and Kinder et al.<sup>[27]</sup>. To avoid sampling bias, we included only the Greene et al.'s<sup>[26]</sup> study in the analysis.

We used Newcastle–Ottawa quality assessment scale of case control studies to evaluate the quality of these studies.<sup>[28]</sup> A study could be awarded a maximum of one star for each numbered item within the Selection and Exposure categories. A maximum of 2 stars could be given for comparability. A study with >5 stars was included in the meta-analysis.

### 2.4. Statistical analysis

Statistical analyses were conducted using STATA software (Stata Corp, TX, version 12.0). All tests were 2-tailed with the significance level set at  $P < .05$ . For analyses of SP-A/SP-D as diagnostic and prognostic biomarkers, Cochran's  $Q$  test was used to assess between-study heterogeneity. Heterogeneity was



**Figure 1.** PRISMA processing map: 2888 publications identified in search strategy (*PubMed* 1353, *EMBASE* 1230, *CNKI* 305). After screening and eligibility process, only 21 articles met the including criteria and were included in ultimate analysis.

presented in the form of the inconsistency index,  $I^2$ , ranging from 0% to 100%.<sup>[29]</sup> To assess heterogeneity, the value of  $I^2$  was divided into 3 groups: <25%, 25% to 75%, and >75%, corresponding to low, moderate, and high heterogeneity. If statistical heterogeneity existed, the potential causes were estimated using sensitivity and subgroup analysis. A random-effect model was applied to reduce the effect of heterogeneity. To estimate the potential publication bias, we used a funnel plot and Egger's test. An asymmetric funnel plot and a  $P < .05$  on the Egger's test identified the existence of publication bias.

When assessing the diagnostic effect of SP-A/SP-D, we treated the biomarkers as continuous, and gave an estimate of the combined overall effect size utilizing standard mean difference (SMD) in the random-effects model. For the evaluation of SP-A/SP-D as prognostic biomarkers of mortality, we used the Z test to test the pooled HR, and presented both a Z and P value. We compared the weight of the individual article in influencing the pooled HR, and the 95% CI. We also did subgroups analysis, stratified by race, for both diagnostic and prognostic analysis.

### 3. Results

#### 3.1. Study inclusion, characteristics, and study quality

We identified articles from three databases: *PubMed* ( $n = 1353$ ), *EMBASE* ( $n = 1230$ ), and *CNKI* ( $n = 305$ ). Nine hundred and

ninety duplicate articles were removed. We excluded 1877 articles that failed to meet the inclusion criteria. A final group of 21 articles met the eligibility criteria<sup>[5,14,22,24,26,30-45]</sup> (Fig. 1). Seven of 21 articles evaluated the prognosis of IPF as an outcome and the observed endpoint was death (Table 1). Eighteen studies compared the serum level of SP-A/SP-D in patients with IPF to patients with non-IPF ILD or pulmonary infection, or healthy controls. All 21 articles were retrospective studies, and none of these studies reported blindness. No relevant prospective studies or large-scale meta-analyses were identified. The main characteristics of the 21 articles are summarized in Table 1. A total of 1289 patients with IPF were included for analyses. The majority of patients (>58%) were Asian. The date of publication ranged from 1992 to 2016. Data reflecting severity included FEV<sub>1</sub>% predicted, FVC% predicted, and DLco% predicted. DLco% predicted values varied from 39.1% to 74.9%. The number of male patients was greater than the number of female patients.

