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CC-chemokine ligand 18, CXC motif chemokine 13 and osteopontin as biomarkers of silicosis and asbestosis: a prospective observational study

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Background Silicosis and asbestosis, distinct forms of pneumoconiosis, manifest progressive interstitial fibrosis due to exposure to silica dust or asbestos fibers. This study aimed to identify potential biomarkers for diagnosing silicosis and asbestosis, while also evaluating disease severity and prognosis. We undertook an prospective observational study involving patients with silicosis or asbestosis. The correlation between baseline CC-chemokine ligand 18 (CCL18), CXC motif chemokine 13 (CXCL13), osteopontin (OPN), periostin, and fibulin-3 and clinical variables was analyzed. Diagnostic sensitivity was evaluated using receiver operating characteristic curves, and correlations between baseline biomarker levels and disease severity were analyzed. Multivariable Cox regression assessed the baseline concentrations' strength in predicting all-cause mortality for silicosis and asbestosis. Of 231 silicosis and 163 asbestosis included in the study, 29 silicosis (12.6%) and 28 (17.2%) asbestosis died within the five years follow-up period. Elevated baseline concentrations of CCL18, CXCL13, and OPN were observed in 231 silicosis patients and 163 asbestosis patients compared to 118 HCs. Diagnostic accuracy for silicosis or asbestosis, in order, was CCL18, OPN, and CXCL13. Combining CCL18, OPN, and CXCL13 enhanced diagnostic accuracy. In silicosis patients, these concentrations were significantly associated with lung function values. However, these biomarkers were not the risk factor for all-cause mortality. CCL18, CXCL13, and OPN stand out as promising biomarkers for diagnosing silicosis and asbestosis. Meanwhile, CCL18, CXCL13, and OPN may be used for the evaluation of silicosis conditions.

Keywords Silicosis, Asbestosis, CC-chemokine ligand 18, CXC motif chemokine 13, Osteopontin

Pneumoconiosis refers to a group of lung diseases caused by long-term inhalation of harmful dust, leading to lung tissue fibrosis[1]. The chemical and physical properties of the dust, as well as the amount of exposure, play a key role in disease development[2]. Silica and asbestos are the most common dust particles responsible for silicosis and asbestosis, respectively. Although these diseases share some similarities, they also differ in their clinical features and underlying mechanisms.

Silicosis results from inhaling crystalline silica dust, causing inflammation, silicotic nodules, and fibrosis, typically in the upper zones of the lungs. These lesions, known as "onion skin lesions," are characterized by concentrically arranged collagen fibers[3]. Asbestosis, on the other hand, is caused by inhaling asbestos fibers. The pathology of asbestosis shows subpleural honeycombing and fibrosis, with HRCT scans revealing diffuse pulmonary fibrosis mainly in the lower lobes, resembling idiopathic pulmonary fibrosis (IPF), and often accompanied by benign pleural abnormalities[4]. The progression of these diseases often leads to irreversible respiratory failure and even death[5]. The Global Burden of Disease study reported over 60,000 new cases of pneumoconiosis in 2017, with 39% attributed to silicosis and 16% to asbestosis[2]. Silicosis, in particular, is highly prevalent in developing countries like China, where rapid industrialization has increased the risk of exposure to silica dust. Additionally, China continues to experience a significant burden of asbestos-related diseases due to ongoing asbestos use and production[6]. Currently, the diagnosis of silicosis and asbestosis primarily depends on

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a history of occupational dust exposure and radiological findings, highlighting the need for reliable biomarkers to assist in diagnosis and disease monitoring.

CC-Chemokine Ligand 18 (CCL18) is a CC-chemokine produced by human myeloid-derived cells, particularly alveolar macrophages and follicular dendritic cells[7]. During the onset of pulmonary fibrosis, alveolar macrophages produced CCL18 stimulates the differentiation of normal lung fibroblasts, leading to collagen production in vitro, revealing a positive feedback loop between alveolar macrophages and lung fibroblasts in which alveolar macrophages produced CCL18 induces collagen expression and fibroblast-produced collagen increases CCL18 in alveolar macrophages[8]. In IPF, CCL18 levels are negatively correlated with pulmonary function test values[9]. The serum levels of CCL18 above 150 ng/ml were independently associated with increased mortality in IPF patients (HR 1.98, $P = 0.005$)[7]. In addition, the expression of CCL18 on alveolar macrophages in IPF can be inhibited by anti-fibrotic drug pirfenidone[10].

The chemokine CXC motif chemokine 13 (CXCL13), also known as B lymphocyte chemoattractant and B cell-attracting chemokine-1, plays a role in the normal organization of secondary lymphoid tissues by facilitating the homing of B and follicular T helper lymphocytes[10]. CXCL13 is expressed in alveolar macrophages of IPF patients and its induction requires the NF-κB and JAK/STAT pathways[11]. CXCL13 has emerged as a robust prognostic biomarker for lung fibrotic diseases[12,13].

Osteopontin (OPN) is a phosphorylated acidic glycoprotein primarily produced by macrophages in response to tissue injury or fibrosis[14]. Immunohistochemical analysis has shown that profibrotic fibroblasts exhibit positive staining for OPN and contribute to the enlargement of fibrotic foci[15]. Increased levels of OPN expression have been observed in both murine models of pulmonary fibrosis and patients with IPF[16,17].

Periostin and fibulin-3 are matricellular proteins that bind to integrins on the cell surface and exert various effects[18–20]. Periostin promotes inflammation and fibrosis, making it potentially involved in fibrogenesis and a promising biomarker for predicting disease progression and therapeutic efficacy in IPF patients[18]. Fibulin-3, a member of the fibulin family and a disulfide-rich glycoprotein encoded by epidermal growth factor-containing fibulin-like extracellular matrix protein 1 (EFEMP1)[21–23], mediates cell signal transduction by bridging fiber-connecting factors and laminin in the extracellular matrix (ECM), and regulates cell proliferation in a contextual manner[23]. Plasma fibulin-3 has been proposed as a potential diagnostic marker for mesothelioma, and workers exposed to asbestos-contaminated minerals have shown elevated levels of fibulin-3[21,22].

This study aimed to compare the plasma concentrations of CCL18, CXCL13, OPN, periostin, and fibulin-3 in patients with silicosis and asbestosis with those in healthy controls (HCs) to evaluate the diagnostic and disease evaluation potential of these proteins.

Materials and methods
Study design and participants
This prospective cohort study was conducted at Beijing Chao-Yang Hospital, focusing on newly diagnosed pneumoconiosis patients. Recruitment spanned from January 2018 to December 2019, with participants sourced from the Department of Occupational Medicine and Toxicology. Beijing Chaoyang Hospital has a regional center for occupational medicine and worker's compensation. Patients with pneumoconiosis who have ever worked or lived in the city or been transferred from other regions will come to the hospital as outpatients or inpatients. The majority of the patients with silicosis were local residents. The jade-processing factories were functional from the 1970s to the 1990s, and the mining plants had been open from the 1970s to the 2000s. The majority of patients with asbestosis were from a district on the east of the city with the asbestos products factories ever opened from 1950s to 1970s located 20 km away from the hospital.

