CC-Chemokine Ligand 18, CXC Motif Chemokine 13, and Osteopontin as Biomarkers of Silicosis and Asbestosis: A Case-Control Study

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Abstract

Background

Silicosis and asbestosis are progressive pneumoconiosis characterized by interstitial fibrosis exposure to silica dust or asbestos fibres. This study aimed to explore the potential biomarkers for diagnosis of silicosis or asbestosis.

Methods

Plasma concentrations of CC-chemokine ligand 18 (CCL18), CXC motif chemokine 13 (CXCL13), osteopontin (OPN), periostin, and fibulin-3 were compared between silicosis, asbestosis, and healthy controls (HCs). The receiver operating characteristic (ROC) curve was used to monitor the diagnostic sensitivity, and their correlation to disease severity was examined.

Results

Plasma CCL18, CXCL13, and OPN concentrations in both patient groups were high. For silicosis patients, these plasma concentrations were significantly correlated with predicted forced vital capacity (FVC%) and lung diffusing capacity for carbon monoxide (DL_{CO} SB%). The order of diagnostic accuracy was CCL18, OPN, and CXCL13 in silicosis or asbestosis. Combining CCL18, OPN, and CXCL13 can improve diagnostic accuracy.

Conclusions

CCL18, CXCL13, and OPN are available biomarkers for the diagnosis of silicosis and asbestosis.

Background

Silicosis and asbestosis are the subtypes of pneumoconiosis which are characterized by progressive interstitial lung fibrosis resulting from the inhalation of respirable crystalline silica (RCS) or asbestos fibres [1, 2]. Disease progression usually leads to irreversible respiratory failure or even death [3]. Silicosis is the most prevalent pneumoconiosis in developing countries including China, in line with the rapid industrialization of the country [4]. In addition, China currently sustains a high level of asbestos use and/or production, resulting in long-term asbestos-related disease burden [5]. The diagnosis of silicosis and asbestosis mainly relies on a history of occupational dust exposure and radiological abnormalities. Hence, it is necessary to identify potential biomarkers that facilitate the detection and evaluation of pneumoconiosis.

CC-Chemokine Ligand 18 (CCL18) is a CC-chemokine produced by human myeloid-derived cells [6]. During the onset of pulmonary fibrosis, CCL18, which is primarily produced by alveolar macrophages in the lungs, stimulate normal lung fibroblast differentiation to produce collagen in vitro [7]. In idiopathic pulmonary fibrosis (IPF), CCL18 is negatively correlated with the pulmonary function test values [8]. In a prospective study, patients with a serum level of CCL18 > 150 ng/ml were independently associated with death in IPF (HR 1.98, 95% CI 2.49–25.51, *P* = 0.005) [6]. Pirfenidone may inhibit the expression of CCL18 on alveolar macrophages in IPF [9].

The chemokine CXC motif chemokine 13 (CXCL13), also known as the B lymphocyte chemoattractant and B cell-attracting chemokine-1, may control the normal organization of secondary lymphoid tissues by stimulating the homing of B and follicular T helper lymphocytes [10]. CXCL13 is expressed in the alveolar macrophages from patients with IPF. The NF- κ B and JAK/STAT pathways are required to induce the expression of the chemokine [10]. CXCL13 is also thought to be a robust prognostic biomarker of lung fibrotic disease[11, 12].

Osteopontin (OPN) is a phosphorylated acidic glycoprotein which is primarily produced from macrophages during tissue-injury or fibrosis [13]. Tsukui et al. reported that profibrotic fibroblasts were immunohistochemically positive for OPN and contributed to the enlargement of fibrotic foci [14]. Elevated levels of OPN expression were observed in both the murine model of pulmonary fibrosis and patients with IPF [15, 16].

Periostin and fibulin-3 are matricellular proteins that exert various effects by binding to integrins on the cell surface [17, 19]. Periostin accelerates inflammation and fibrosis; hence, it may be involved in fibrogenesis and could be a potential biomarker for predicting disease progression and therapeutic efficacy in IPF patients [17]. Fibulin-3 is a member of the fibulin family and a disulfide-rich glycoprotein encoded by epidermal growth factor-containing fibulin-like extracellular matrix protein 1 (EFEMP1) [20–22]. Fibulin-3 may mediate cell signal transduction by bridging fiber-connecting factors and laminin in the extracellular matrix (ECM) and regulate cell proliferation in a contextual manner [22]. Plasma fibulin-3 was reported to be a potential diagnostic marker for mesothelioma and the workers exposed to asbestos-contaminated minerals also showed high levels of fibulin-3 [20, 21].