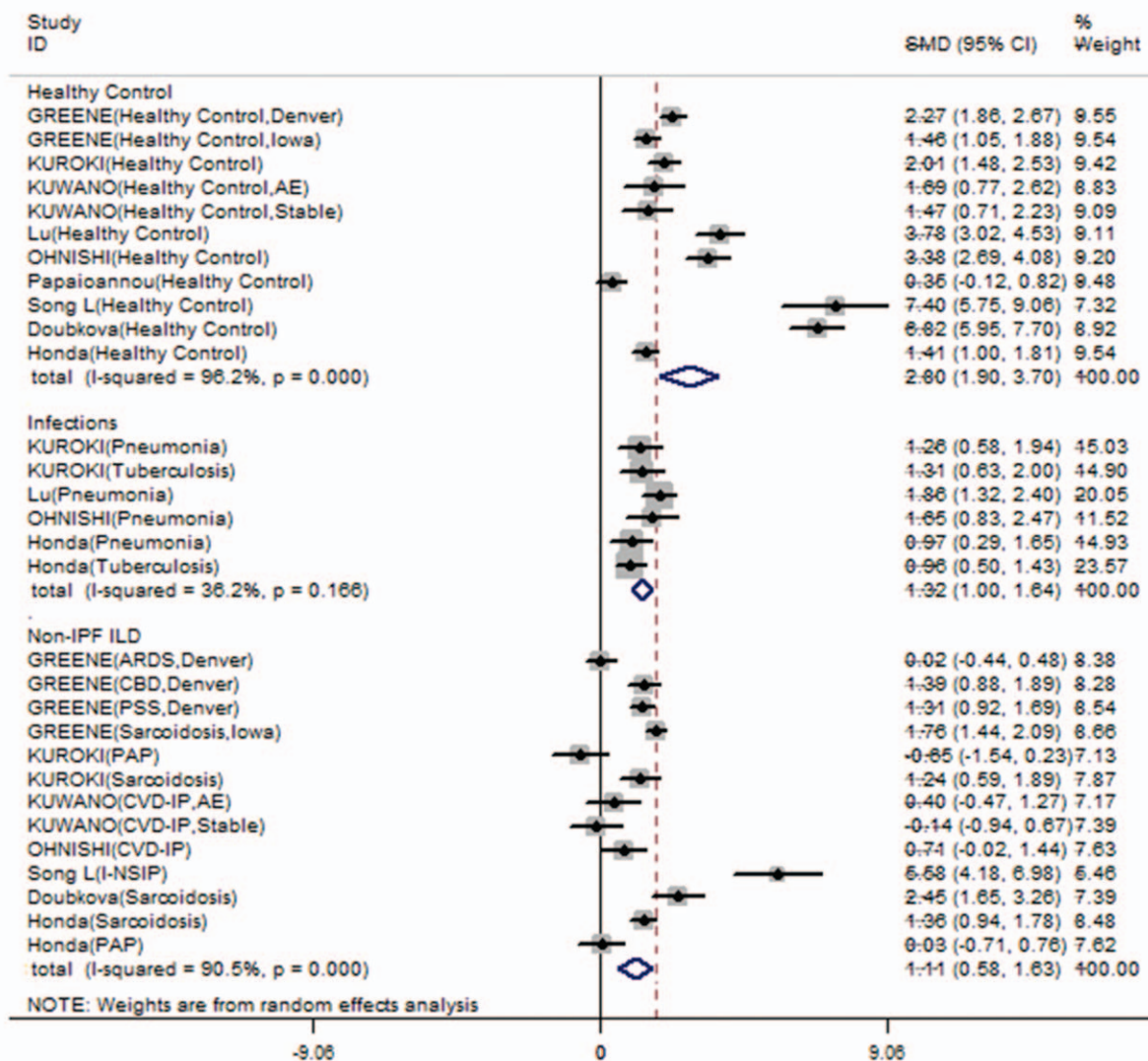
According to study quality assessment criteria (Supplemental Table 1, <http://links.lww.com/MD/B723>), the study participation was adequate and the baseline study sample was completely delineated in all included articles. Every study that assessed the prognostic effect of SP-A/SP-D included patient follow-up. All studies assessed SP-A and (or) SP-D levels; however, different cutoff values were used, as described in Table 1. SP-A cutoff values ranged from 53 to 83.5 except Papaioannou's (280), and SP-D cutoff values ranged from 225 to 287 except Barlo's (460).