Inclusion criteria involved consecutively enrolling patients included silicosis and asbestosis, and all underwent baseline assessments comprising chest X-ray and high-resolution computed tomography (HRCT). Pneumoconiosis diagnoses adhered to the 2011 International Labour Organization (ILO) classification's radiological criteria[24]. Exclusion criteria included patients with other pneumoconiosis types, tuberculosis, pneumonia, autoimmune diseases, liver or kidney dysfunction, and malignant tumors. Ultimately, 231 silicosis patients and 163 asbestosis patients met the inclusion criteria (refer to Fig. 1 for details).

The Healthy Controls (HCs) group comprised 118 volunteers selected from the health examination center of Beijing Chao-yang Hospital during the same time frame. None of whom had been exposed to occupational dust including crystalline silica or asbestos.

All patients completed a standardized questionnaire to collect information on their occupational history. All jobs throughout the individuals' working life were taken into account. Clinical data were uniformly collected from each participant's medical records during their initial clinical visit. Demographic information encompassed age, sex, BMI, and smoking status and other clinical information. This meticulous approach ensured a comprehensive dataset for subsequent analysis. The smoking status of all patients was carefully determined, and they were categorized as non-smokers, ex-smokers (had quit smoking ≥ 12 months before the study period), and smokers (currently smoking or had quit smoking < 12 months before the study period). Cigarette smoking is shown by pack-years.

This study was conducted in accordance with the principles of Helsinki (as revised in 2013) and was approved by the Institutional Ethics Committee for Human Research, Beijing Chao-Yang Hospital. Written informed consent was obtained from all the participants involved in the research.

ELISA assay
The plasma samples were collected from each participant at baseline study and stored at − 80 °C for analysis. All samples were measured within two weeks of storage. The thawed plasma samples were removed from storage on the morning of the experiment day, gently mixed, and then centrifuged at 3000 rpm for 10 min at 4 °C using a low-temperature high-speed centrifuge. The supernatant was then carefully collected for immediate
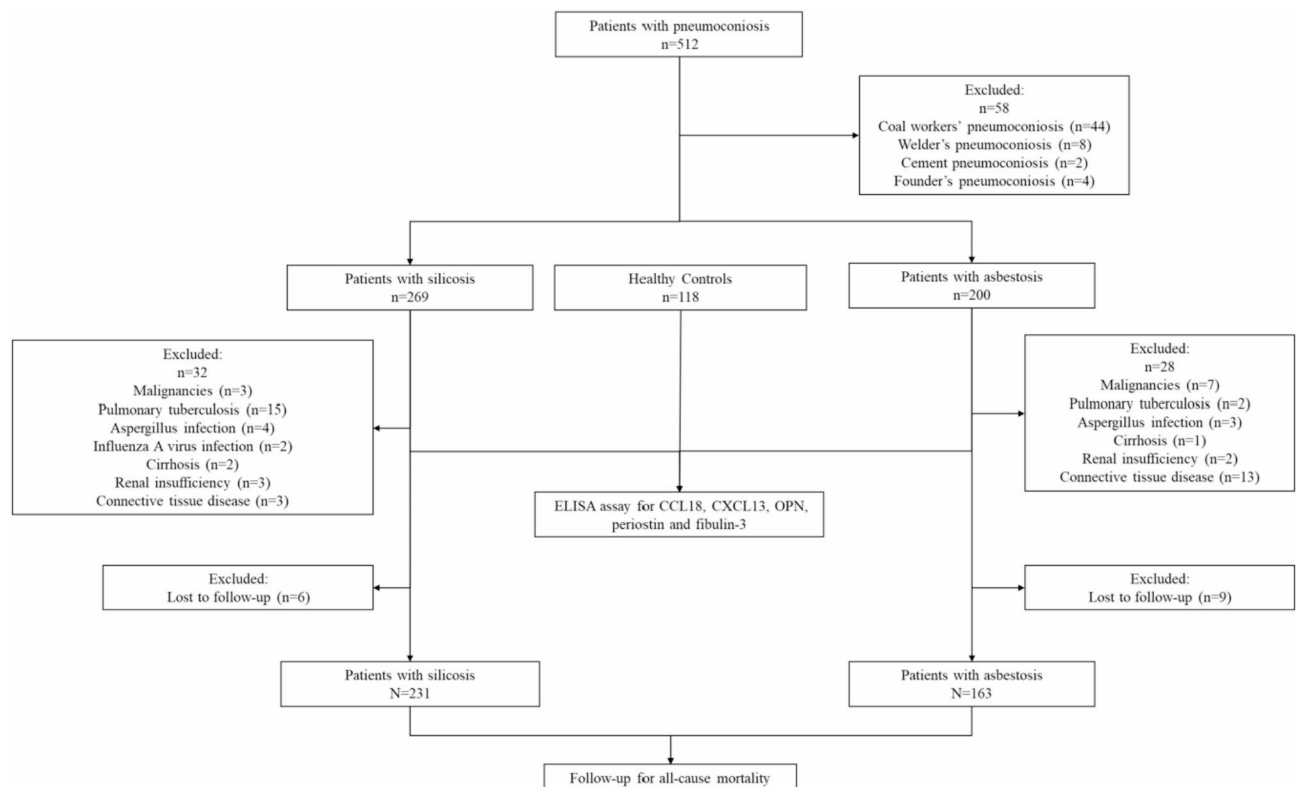
Fig. 1. Flow chat of the enrolled participants.

analysis. The plasma concentrations of CCL18, CXCL13, and OPN were measured using commercially available human ELISA kits (R&D Systems, Inc., Minneapolis, MN, USA), with a sensitivity of 1.77 pg/ml, 3.97pg/ml, and 0.024ng/ml, respectively. The plasma periostin concentrations were measured using commercially available ELISA kits (Bluegene, Shanghai, China) with a sensitivity of 10 pg/ml. The plasma fibulin-3 concentrations were measured using commercially available ELISA kits (Abcam, Waltham, USA) with a sensitivity of 0.63 ng/ml.

HRCT scans and chest radiograph
HRCT scans of patients were performed with 0.625-mm sections, a 1-s scan time, and a 10-mm interval in the apex base scans with the inclusion of both lungs in the field of view. The signs of the patients were assessed by two occupational specialists who were blinded to the clinical data. In patients of disagreement between the occupational specialists after the initial assessment, consultation was sought from an occupational expert. Further details were presented in the Supplementary online material.

Each patient underwent chest radiograph which were independently assessed by two experienced clinicians according to the International Labor Organization classification, with interobserver correlation value 0.82. Pneumoconiosis was classified as stage I, II or III based on the density and distribution of small nodules and/or large opacities showed on chest radiograph. Briefly, each lung field was divided into three zones (upper, middle, lower) on the posterior chest radiographs. When the highest density of small opacities was ≥ 1/0, the distribution affected two or more zone, the patients were diagnosed as Stage I. When the highest density of small opacities was ≥ 2/1 and the distribution affected more than four zones, or the highest density of small opacities was ≥ 3/2 and the distribution affected four or more zones, the patients were diagnosed as Stage II. When the highest density of small opacities was ≥ 3/2 and the distribution affected four or more zones with aggregation of small or large opacities, or the diameter of the largest opacity was ≥ 20 × 10 mm, the patients were diagnosed as Stage III.