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

Methods

Study design and population

A case–control study of HCs and patients with silicosis and asbestosis was conducted using commercially available enzyme-linked immunosorbent assay (ELISA) to detect the plasma concentrations of CCL18, CXCL13, OPN, periostin, and fibulin-3. All participants underwent chest X-ray, chest high-resolution computed tomography (HRCT), and pulmonary function test.

Over approximately two years (from January 2018 to December 2019), patients from the Department of Occupational Medicine and Toxicology, Beijing Chao-Yang Hospital, were inducted into the study. A total of 231 and 163 outpatients had been recently diagnosed with silicosis and asbestosis, respectively. They were diagnosed according to the radiological criteria of pneumoconiosis based on the 2011 International Labour Organization (ILO) classification.²³ Patients with bronchial asthma, tuberculosis, pneumonia, autoimmune diseases, liver or kidney dysfunction, and malignant tumors were excluded. The HCs group comprised 118 age-, sex-, and body mass index (BMI)-matched healthy volunteers from the health examination center of Beijing Chao-yang Hospital during the same time period.

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[A1]), and smokers (currently smoking or had

quit smoking <12 months before the study period). Cigarette smoking is shown by pack-years.

This study was approved by the Institutional Ethics Committee for Human Research, Beijing Chao-Yang Hospital, Capital Medical University, China. Written informed consent was obtained from all the participants involved in the research.

Occupational dust exposure

All the patients provided information on their occupational histories through a standardized questionnaire. The 231 silicosis patients were local residents who had been exposed to silica dust during the processing of jade (71, 30.7%), excavation and digging (68, 29.4%), mining and quarrying (49, 21.2%), sand blasting (13, 5.6%), and raw material handling (30, 12.9%). The 163 asbestosis patients were local residents who had been exposed to chrysotile dust or fibers; among them, 127 (77.9%) and 36 (22.1%) patients were exposed to asbestos products during textile production and in a working atmosphere (e.g., heat insulation workers and boiler maintenance workers), respectively.

Evaluation of CCL18, CXCL13, OPN, periostin, and fibulin-3 concentrations

The plasma samples were collected from each participant and stored at -80°C for analysis. All samples were measured within two weeks of storage. 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.

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).²⁴ Pulmonary function parameters were measured using spirometry, whole body plethysmography, and single-breath diffusing capacity for carbon monoxide (DL_{CO} SB) measurements.

HRCT Scan

HRCT scans of patients were performed with 0.625-mm sections, a 1-s scan time, and a 10-mm interval in the apex basescans with the inclusion of both lungs in the field of view.

Statistical analysis

Data are expressed as mean $\pm$ 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. Correlations between parameters were assessed using 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. A P value of < 0.05 was considered statistically significant. Statistical analyses were performed using GraphPad Prism 6 (GraphPad Software Inc, San Diego, CA, USA) and SPSS Statistics version 23 (IBM Inc, Chicago, IL, USA).

Results

Demographics of the participants

The demographics of each participant group are summarized in Table 1. There were no significant differences in sex, age, or body mass index (BMI) among the groups. There were significant differences in 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 DL_{CO} SB showed significant differences among the groups (Table 1).

Plasma concentrations of CCL18, CXCL13, OPN, periostin, and fibulin-3

As shown in Fig. 1, 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.

The plasma concentrations of CCL18 and OPN were significantly different among the various stages of silicosis (Table 2). The plasma concentrations of CCL18, CXCL13, and OPN were not significantly different among the various stages of asbestosis.

Correlations between the CCL18, CXCL13, and OPN concentrations and pulmonary function parameters in silicosis or asbestosis

Pulmonary function was assessed for each patient in the validation study. As shown in Fig. 2, among the silicosis patients, the plasma CCL18, CXCL13, and OPN concentrations were negatively correlated with the predicted values of FVC% and DL_{CO} SB% (both $P < 0.05$).

No correlations were observed between the CCL18, CXCL13, and OPN concentrations and pulmonary function parameters in asbestosis patients.

ROC curve analysis 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 between silicosis or asbestosis patients and HCs (Table 3). Based on the area under the curve (AUC), the order of the diagnostic accuracy was observed to be CCL18, OPN, and CXCL13 in either silicosis alone

or asbestosis alone. The combined sensitivity and specificity of these three biomarkers were 93.1% and 89.1% respectively for diagnosing silicosis; and 90.1% and 89.3% respectively for diagnosing asbestosis.