**Table 1**

**Baseline information for all articles included.**

Baseline information										IPF patients			Non-IPF control			IPF AE		IPF prognosis			
Author	Year	Race	Quality assessment	IPF patients	Mean age (y)	Male/female	Former or current smokers	% Pred FEV <sub>1</sub>	% Pred VC	% Pred DLco	Non-IPF patients	IPF AE patients	End point	Death rate	SP-A	SP-D	HR estimate	Follow-up period			
Hamai	2016	Asian	6	65	69.3	50/15	N/A	N/A	74.5	47.1	I 31/HC 101	N/A	Death	53.0	225.0	UV/MV	Median 31 mo				
Papadonnoou	2016	Caucasian	5	62	72.0	43/19	57	76.4	68.3	46.8	HC 25	No AE	Death	280.0	287.0	UV	1 y				
Song	2012	Asian	5	118	62.8	95/23	88	N/A	75.0	65.0	Only IPF patients included	N/A	Death	80.3	N/A	UV/MV	Median 24 mo				
Barlo	2009	Caucasian	6	72	62.9	56/16	53	77.0	75.0	43.0	HC 305	N/A	Death	N/A	460.0	UV/MV	39				
Collard	2009	Asian	5	67	N/A	52/15	58	N/A	Stable 84.0 AE 75.0	Stable 74.0 AE 64.0	Only IPF patients included	Stable 20/AE 47	Death	N/A	N/A	N/A	N/A	N/A			
Takahashi	2006	Asian	5	82	N/A	65/17	N/A	N/A	N/A	N/A	Only IPF patients included	N/A	Death	83.5	253.0	UV/MV	N/A				
Greene (Denver)	2002	83.3% Caucasian	6	142	48.4	93/59	122	N/A	N/A	N/A	ILD 77/HC 46	N/A	Death/lung transplant	N/A	N/A	UV	N/A				
Greene (Iowa)	2002	89.9% Caucasian	6	68	62.5	35/33	48	N/A	N/A	N/A	ILD 160/HC 49	N/A	Death/lung transplant	N/A	N/A	UV	N/A				
Doubková	2016	Caucasian	6	18	68.5	8/10	7	85.0	68.0	52.0	ILD 25/HC 155	N/A	Prognosis information not available	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis			
Möllerken	2016	Caucasian	6	91	68.0	65/26	28	69.0	68.0	47.0	ILD 22/HC 31	N/A	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis			
White	2016	Caucasian	6	86	63.0	62/24	60	68.0	62.0	44.0	ILD 41/HC 127	N/A	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis			
Lu Wei	2016	Asian	6	38	60.6	23/15	N/A	N/A	N/A	N/A	I 38/HC 38	N/A	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis			
Song Lei	2015	Asian	6	18	51.0	10/8	6	N/A	56.0	47.0	ILD 22/HC 28	N/A	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis			
Kennedy	2015	Caucasian	6	13	72.6	10/3	11	83.4	83.3	39.1	ILD 6/HC 10	N/A	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis			
Okamoto	2015	Asian	5	54	66.0	42/12	44	N/A	91.3	56.3	ILD 206	N/A	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis			
Okuda	2015	Asian	6	30	72.3	25/5	23	N/A	Stable 78.4	Stable 74.9	ILD 7	Stable 30/AE 11	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis			
Kakugawa	2013	Asian	5	53	N/A	39/14	44	N/A	N/A	N/A	Only IPF patients included	Stable 33/AE 20	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis			
Kuwano	2002	Asian	6	35	N/A	29/6	29	N/A	Active 56.0 Inactive 73.0	Active N/A Inactive 57.0	ILD 17/HC 13	Active 12/inactive 23	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis			
Omishi	2002	Asian	5	21	56.2	15/6	N/A	N/A	N/A	N/A	ILD 12/1 12/HC 70	N/A	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis			
Honda	1995	Asian	5	59	N/A	N/A	N/A	N/A	N/A	N/A	ILD 56/1 35/HC 179	N/A	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis			
Honda	1994	Asian	5	65	N/A	N/A	N/A	N/A	N/A	N/A	ILD 55/1 38/HC 53	N/A	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis			
Kuroki	1992	Asian	6	32	N/A	N/A	N/A	N/A	N/A	N/A	ILD 22/1 28/HC 57	N/A	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis	Research irrelevant to prognosis			

ATS = ATS criteria or ATS/ERS statement or ATS/ERS/JRS/ALAT statement, CCP = clinical features, CT scan, and pathology, HC = healthy control, HR = hazard ratio, I = infection, ILD = non-IPF ILD, MV = multivariate analysis, N/A = not available, Pathology = thoracoscopic lung biopsy, autopsy, UV = univariate analysis.



**Figure 2.** Serum SP-A levels comparisons between IPF and other groups (non-IPF ILD, pulmonary infections or healthy controls). Serum SP-A levels in IPF patients were higher than non-IPF ILD patients (SMD: 1.108 [0.584, 1.632], Z value=4.15, P<.001), pulmonary infections (SMD: 1.320 [0.999, 1.640], Z value=8.07, P<.001) or healthy controls (SMD: 2.802 [1.901, 3.702], Z value=6.10, P<.001).

Moreover, 5-year survival rates based on these cutoff points were listed in Supplemental Table 2, <http://links.lww.com/MD/B723>.

### 3.2. Meta-analysis

**3.2.1. Comparative analysis.** Eighteen publications that reported serum levels of SP-A and (or) SP-D were included in these analyses. We compared the serum levels of SP-A and (or) SP-D of patients with IPF to patients with non-IPF ILD or pulmonary infection, or healthy controls (Figs. 2 and 3).

Serum SP-A levels among patients with IPF were significantly higher than patients with non-IPF ILD (SMD: 1.108 [0.584, 1.632], Z value=4.15, P<.001, I<sup>2</sup>=90.5%, P<.001); patients with pulmonary infection (SMD: 1.320 [0.999, 1.640], Z value=8.07, P<.001, I<sup>2</sup>=36.2%, P=.166); and healthy controls (SMD: 2.802 [1.901, 3.702], Z value=6.10, P<.001, I<sup>2</sup>=96.2%, P<.001; Fig. 2). The I<sup>2</sup> values suggest high heterogeneity across healthy and non-IPF ILD comparisons. Serum SP-A levels in patients with ILD and pulmonary infections were significantly higher than controls (SMD: 2.200 [1.627, 2.773]; 1.097 [0.420, 1.774], Supplemental Fig 1, <http://links.lww.com/MD/B724>).