Pulmonary function test
Pulmonary function tests were carried out by certified technicians according to the hospital guidelines, which met the recommendations of the American Thoracic Society (ATS)/European Respiratory Society (ERS)[25]. Pulmonary function parameters were measured using spirometry, whole body plethysmography, and single-breath diffusing capacity for carbon monoxide (DLCO SB) measurements.

Follow-up
Patients underwent regular follow-up assessments at intervals of 6–12 months after the initial diagnosis. The primary endpoint of interest was all-cause mortality from the time of diagnosis through December 2022. Vital status data were obtained through a comprehensive review of patients' medical records and supplemented by

follow-up phone calls to either the patients or their family members. This meticulous approach ensured accurate and up-to-date information for the assessment of the primary endpoint.

Statistical analysis

Data were expressed as mean ± standard deviation and/or interquartile range, and the differences among the three groups were analyzed using the Kruskal–Wallis test or one-way analysis of variance. The differences among the two groups were evaluated using t test. Counting data were analyzed using the chi-square test. The association of these variables with silicosis or asbestosis was further checked with univariate and multiple Logistic regression. The results were presented as estimates of relative risk by OR with a 95% CI. A total of 173 silicosis patients and 121 asbestosis patients had complete data and were included in the logistic regression analysis and Pearson's correlation coefficient. The levels of plasma biomarkers were further analyzed by a receiver operating characteristic (ROC) curve to determine the cut-off levels that resulted in the optimal diagnostic accuracy for each marker between the patients and controls. These cut-off levels were used to determine the sensitivity and specificity. The impact of the predictor variables on mortality was assessed using univariable and multivariable Cox regression analyses. A P value < 0.05 was considered statistically significant. Statistical analyses were performed using GraphPad Prism 6 (GraphPad Software Inc, SanDiego, CA, USA) and SPSS Statistics version 23 (IBM Inc, Chicago, IL, USA).

Results

Demographics of the participants

The demographics of each participant group were summarized in Table 1. There were significant differences in sex, age, BMI and the smoking status among the groups. The pulmonary function values, including the predicted percentages of forced vital capacity (FVC), forced expired volume in 1 s (FEV_1), the FEV_1/FVC ratio, and DLCO SB% showed significant differences among the groups.

Occupational history of the enrolled patients

The 231 patients with silicosis were local residents who had been exposed to crystalline silica through the processing of excavation and digging underground in the iron ores or the gold mines ($n = 136$, 58.9%), polishing and buffing stones or jade with grinding wheels ($n = 47$, 20.3%), abrasive blasting and sand blasting ($n = 31$, 13.4%), handling raw iron ores ($n = 17$, 7.4%). The 163 patients with asbestosis were exposed to chrysotile dust

	Silicosis	Asbestosis	HCs	*P*-value*
N	231	163	118	
Male: Female	204:27	55:108	79:39	<0.001
Age (yrs)	63.0 ± 12.5	73.2 ± 8.0	60.0 ± 11.2	<0.001
BMI (kg/m²)	24.3 ± 4.0	25.7 ± 4.2	25.5 ± 3.1	0.002
Smoking status				
Current smoker (n)	116	32	25	<0.001
Cumulative pack-yrs, median(IQR)	17.8(1.8–41.3)	15.0(1.5–50.0)	13.0(0.5–23)	0.006
Ex-smokers (n)	34	27	19	<0.001
Non-smokers (n)	81	104	74	<0.001
Years of exposure to crystalline silica/asbestos(yrs)	13.0(8.0–20.0)	15 (6.0–27.0)	NA	0.539
Pulmonary function values				
FVC (predicted %)	68.7 ± 42.4	71.5 ± 21.6	83.7 ± 16.4	<0.001
FEV₁ (predicted %)	56.8 ± 40.1	68.3 ± 21.8	81.6 ± 11.3	<0.001
FEV₁/FVC (%)	69.2 ± 23.6	83.3 ± 21.1	83.8 ± 10.6	<0.001
TLC (predicted %)	72.4 ± 39.4	76.2 ± 21.4	86.7 ± 9.2	0.002
DLCO SB (predicted %)	73.7 ± 24.4	63.8 ± 20.7	80.3 ± 8.7	0.010
PH	49(21.2%)	6(3.7%)	0	<0.001
COPD	63(27.3%)	27(16.5%)	0	0.012
Respiratory failure	35(15.3%)	20(12.3%)	0	<0.001
Stages of pneumoconiosis				
Stage I (n)	68	98	NA	0.000
Stage II (n)	61	47	NA	0.356
Stage III (n)	102	18	NA	0.000

Table 1. Demographics of the study participants. Data were presented as n, percentage, or mean ± SD. Abbreviations: BMI, body mass index; HCs, healthy controls; FVC, forcedvital capacity; FEV_1 ,forced expiratory volume in first second; DLCOSB,diffusing capacity of the lung for carbon monoxide; PH, pulmonaryhypertension; COPD,chronic obstructive pulmonary disease; NA, notavailable. * P-values were computed by chi-square test for sex and smoking status,one-way analysis of variance for age, BMI, FVC, FEV_1, FEV_1/FVC ratio,DLCOSB. t test was used to estimate stages of pneumoconiosis.

or fibers in asbestos products during the manufacture of asbestos textiles ($n = 112$, 68.7%) or asbestos-based products ($n = 51,31.3\%$) during heat insulation or boiler maintenance work. Because of the lack of atmospheric measurements and detailed information on the frequency of exposure for each job potentially associated with silica or asbestos fiber exposure, the duration of occupational dust exposure (years) was determined as a proxy measure.

Peripheral concentrations of CCL18, CXCL13, OPN, Periostin and fibulin-3

As shown in Fig. 2, there was no significant difference in the plasma concentrations of CCL18 and CXCL13 in silicosis and asbestosis patients, but the values were significantly higher compared to that of HCs ($P < 0.01$). The plasma OPN concentration was significantly higher in silicosis and asbestosis patients compared to HCs (both $P < 0.01$). Meanwhile, the plasma OPN concentration was significantly higher in silicosis patients than in asbestosis patients ($P < 0.01$). The plasma periostin concentration was significantly higher in silicosis patients compared to that in HCs ($P < 0.01$). No significant differences were found in the plasma fibulin-3 concentration among silicosis and asbestosis patients, and HCs (Table S1).

The plasma concentrations of CCL18 and OPN were statistically significantly higher in Stage III of silicosis than in Stage I or Stage II of silicosis (Table 2). The plasma concentrations of CCL18, CXCL13, and OPN were not significantly different among the various stages of asbestosis (Table S2).

Peripheral biomarkers for detecting silicosis or asbestosis

As shown in Table 3, compared with HCs, univariate logistic regression analysis showed that CCL18, CXCL13, OPN and periostin were positively correlated with silicosis, and the correlation was more strongly after adjusted for other confounding factors. The adjusted odds ratios (OR) of CCL18, CXCL13, OPN and periostin for silicosis were 1.13(95% CI 1.081–1.190), 1.08(95% CI 1.045–1.113), 1.05(95% CI 1.028–1.068) and 1.21(95% CI 1.078–1.368), respectively.