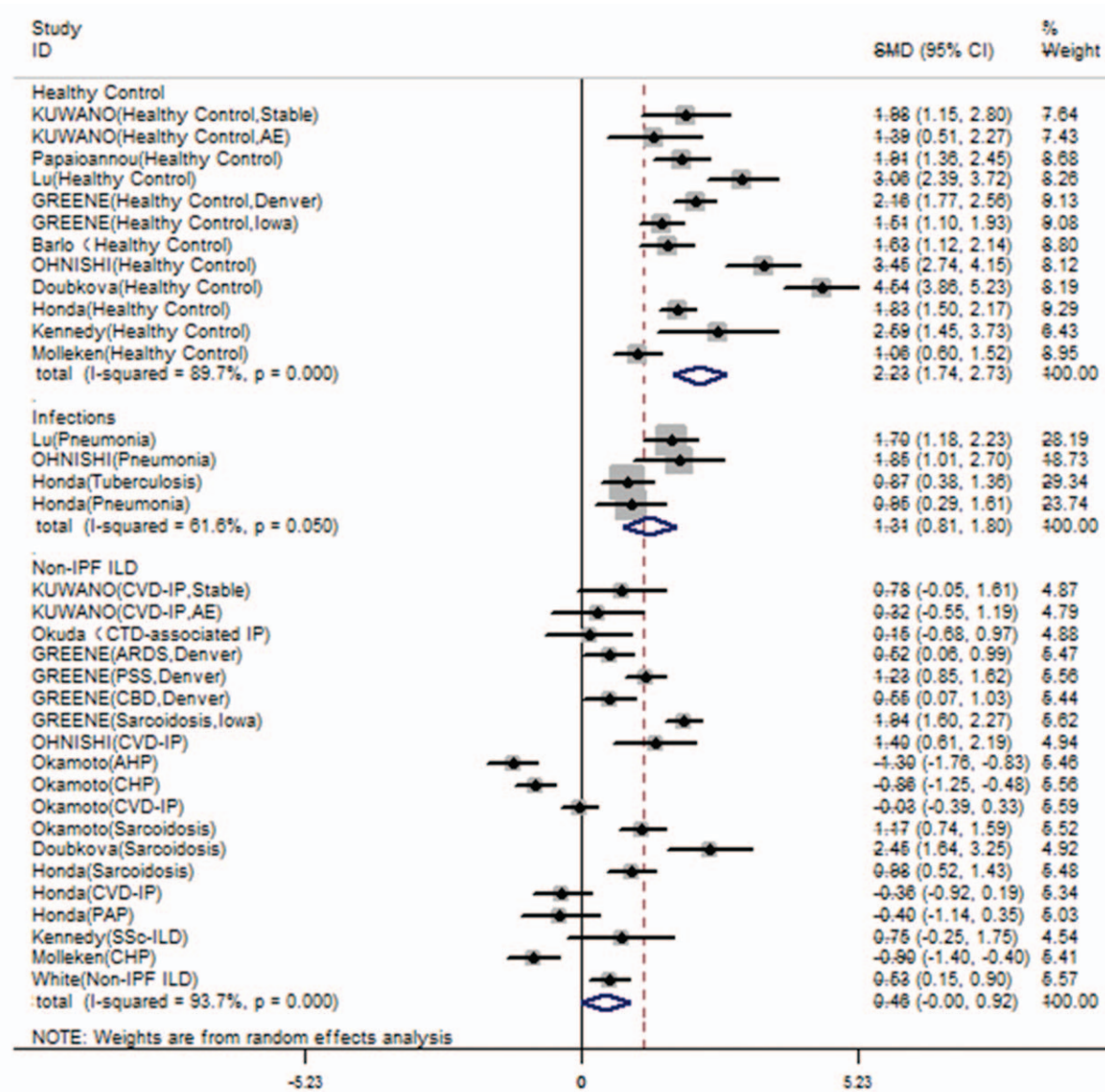
These comparisons suggest a high level of heterogeneity (I<sup>2</sup>=95.8%, 87.0%, respectively, with P<.001, Supplemental Fig 1, <http://links.lww.com/MD/B724>). To further evaluate the cause of heterogeneity, we repeated the serum SP-A level comparisons in specific diseases. The SP-A levels were significantly higher in patients with IPF than in patients with sarcoidosis and pneumonia, but not collagen vascular disease-associated interstitial pneumonia (SMD: 0.344 [-0.158, 0.846], Z value=1.34, P=.180; Table 2).