Compared with HCs, univariate logistic regression analysis showed that CCL18, CXCL13 and OPN were positively correlated with asbestosis, and the correlation was more strongly after adjusted for other confounding factors. The adjusted OR of CCL18, CXCL13 and OPN for asbestosis were 1.14(95% CI 1.078-1.200), 1.09(95% CI 1.053–1.136) and 1.05(95% CI 1.028–1.076), respectively.

ROC curve for the identification of silicosis or asbestosis

ROC curve analysis was used to evaluate the ability of the plasma CCL18, CXCL13, and OPN concentrations to differentiate patients with silicosis or asbestosis from HCs (Table 4). Based on the area under the curve (AUC), the order of the diagnostic accuracy was observed to be CCL18, OPN, and CXCL13 (Fig. 3). The sensitivity and specificity of the combination of all three biomarkers for the diagnosis of silicosis were 93.1% and 89.1%; and 90.1% and 89.3% respectively for diagnosing asbestosis.

Correlations between CCL18, CXCL13, and OPN levels and lung function values

Due to dysaudia, older age, dyspnea on exertion or severe cough, some patients could not cooperate to complete lung function tests. In silicosis group, 58 patients did not take the examination, including 9 cases of dysaudia, 17 cases of older age, and 32 cases of respiratory distress. In the asbestosis group, 42 patients did not take the examination, including 7 cases of dysaudia, 19 cases of older age, and 16 cases of exertion or severe cough. Ultimately, pulmonary function was assessed for 173 silicosis patients and 121 asbestosis patients in the validation study. As shown in Fig. 4, among the silicosis patients, the plasma CCL18, CXCL13, and OPN concentrations were negatively correlated with the predicted values of FVC% and DLCO SB% (both $P < 0.05$). There were no correlation between CCL18, CXCL13, and OPN levels and lung function values among the asbestosis patients. In HCs group, no correlation was noticed between CCL18, CXCL13, and OPN levels and lung function values.

Risk prediction of death by biomarkers

By the end of December 2022, over a median follow-up period of 41.9 (range 1–60) months. Among the 231 silicosis, 29 died and 6 patients were lost to follow-up. Among the 163 asbestosis, 28 died and 9 patients were lost to follow-up. Further details of death records were presented in the online Supplementary materials. Cox proportional hazards analysis for silicosis and Cox proportional hazards analysis for asbestosis were presented in the online Supplementary materials (Table S3 and Table S4). According to univariable analysis, age, BMI, FVC%, DLCO SB%, PH, respiratory failure and OPN levels at baseline were significantly associated with an increased risk of death in patients with silicosis or asbestosis (Table S5). After adjustm ent for covariates, age and respiratory failure were the risk factor for all-cause mortality, but OPN was not the risk factor for all-cause mortality. Kaplan–Meier survival curves demonstrated no significantly survival rate in the silicosis patients compared to the asbestosis patients (Figure S1).

Discussion

In this study, we have demonstrated that plasma biomarkers, specifically CCL18, CXCL13, and OPN, exhibited higher concentrations in patients diagnosed with silicosis or asbestosis when compared to HCs. Within the silicosis patient group, significant variations in plasma CCL18 and OPN concentrations were observed across different disease stages. Among silicosis patients, we observed a negative correlation between plasma concentrations of CCL18, CXCL13, and OPN with predicted values for FVC% and DLCO SB%. In terms of diagnostic accuracy, the ROC curve analysis revealed that, for both silicosis and asbestosssssis patients, CCL18 exhibited the highest diagnostic accuracy, followed by OPN and CXCL13. Importantly, when combined these biomarkers (CCL18, CXCL13, and OPN), it significantly enhanced the diagnostic sensitivity for detecting
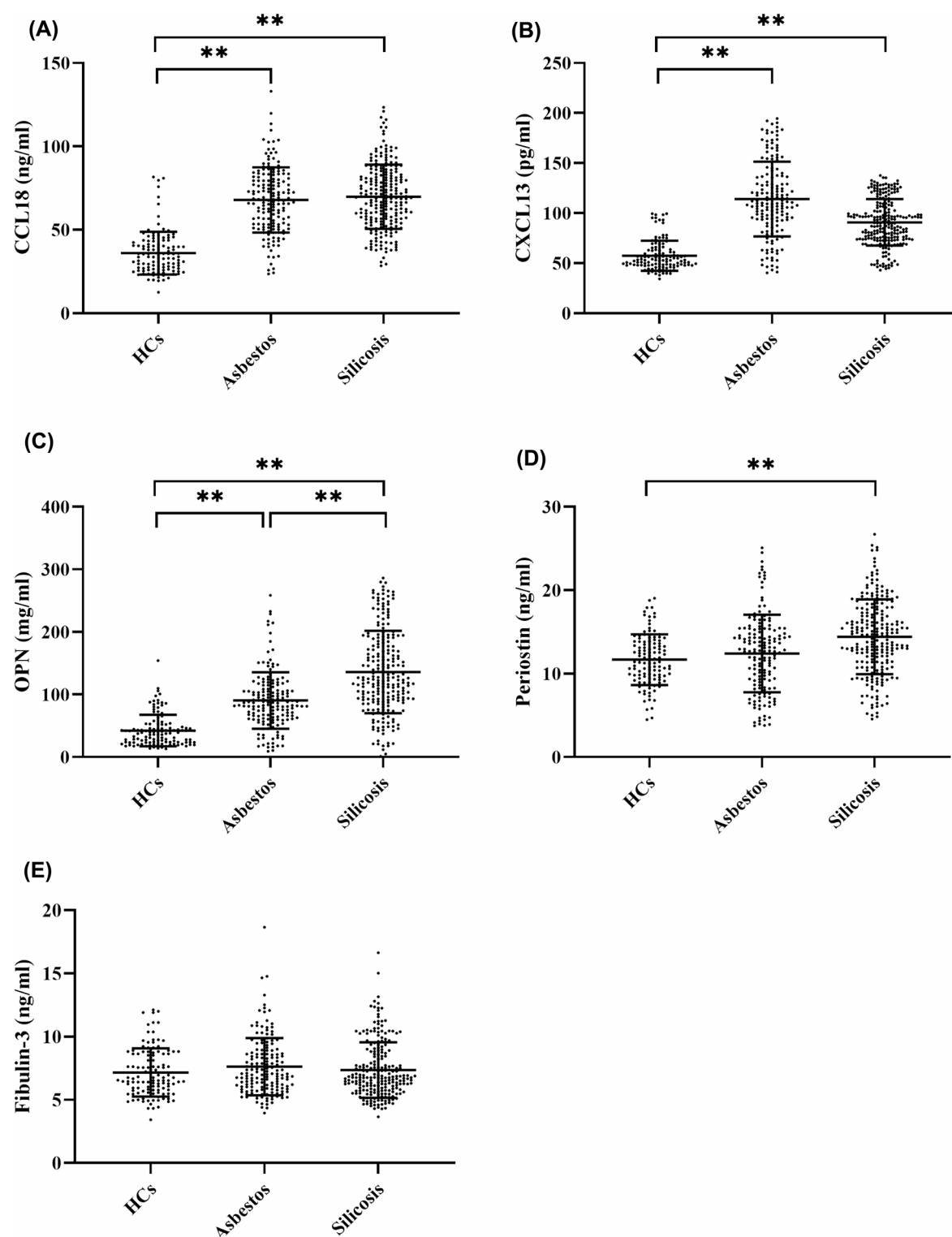
Fig. 2. Comparisons of plasma concentrations of CCL18, CXCL13 OPN, periostin and fibulin-3 among the study groups using the Kruskal–Wallis test. (**A**) CCL18; (**B**) CXCL13; (**C**) OPN; (**D**) periostin; (**E**) fibulin-3 * $P < 0.05$, **< 0.01.

silicosis or asbestosis. These findings highlight the potential utility of these biomarkers for improved diagnosis and assessment of pneumoconiosis.