Serum SP-D levels in patients with IPF were significantly higher than SP-D in patients with pulmonary infection (SMD: 1.308 [0.813, 1.803], Z value=5.18, P<.001; Fig. 3) and healthy controls (SMD: 2.235 [1.739, 2.731], Z value=8.83, P<.001; Fig. 3). There was no significant difference in SP-D levels between patients with IPF and non-IPF ILD (SMD: 0.459 [-0.000, 0.919], Z value=1.96, P=.050; Fig. 3). Surfactant protein-D levels are significantly elevated in patients with ILD (SMD: 1.935 [1.504, 2.367], Supplemental Fig 2, <http://links.lww.com/MD/B724>), as well as pulmonary infections (SMD: 2.466 [0.558, 4.374], Supplemental Fig. 2, <http://links.lww.com/MD/B724>). Further-

**Table 2**  
**Role of serum SP-A/D levels in specific disease comparisons.**

SP-A/D and diseases	Comparisons	No.	SMD	SMD LL	SMD UL	Z	P	I <sup>2</sup>
SP-A								
IPF vs sarcoidosis	4	183/248	1.647	1.242	2.052	7.97	<.001	0.607
IPF vs CVD-IP	3	56/29	0.344	-0.158	0.846	1.34	.180	0.156
IPF vs pneumonia	4	156/74	1.457	1.040	1.875	6.84	<.001	0.356
IPF (AE vs stable)	2	52/29	0.770	0.300	1.240	3.21	.001	0.000
SP-D								
IPF vs sarcoidosis	4	199/264	1.582	0.993	2.172	5.26	<.001	0.844
IPF vs CVD-IP	5	169/112	0.360	-0.212	0.931	1.23	.218	0.752
IPF vs pneumonia	3	118/61	1.489	0.954	2.023	5.46	<.001	0.484
IPF (AE vs stable)	4	106/90	0.684	0.265	1.104	3.20	.001	0.425

AE=acute exacerbation, CVD-IP=collagen vascular disease-associated interstitial pneumonitis, IPF=idiopathic pulmonary fibrosis, LL=lower limit, UL=upper limit.



**Figure 3.** Serum SP-D levels comparisons between IPF and other groups (non-IPF ILD, pulmonary infections or healthy controls). Serum SP-D levels in IPF patients were higher than pulmonary infections (SMD: 1.308 [0.813, 1.803], Z value=5.18, P<.001) or healthy controls (SMD: 2.235 [1.739, 2.731], Z value=8.83, P<.001). However, there was no significant difference of serum SP-D levels between IPF and non-IPF ILD patients (SMD: 0.459 [-0.000, 0.919], Z value=1.96, P=.050).

more, we compared the serum SP-D level in specific diseases. The SP-D serum level in patients with IPF patients was significantly higher than in patients with sarcoidosis (SMD=1.582 [0.993, 2.172], Z value=5.26,  $P < .001$ ; Table 2), and the heterogeneity was high ( $I^2=84.4\%$ ). There was no significant difference between patients with collagen vascular disease-associated interstitial pneumonia and IPF (SMD: 0.360 [-0.212, 0.931], Z value=1.23,  $P = .218$ ; Table 2). Moreover, elevated SP-A and SP-D might indicated AE of IPF (SP-A SMD: 0.770 [0.300, 1.240], SP-D SMD: 0.684 [0.265, 1.104]; Table 2). Only Kakugawa's research reported elevated SP-A and potentially elevated SP-D level in AE phase compared to stable phase in the same patients.<sup>[35]</sup>

**3.2.2. Prognostic analysis.** Seven publications were included to evaluate the effect of serum levels of SP-A and SP-D on the death of patients with IPF. A higher SP-A level was associated with a significantly higher risk of death, and there was no heterogeneity (pooled HR: 1.39 [1.10, 1.75], Z value=2.80,  $P = .005$ ,  $I^2 = 0.0\%$ ; Fig. 4A). There was a significant association between higher SP-D level and increased risk of death, without heterogeneity (HR: 2.11 [1.60, 2.78], Z value=5.31,  $P < .001$ ,  $I^2 = 0.0\%$ ; Fig. 4B). Sensitivity analysis indicated that no specific study influenced the pooled HR (Supplemental Fig. 3, <http://links.lww.com/MD/B724>).

No obvious asymmetry was observed in the funnel plot regarding the association between SP-A and death (Egger's test:  $P = .388$ ; Fig. 4C), indicating no evidence of publication bias.

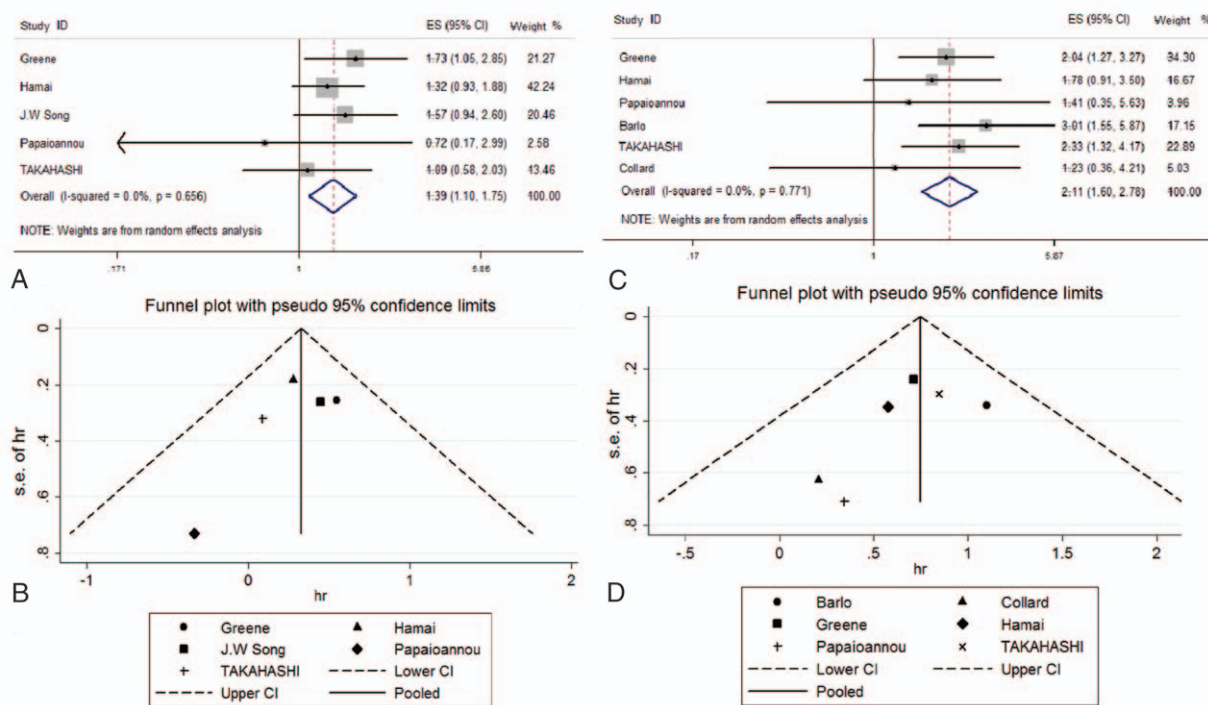
Similar asymmetry was observed for the association between SP-D and death (Egger's test:  $P = .276$ ; Fig. 4D).

**3.3. Race subgroup analysis**

Both Asian (SMD 2.849 [1.862, 3.836],  $P < .001$ ) and Caucasian (SMD 2.685 [0.832, 4.538],  $P = .005$ ; Table 3) patients with IPF had significantly higher serum levels of SP-A when compared with healthy controls. Similar results were observed for SP-D (Table 3). Significantly higher SP-A serum levels (SMD 1.355 [0.665, 2.044],  $P < .001$ ) and SP-D (SMD 0.871 [0.202, 1.540],  $P = .011$ ) were identified in Caucasian patients with IPF, when compared to Caucasian patients with non-IPF ILD. However, no significant differences in serum levels of SP-D (SMD 0.150 [-0.401, 0.701],  $P = .593$ ) were identified when Asian patients with IPF were compared to Asian patients with non-IPF ILD (Table 3). Higher levels of SP-A (pooled HR 1.336, 95% CI [1.027, 1.738],  $P = .031$ ) and SP-D (pooled HR 1.961, 95% CI [1.299, 2.961],  $P = .001$ ) were associated with increased risk of death among Asian patients with IPF. In Caucasian patients with IPF, a higher level of SP-D (pooled HR 2.243, 95% CI [1.547, 3.252],  $P < .001$ ) was a risk factor for increased mortality, while a higher SP-A was not associated with increased mortality (pooled HR 1.459, 95% CI [0.740, 2.879],  $P = .276$ ).

**4. Discussion**

In this study, serum levels of SP-A could be used to differentiate patients with IPF from patients with non-IPF ILD, pulmonary



**Figure 4.** Forest plot and funnel plot for the association between serum SP-A/D levels and IPF patients' prognosis. (A) Forest plot for the role of serum SP-A levels in IPF prognosis. HR and heterogeneity were shown (HR: 1.39 [1.10–1.75], Z value: 2.80,  $P = .005$ ,  $I^2 = 0.0\%$ ). P value of Q statistic test was also presented ( $P = .656$ ). (B) Funnel plot for role of serum SP-A levels in IPF prognosis. No obvious asymmetry could be observed (Egger's test:  $P = .388$ ). (C) Forest plot for the role of serum SP-D levels in IPF prognosis. HR and heterogeneity were shown (HR: 2.11 [1.60–2.78], Z value: 5.31,  $P < .001$ ,  $I^2 = 0.0\%$ ). P value of Q statistic test was also presented ( $P = .771$ ). (D) Funnel plot for role of serum SP-D levels in IPF prognosis. No obvious asymmetry could be observed (Egger's test:  $P = .276$ ).