The common feature of asbestosis and silicosis are characterized by a persistent inflammatory response and generation of proinflammatory and profibrotic mediators[1]. Inflammation and epithelial mesenchymal transition (EMT) play important roles in the pathogenesis of pulmonary fibrosis[26]. Several in vitro studies indicated

	Stage I	Stage II	Stage III	P-value*
N	68	61	102	
OPN(ng/ml)	72.2 ± 48.8	88.1 ± 43.9	145.0 ± 76.7	0.000
CCL18(ng/ml)	67.0 ± 22.9	67.0 ± 21.7	80.8 ± 29.7	0.023
CXCL13(pg/ml)	80.6 ± 66.5	79.8 ± 55.9	99.2 ± 70.2	0.209
Periostin(ng/ml)	15.2 ± 9.7	13.2 ± 7.9	16.2 ± 4.6	0.148

Table 2. Plasma concentrations of CCL18, CXCL13, OPN and Periostin in the patients with various stages of silicosis. Data were presented as mean ± SD. Abbreviations: CCL18,CC-chemokine ligand 18; CXCL13,CXC motif chemokine 13; OPN, osteopontin. *P-values were computed by one-way analysis of variance for stages of silicosis.

Characteristics	Model 1		Model 2		Model 3	
	OR (95% CI) P-value		OR (95% CI) P-value		OR (95% CI) P-value	
Silicosis vs. HCs						
OPN	1.05(1.037–1.059)	<0.001	1.04(1.031–1.055)	<0.001	1.05(1.028–1.068)	<0.001
CCL18	1.14(1.108–1.172)	<0.001	1.14(1.097–1.176)	<0.001	1.13(1.081–1.190)	<0.001
CXCL13	1.09(1.070–1.110)	<0.001	1.09(1.067–1.122)	<0.001	1.08(1.045–1.113)	<0.001
Periostin	1.19(1.118–1.267)	<0.001	1.20(1.107–1.306)	<0.001	1.21(1.078–1.368)	<0.001
Fibulin-3	1.05(0.938–1.650)	0.419	1.04(0.903–1.203)	0.570	1.02(0.819–1.271)	0.855
Asbestosis vs. HCs						
OPN	1.04(1.033–1.055)	<0.001	1.04(1.025–1.055)	<0.001	1.05(1.028–1.076)	<0.001
CCL18	1.12(1.093–1.153)	<0.001	1.12(1.118–1.287)	<0.001	1.14(1.078-1.200)	<0.001
CXCL13	1.08(1.061–1.098)	<0.001	1.09(1.056–1.120)	<0.001	1.09(1.053–1.136)	<0.001
Periostin	1.05(0.986–1.111)	0.138	1.05(0.955–1.145)	0.336	1.01(0.901–1.130)	0.880
Fibulin-3	1.11(0.988–1.250)	0.077	1.07(0.893–1.292)	0.447	0.95(1.770–1.168)	0.617

Table 3. Associations between biomarkers and silicosis or asbestosis. Model 1 was univariate analysis. Model 2 adjusted for age, BMI and smoking index; Model 3 adjusted for age, BMI and smoking index and pulmonary function. Abbreviations: CI, confidence interval; CCL18, CC-chemokine ligand 18; CXCL13, CXC motif chemokine 13; HCs, healthy controls; OPN, osteopontin; OR, odds ratio.

	Identifying silicosis			Identifying asbestosis		
	CCL18	OPN	CXCL13	CCL18	OPN	CXCL13
AUC	0.919	0.899	0.717	0.910	0.827	0.788
95%CI	0.874–0.966	0.856–0.943	0.638–0.791	0.862–0.957	0.761–0.892	0.720–0.856
Cut-off value	49.8ng/ml	86.6ng/ml	75.2pg/ml	48.4ng/ml	58.3ng/ml	78.4pg/ml
Sensitivity, %	84.1	76.4	53.3	84.6	81.5	62.3
Specificity, %	90.9	92.7	87.3	89.3	80.4	89.3

Table 4. Cut-off values and the identifying ability of CCL18, OPN and CXCL13 by ROC curve analysis. Abbreviations: AUC, area under curve; CI, confidence interval; CCL18, CC-chemokine ligand 18; CXCL13, CXC motif chemokine 13; OPN, osteopontin.

that transforming growth factor-beta (TGF-β) can induce EMT in human alveolar type II cells in a time and concentration-dependent manner[27]. Studies have shown that OPN is highly induced in the lungs of mice exposed to carbon nanotubes, and plays a key role in TGF-β signaling activation and myofibroblast differentiation, and promotes the formation of pulmonary fibrosis[28]. M2 macrophages contribute to fibrosis via the production of fibrotic mediators, such as TGF-β and CCL18[29]. CXCL13 modulated cell proliferation, the EMT of epithelial cells, and induced the fibrosis of prostatic stromal cells via a variety of factors[30].

OPN is a phosphoprotein secreted by a variety of cells such as activated macrophages, vascular smooth muscle cells, and epithelial cells, mediating cell chemotaxis, adhesion, proliferation, and migration[16]. OPN expression is associated with fibrosis, acting as a chemotactic factor for fibroblasts, modulating the secretion of metalloproteinases (MMPs), and regulating the production of the transforming TGF-β[31]. OPN is a potential diagnostic marker of IPF; plasma surfactant protein (SP)-D > 31ng/ml, MMP-7 > 1.75 ng/ml, and OPN > 6 ng/ml each significantly distinguished patients with IPF from patients with alternative idiopathic interstitial lung disease (ILD)[32]. The elevated levels of OPN in the serum may be a useful indicator in identifying the patients
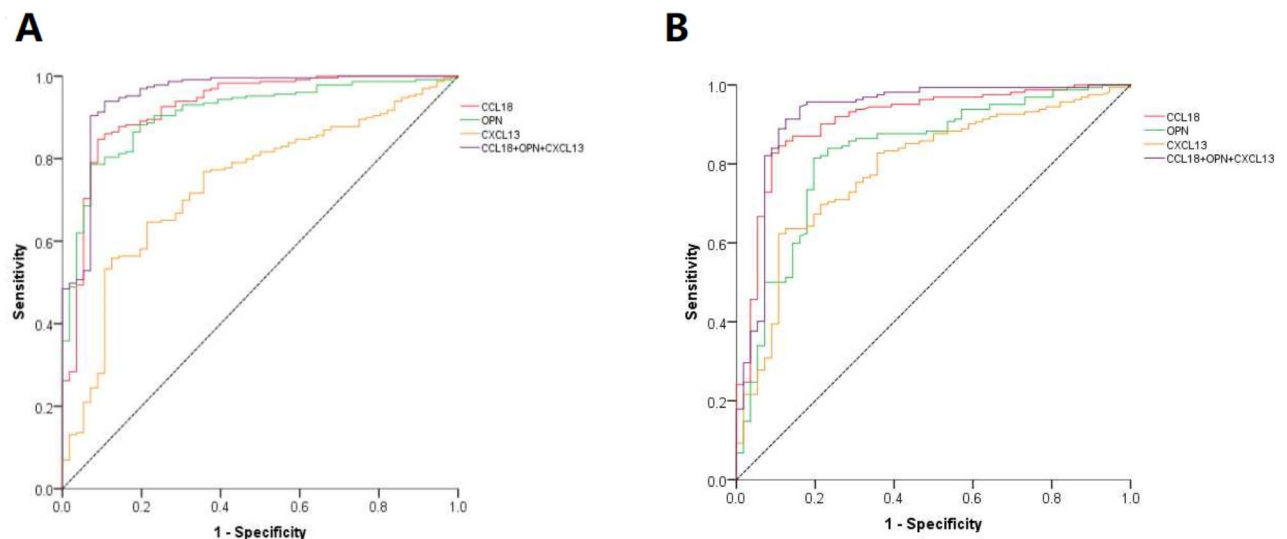
Fig. 3. (**A**) ROC curve analysis to differentiate patients with silicosis from healthy controls; (**B**) ROC curve analysis to differentiate patients with asbestosis from healthy controls.

with ILD who are more likely to experience poor outcomes[31,33]. Meanwhile, there was a weak but statistically significant negative correlation between serum OPN levels and FVC% at baseline[31]. In addition, recent studies revealed that OPN has a high value in the diagnosis of coal workers' pneumoconiosis[34]. In the present study, we found that OPN may be an available biomarker for the adjuvant diagnosis of silicosis or asbestosis. Moreover, the OPN level was higher in Stage III silicosis than in Stage I or Stage II silicosis and was negatively associated with the predicted values of FVC% and DLCO SB% in the silicosis group. The plasma levels of OPN may be used to evaluate the severity of silicosis.

CCL18 is predominantly expressed by alveolar macrophages and occurs at relatively high levels in the lung tissue, which is also produced in the germinal centers of the secondary lymphoid organs by the dendritic cells (DCs)[7]. Previous studies have found that DCs accumulated in lung tissues of silica dust exposure rats and regulated the polarization of Th1/Th2 cells via CD80, CD86, MHC-II and IL-12 expressions, indicating that DCs may play a critical role in modulating immune homeostasis during silicosis in rats[35]. Serum CCL18 level is associated with the CCL18 rs2015086 genotype and predicts mortality and disease progression of IPF patients[36]. The potential relationships between the elevated serum level of CCL18 and clinical outcomes including acute exacerbation and survival in IPF patients have been demonstrated[36], and has been confirmed through data from two randomized controlled trials[37]. A close negative correlation between serum CCL18 concentration and pulmonary functions was observed in patients with fibrotic lung diseases[37]. In the present study, we found that CCL18 can be an available biomarker for the adjuvant diagnosis of silicosis or asbestosis. Moreover, the CCL18 level was higher in Stage III silicosis than in Stage I or II silicosis and was negatively associated with the predicted values of FVC% and DLCO SB% in the silicosis group. The plasma levels of CCL18 may be used to evaluate the severity of silicosis.

CXCL13 is produced by alveolar macrophages in patients with IPF and TNF-a and IL-10 regulate its production[11]. Studies have indicated that CXCL13 expression is upregulated by Fli1 deficiency in macrophages, potentially contributing to the development of tissue fibrosis, vasculopathy, and immune activation in systemic sclerosis (SSc)[38]. Studies have reported that the expression of CXCL13 mRNA in IPF lungs and the level of plasma CXCL13 were both significantly higher than those in chronic obstructive pulmonary disease (COPD) patients and healthy controls[13]. The plasma CXCLl3 level increases significantly when IPF is accompanied by pulmonary hypertension or acute exacerbation[13]. When the plasma CXCL13 concentration increases by more than 50%, it indicates that the patient may have respiratory failure and a poor prognosis[14]. CXCL13 is associated with disease severity and survival in IPF patients[12]. Serum CXCL13 levels were elevated in SSc patients compared with healthy controls and correlated positively pulmonary function test results[38]. A pooled, multicenter, propensity-matched analysis of IPF patients with and without antifibrotic exposure found that cancer antigen 125(CA-125), CXCL13, MMP7, chitinase-3-like protein-1(YKL-40), and OPN predicted differential transplant-free survival in antifibrotic-exposed patients but at higher thresholds than in antifibrotic-nonexposed individuals[39]. Plasma biomarker level generally increased over time in nonexposed patients but remained unchanged in antifibrotic-exposed patients[39]. Our data showed that plasma CXCL13 may be an available biomarker for the adjuvant diagnosis of silicosis or asbestosis. Moreover, the level of CXCL13 was negatively associated with the predicted values of FVC% and DLCO SB% in the silicosis group.