**Table 3****Race subgroups analysis in IPF comparisons and prognosis.**

Comparisons	Asian			Caucasian		
	<i>n</i>	SMD/HR (95% CI)	<i>P</i>	<i>n</i>	SMD/HR (95% CI)	<i>P</i>
SP-A						
IPF vs healthy controls	7	2.849 (1.862–3.836)	<.001	4	2.685 (0.832–4.538)	.005
IPF vs Non-IPF ILD	8	0.966 (0.123–1.810)	.025	4	1.355 (0.665–2.044)	<.001
High vs low SP-A in IPF prognosis	3	1.336 (1.027–1.738)	.031	2	1.459 (0.740–2.879)	.276
SP-D						
IPF vs healthy controls	5	2.349 (1.613–3.085)	<.001	7	2.164 (1.448–2.881)	<.001
IPF vs non-IPF ILD	11	0.150 (-0.401 to 0.701)	.593	8	0.871 (0.202–1.540)	.011
High vs low SP-D in IPF prognosis	3	1.961 (1.299–2.961)	.001	3	2.243 (1.547–3.252)	<.001

CI=confidence interval, HR=hazard Ratio, ILD=interstitial lung disease, IPF=idiopathic pulmonary fibrosis, SMD=standard mean difference.

infection, and healthy controls. Serum SP-D levels could be used to differentiate patients with IPF from those with pulmonary infections and healthy controls, but not from patients with non-IPF ILD. Among Caucasian patients, both SP-A and SP-D levels differentiated patients with IPF from those with non-IPF ILD, and healthy controls. However, among Asian patients, higher level of SP-D differentiated patients with IPF only when compared to healthy controls. SP-A and SP-D could predict prognosis. Patients with IPF and elevated levels of SP-A had a 1.39-fold (95% CI 1.10, 1.75) increased risk of poor prognosis. Patients with IPF and elevated levels of SP-D had 2.11-fold (95% CI 1.60, 2.78) increased risk of poor prognosis. The cutoff points of SP-A (or SP-D) were the same order of magnitude except particular research. Different cutoff points may due to different calculation methods, race, severity, and so on.

Higher SP-A and SP-D serum levels among patients with IPF may be a result of genetic and environmental susceptibility. Pulmonary fibrosis may occur in genetically susceptible individuals after exposure to one of several environmental stressors. These stressors cause abnormal surfactant synthesis, secretion, and recycling, in both children and adults.<sup>[46]</sup> Serum levels of SP-A and SP-D are significantly elevated in patients with IPF, bacterial pneumonia, and tuberculosis, compared to the healthy controls. Kati et al.<sup>[47]</sup> suggested that serum SP-D levels were significantly higher in patients with IPF and submassive pulmonary embolism than in controls and SP-D may have a diagnostic role in sub-massive pulmonary embolism.

AE of IPF is characterized by an acute worsening of dyspnea, with the potential of hypoxemic respiratory failure, and new or progressive pulmonary infiltrates on radiologic images.<sup>[48]</sup> There is a difference in levels of serum SP-A and SP-D between stable and AE of IPF. In this study, we found that serum levels of SP-A and SP-D increased significantly in an AE of IPF, when compared to levels in stable IPF. Pathologically, it may result from functional abnormalities of type II alveolar epithelial cells, including endothelial cells injuries and coagulation abnormalities.<sup>[49]</sup> Therefore, SP-A and SP-D, reflecting type II alveolar epithelial cell injury and proliferation, will increase with an AE of IPF. However, in clinical practice, the combination of serum SP-A/SP-D with other predictors (eg, symptoms or HRCT) will enhance diagnostic accuracy of AE.

Elevated SP-A and SP-D in patients with IPF is likely the result of increased secretion by type II alveolar epithelial cells. An increase in total number of type II cells, resulting from diffuse hyperplasia, leads to increased leakage from the alveoli to the interstitium, and decreased clearance from vascular compartment. Selman and Pardo<sup>[50]</sup> reported that IPF was not an

inflammatory disorder, and that alveolar epithelial cell damage and activation were a result of constant microscopic injury. Hyperplasia of type II alveolar epithelial cells results in loss of type I alveolar epithelial cells. In patients with IPF, Nishikiori et al.<sup>[20]</sup> identified thickened matrix, near the alveolar space, and capillary hyperplasia was detected using CD34.<sup>[51,52]</sup> These capillaries were near the regenerated type II alveolar epithelial cells, and were SP-A and SP-D positive. Surfactant proteins can leak directly into the bloodstream through capillaries instead of lymphatic ducts.