Some limitations of this study should be mentioned. First, selection bias may exist in the present study. This study recruited patients from a single medical centre; hence, the cohort may not be fully representative of all silicosis and asbestosis patients. Second, since the patients in the study were employed by different industries, and many plants were no longer in operation, it was difficult to collect age- and sex-matched dust-exposed workers
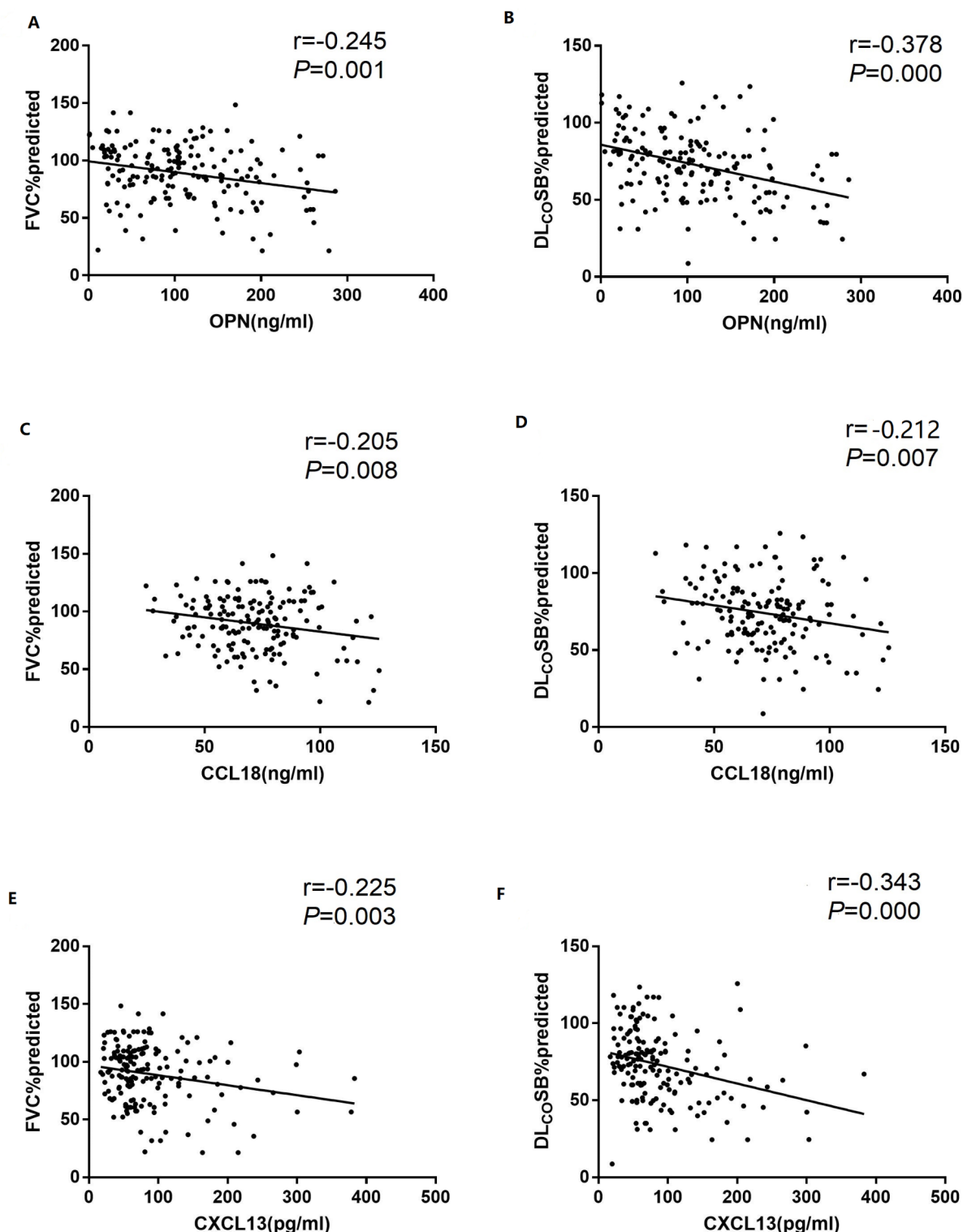
Fig. 4. Correlations between OPN, CCL18 and CXCL13 and pulmonary function parameters in the patients with silicosis. (**A**) OPN and FVC % predicted; (**B**) OPN and DL_{CO} SB% predicted; (**C**) CCL18 and FVC % predicted; (**D**) CCL18 and DLCO SB% predicted; (**E**) CXCL13 and FVC % predicted; (**F**) CXCL13 and DLCO SB%.

without silicosis or asbestosis as a control group. Meanwhile, the lack of atmospheric measurements and detailed information on the frequency of exposure for each job, the level of the exposure could not be accurately assessed. Third, our study primarily focused on investigating the relationship between baseline marker concentrations and prognosis, we did not continuously monitor the biomarker concentrations in every patient to evaluate the

dynamic changes in the progression of asbestosis and silicosis. Finally, there may be other effective biomarkers for monitoring and evaluating disease progression and prognosis. Further research is warranted to explore the potential biomarkers to detect and evaluate pneumoconiosis.

Conclusions

This study has established that CCL18, CXCL13, and OPN serve as viable biomarkers for assisting in the diagnosis of silicosis or asbestosis. Moreover, these biomarkers showed promise in assessing the severity of silicosis. However, our research did not find that these biomarkers can be risk factors for all-cause mortality. Further research is warranted to validate whether the levels of CCL18, CXCL13, and OPN can effectively indicate the presence of early occupational lung fibrosis in individuals exposed to dust in their workplaces. The utilization of these biomarkers has the potential to enhance the identification of active prevention and management for pneumoconiosis.

Data availability

The datasets used during the current study are available from the corresponding author on reasonable request.