The blood-air barrier of lung comprises the alveolar epithelium, alveolar epithelial basal layer, substantia propria, capillary basement membrane, and capillary endothelial cells. Injury to any part of the barrier will increase permeability, resulting in leakage of SP-A and SP-D from the alveolus to the capillaries. Barbas-Filho et al.<sup>[53]</sup> conducted a retrospective study including 55 patients with histologically confirmed IPF. Using electron microscopy, they detected apoptosis in 89% of the patients with IPF/UIP.

In animal experiments, alveolar epithelial cell death was observed, and bleomycin was used to induce pulmonary fibrosis.<sup>[54,55]</sup> Repeated injuries of alveolar epithelial cells resulted in cell death, but the exact mechanism is unclear. Angiotensin II and Fas signaling were associated with alveolar epithelial cell death in bleomycin-induced lung injury.<sup>[56,57]</sup>

The inflammatory reaction may be involved in the pathogenesis of IPF. There is evidence of immune cell infiltration and inflammatory cytokine production in patients with IPF. Carre et al.<sup>[58]</sup> reported that the levels of interleukin-8 (IL-8) mRNA in alveolar macrophages, and the level of IL-8 protein in bronchoalveolar lavage, were significantly higher in patients with IPF than in healthy subjects. Both IL-8 mRNA and IL-8 protein were associated with disease severity. Other cytokines and chemokines, including tumor necrosis factor alpha (TNF- $\alpha$ ), IL-1 $\beta$ , IL-4, and IL-5, are overexpressed in patients with IPF.<sup>[59,60]</sup> In an inflammatory environment, capillary permeability increases, and SP-A and SP-D leakage from the alveolus to the capillary increases. Mechanisms include damage of the junction of capillary endothelial cells, dysfunction of endothelial cell signal transduction, and release of inflammatory cytokines.

In addition, the concentration difference between alveolar airspace and the blood and reduction of SP clearance contribute to the elevation of the serum levels of SP-A and SP-D. IPF and non-IPF ILD have a similar pathogenesis. We found no significant differences in the serum concentration of SP-A and SP-D between patients with IPF and non-IPF ILD (including progressive systemic sclerosis, pulmonary alveolar proteinosis, idiopathic

nonspecific interstitial pneumonia, and sarcoidosis). When comparing patients with IPF and sarcoidosis, there is no difference in SP-A, but there is a difference in SP-D, and the reason is unclear.

The median survival of patients with IPF patients is three years, indicating a poor prognosis.<sup>[61]</sup> Despite the potential use of SP-A/SP-D as diagnostic aids, the diagnosis of IPF is confirmed by surgical biopsy or HRCT. According to guidelines, serum SP-A/SP-D cannot replace biopsy or HRCT.<sup>[4]</sup> However, SP-A/SP-D has the following advantages. First, SP-A/SP-D serum levels could be preliminarily evaluated in patients apprehensive of traumatic examination and surgery. Second, image findings in HRCT are not typical, which complicate the diagnosis, and may delay early diagnosis and treatment. Other physiological measurements have been suggested for predicting the severity and prognosis of patients with IPF, including the GAP index<sup>[62]</sup> (gender, age, and 2 lung physiology variables, FVC and DLco) and the CPI<sup>[13]</sup> (composite physiologic index). Ley et al.<sup>[62]</sup> reported that the GAP index and staging system and the GAP calculator were better predictors of mortality in patients with IPF than previously developed prediction models. This method has important utility for both clinical practice and trials. Our analyses indicate that the use of serum biomarkers should not replace the existing physiological measurements for predicting prognosis, as each method has its own limitation. Combining these methods (eg, GAP models, HRCT, and serum biomarkers) could increase the accuracy and sensitivity in determining the prognosis of patients with IPF. Several studies suggested that serum SP-A and/or SP-D can combine with existing physiologic parameters to enhance the ability of predicting survival. Those clinical variables include age, smoking status, FVC, Dlco, and so on.<sup>[27,44]</sup>

Our study still has several limitations. Although we tried to control the study quality by the Newcastle–Ottawa quality assessment scale, the included studies were all retrospective, which intrinsically presents difficulty in causality inference. Only Chinese and English articles were included in this analysis. Statistical heterogeneity was prevalent among our included studies in the overall analyses. The cutoff of high versus low serum levels was inconsistent among the included publications. In the articles included, 3 did not provide a specific cutoff value, and we were unable to obtain the original data. Among the cutoff values provided by the author, the numbers in each article were different.

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