References

1. Hua, J. T. et al. Pathology and mineralogy of the pneumoconioses. *Semin Respir Crit. Care Med.* **44**, 327–339. https://doi.org/10.1055/s-0043-1764406 (2023).
2. Barnes, H. et al. Occupational interstitial lung diseases. *Immunol. Allergy Clin. North. Am.* **43**, 323–339. https://doi.org/10.1016/j.iac.2023.01.006 (2023).
3. Zhou, M. et al. Plasma metabolic profiling in patients with silicosis and asbestosis. *J. Occup. Environ. Med.* **63**, 787–793. https://doi.org/10.1097/JOM.0000000000002232 (2021).
4. Ma, R. et al. High-resolution computed tomography features of asbestosis versus fibrotic hypersensitivity pneumonitis: an observational study. *BMC Pulm Med.* **22**, 207. https://doi.org/10.1186/s12890-022-01967-3 (2022).
5. Wu, N. et al. Artificial stone-associated silicosis in China: A prospective comparison with natural stone-associated silicosis. *Respirology* **25**, 518–524. https://doi.org/10.1111/resp.13744 (2020).
6. Wang, J. et al. The incidence of malignancies in asbestosis with Chrysotile exposure: a large Chinese prospective cohort study. *Front. Oncol.* **13** https://doi.org/10.3389/fonc.2023.1172496 (2023).
7. Korbecki, J. et al. CCL18 in the progression of Cancer. *Int. J. Mol. Sci.* **21** https://doi.org/10.3390/ijms21217955 (2020).
8. Prasse, A. et al. A vicious circle of alveolar macrophages and fibroblasts perpetuates pulmonary fibrosis via CCL18. *Am. J. Respir Crit. Care Med.* **173**, 781–792. https://doi.org/10.1164/rccm.200509-1518OC (2006).
9. Prasse, A. et al. CCL18 as an indicator of pulmonary fibrotic activity in idiopathic interstitial pneumonias and systemic sclerosis. *Arthritis Rheum.* **56**, 1685–1693. https://doi.org/10.1002/art.22559 (2007).
10. Saito, Y. et al. Pirfenidone exerts a suppressive effect on CCL18 expression in U937-derived macrophages partly by inhibiting STAT6 phosphorylation. *Immunopharmacol. Immunotoxicol.* **3**, 464–471. https://doi.org/10.1080/08923973.2016.1247852 (2016).
11. Bellamri, N. et al. TNF-α and IL-10 control CXCL13 expression in human macrophages. *J. Immunol.* **204**, 2492–2502. https://doi.org/10.4049/jimmunol.1900790 (2020).
12. Vuga, L. J. et al. C-X-C motif chemokine 13 (CXCL13) is a prognostic biomarker of idiopathic pulmonary fibrosis. *Am. J. Respir Crit. Care Med.* **189**, 966–974. https://doi.org/10.1164/rccm.201309-1592OC (2014).
13. Bowman, W. S. et al. Biomarkers in progressive fibrosing interstitial lung disease: optimizing diagnosis, prognosis, and treatment response. *Front. Med. (Lausanne).* **8**, 680997. https://doi.org/10.3389/fmed.2021.680997 (2021).
14. Shimodaira, T. et al. Upregulation of osteopontin expression via the interaction of macrophages and fibroblasts under IL-1b stimulation. *Cytokine* **110**, 63–69. https://doi.org/10.1016/j.cyto.2018.04.025 (2018).
15. Tsukui, T. et al. Qualitative rather than quantitative changes are hallmarks of fibroblasts in bleomycin-induced pulmonary fibrosis. *Am. J. Pathol.* **183**, 758–773. https://doi.org/10.1016/j.ajpath.2013.06.005 (2013).
16. Hatipoglu, O. F. et al. Osteopontin Silencing attenuates bleomycin-induced murine pulmonary fibrosis by regulating epithelial-mesenchymal transition. *Biomed. Pharmacother.* **139**, 111633. https://doi.org/10.1016/j.biopha.2021.111633 (2021).
17. Gui, X. et al. Prognostic value of serum osteopontin in acute exacerbation of idiopathic pulmonary fibrosis. *Biomed. Res. Int.* **10**, 3424208. https://doi.org/10.1155/2020/3424208 (2020).
18. Izuhara, K. et al. Periostin in inflammation and allergy. *Cell. Mol. Life Sci.* **74**, 4293–4303. https://doi.org/10.1007/s00018-017-2648-0 (2017).
19. Yoshihara, T. et al. Periostin plays a critical role in the cell cycle in lung fibroblasts. *Respir Res.* **21** https://doi.org/10.1186/s12931-020-1299-0 (2020).
20. Okamoto, M. et al. Ability of Periostin as a new biomarker of idiopathic pulmonary fibrosis. *Adv. Exp. Med. Biol.* **1132**, 79–87. https://doi.org/10.1007/978-981-13-6657-4_9 (2019).
21. Pass, H. I. et al. Fibulin-3 as a blood and effusion biomarker for pleural mesothelioma. *N Engl. J. Med.* **367**, 1417–1427. https://doi.org/10.1056/NEJMoa1115050 (2012).
22. Jiang, Z. et al. Plasma Fibulin-3 as a potential biomarker for patients with Asbestos-Related diseases in the Han population. *Dis. Markers.* **2017** https://doi.org/10.1155/2017/1725354 (2017).
23. Panou, V. et al. The established and future biomarkers of malignant pleural mesothelioma. *Cancer Treat. Rev.* **41**, 486–495 (2015). https://doi.org/10.1016/j.ctrv.2015.05.001
24. Muszyńska-Graca, M. et al. Guidelines for the use of the international classification of radiographs of pneumoconioses of the international labour office (ILO): substantial changes in the current edition. *Med. Pr.* **67**, 833–837. https://doi.org/10.13075/mp.5893.00493 (2016).
25. Cotes, J. E. et al. Standardization of the measurement of transfer factor (diffusing capacity). Report working party standardization of lung function tests, European community for steel and coal. Official statement of the European respiratory society. *Eur. Respir J. Suppl.* **16**, 41–52 (1993).
26. Inui, N., Sakai, S. et al. Molecular pathogenesis of pulmonary fibrosis, with focus on pathways related to TGF-β and the Ubiquitin-Proteasome pathway. *Int. J. Mol. Sci.* **22**, 6107. https://doi.org/10.3390/ijms22116107 (2021).
27. Lee, J. H. et al. TGF-β in developmental and fibrogenic EMTs. *Semin Cancer Biol.* **86**, 136–145. https://doi.org/10.1016/j.semcancer.2022.09.004 (2022).
28. Dong, J. et al. Osteopontin enhances multi-walled carbon nanotube-triggered lung fibrosis by promoting TGF-β activation and myofibroblast differentiation. *Part. Fibre Toxicol.* **14**. https://doi.org/10.1186/s12989-017-0198-0 (2017).

29. Isshiki, T. et al. Therapeutic strategies targeting pro-fibrotic macrophages in interstitial lung disease. *Biochem. Pharmacol.* **211** https://doi.org/10.1016/j.bcp.2023.115501 (2023).
30. Liu, D. et al. Changes in the expression and functional activities of C-X-C motif chemokine ligand 13 (CXCL13) in hyperplastic prostate. *Int. J. Mol. Sci.* **24** https://doi.org/10.3390/ijms24010056 (2022).
31. Iturbe-Fernández, D. et al. Osteopontin as a biomarker in interstitial lung diseases. *Biomedicines* **12** (1108). https://doi.org/10.3390/biomedicines12051108 (2024).
32. White, E. S. et al. Plasma surfactant Protein-D, matrix Metalloproteinase-7, and osteopontin index distinguishes idiopathic pulmonary fibrosis from other idiopathic interstitial pneumonias. *Am. J. Respir Crit. Care Med.* **194**, 1242–1251. https://doi.org/10.1164/rccm.201505-0862OC (2016).
33. Ji, J. et al. Increased expression of OPN contributes to idiopathic pulmonary fibrosis and indicates a poor prognosis. *J. Transl Med.* **21** https://doi.org/10.1186/s12967-023-04279-0 (2023).
34. Hou, Z. et al. KL-6, and Syndecan-4 as potential biomarkers in the diagnosis of coal workers' pneumoconiosis: A Case-Control study. *Pharmgenomics Pers. Med.* **16**, 537–549. https://doi.org/10.2147/PGPM.S409644 (2023).
35. Bao, L. et al. Dendritic cells trigger imbalance of Th1/Th2 cells in silica dust exposure rat model via MHC-II, CD80, CD86 and IL-12. *RSC Adv.* **20**, 26108–26115. https://doi.org/10.1039/c8ra03970d (2018).
36. Wiertz, I. A. et al. Genetic variation in CCL18 gene influences CCL18 expression and correlates with survival in idiopathic pulmonary fibrosis: part A. *J. Clin. Med.* **9** https://doi.org/10.3390/jcm9061940 (2020).
37. Neighbors, M. et al. Prognostic and predictive biomarkers for patients with idiopathic pulmonary fibrosis treated with Pirfenidone: post-hoc assessment of the CAPACITY and ASCEND trials. *Lancet Respir Med.* **6**, 615–626. https://doi.org/10.1016/S2213-2600(18)30185-1 (2018).
38. Taniguchi, T. et al. CXCL13 produced by macrophages due to Fli1 deficiency May contribute to the development of tissue fibrosis, vasculopathy and immune activation in systemic sclerosis. *Exp. Dermatol.* **27**, 1030–1037. https://doi.org/10.1111/exd.13724 (2018).
39. Adegunsoye, A. et al. Circulating plasma biomarkers of survival in Antifibrotic-Treated patients with idiopathic pulmonary fibrosis. *Chest* **158**, 1526–1534. https://doi.org/10.1016/j.chest.2020.04.066 (2020).

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N.W., C.X., Y.W., S.Y. and S.D. collected the clinical data. N.W. analyzed the data and drafed the manuscript. Q.Y. revised the manuscript. All authors reviewed the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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