Discussion

In the present study, we showed that the concentrations of the plasma biomarkers CCL18, CXCL13, and OPN were higher in silicosis or asbestosis patients than in HCs. The plasma concentrations of CCL18 and OPN were also significantly different among the various stages of silicosis. In silicosis patients, the plasma CCL18, CXCL13, and OPN concentrations were negatively correlated with the predicted values of FVC% and DL_{CO} SB%. The order of diagnostic accuracy according to the ROC curve was CCL18, OPN, and CXCL13 in silicosis or asbestosis patients. The combination of CCL18, CXCL13, and OPN may improve the diagnostic sensitivity to silicosis or asbestosis.

CCL18 is a small protein derived from alveolar macrophages that acts as a chemoattractant, which is predominantly expressed by alveolar macrophages and occurs at relatively high levels in the lung tissue[25, 26]. The alveolar macrophages of the pulmonary fibrosis patients showed an alternatively activated phenotype, which upregulated the collagen production by lung fibroblasts through the production of CCL18[26]. The potential relationships between the elevated serum level of CCL18 and clinical outcomes including acute exacerbation and survival in IPF patients have been demonstrated [6, 27], and has been confirmed through data from two randomized controlled trials [28]. The elevated serum levels of CCL18 were also found to be correlated with death or pulmonary disease progression in the patients with systemic sclerosis (SSc)-associated interstitial lung diseases (ILD) [29]. 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 DL_{CO} SB% in the silicosis group. The plasma levels of CCL18 may be used to evaluate the severity of silicosis.

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, 30]. The bronchial epithelial cells and alveolar macrophages of the lungs may produce OPN. In the murine model of lung fibrosis, the elevated OPN levels may accelerate the progression of fibrosis by mediating fibroblast migration, adhesion, and proliferation [30]. OPN is able to regulate the expression of matrix metalloproteinase (MMP) to promote the accumulation of extracellular matrix (ECM) [31]. OPN is a potential diagnostic marker of IPF; plasma surfactant protein (SP)-D > 31 ng/ml, MMP-7 > 1.75 ng/ml, and OPN > 6 ng/ml each significantly distinguished patients with IPF from patients with alternative idiopathic ILD (a-ILD) [32]. In addition, elevated OPN can potentially predict the acute exacerbation and patient survival in IPF[16]. 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 DL_{CO} SB% in the silicosis group. The plasma levels of OPN may be used to evaluate the severity of silicosis.

CXCL13 is a critical agent for B-cell homing to inflammatory foci, which is implicated in the pathogenesis of several immunologic disorders [10–11, 13]. CXCL13 is produced by alveolar macrophages (AM) in patients with IPF and TNF- α and IL-10 regulate its production [10]. CXCL13 is associated with disease severity and survival in IPF patients [11]. 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. The plasma CXCL13 level increases significantly when IPF is accompanied by pulmonary hypertension or acute exacerbation [11]. When the plasma CXCL13 concentration increases by more than 50%, it indicates that the patient may have respiratory failure and a poor prognosis [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 SSc, especially ILD and digital ulcers [34]. 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 DL_{CO} 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 without silicosis or asbestosis as a control group. Third, the cross-sectional design did not disclose the association between biomarkers and disease progression or survival, which are clinically significant. 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 demonstrated that CCL18, CXCL13, and OPN are available biomarkers for the adjuvant diagnosis of silicosis or asbestosis, and may be used to assess the severity of silicosis. Further research is required to confirm that the levels of CCL18, CXCL13, and OPN can indicate early occupational lung fibrosis in workers exposed to dusts. The use of these biomarkers can potentially facilitate suitable therapeutic interventions against the disease.

Abbreviations

AUC: The area under the curve; CCL18:CC-chemokine ligand 18; CXCL13:CXC motif chemokine 13; DLCO: Diffusing capacity of the lung for carbon monoxide; ECM:extracellular matrix; ELISA: Enzyme-linked immunosorbent assay; FEV₁: Forced expired volume in the first second; FVC: Forced vital capacity; HCs: Healthy controls; HRCT: High-resolution computed tomography; ILD: interstitial lung diseases; IPF: Idiopathic pulmonary fibrosis; MMP: Matrix metalloproteinase; OPN:osteopontin; ROC: Receiver operating characteristic; SP-D: Surfactant protein D

Declarations

Ethics approval and consent to participate

All investigations were conducted in accordance with the ethical standards of Beijing Chao-Yang Hospital and the World Medical Association Declaration of Helsinki. This work was conducted at Beijing Chao-Yang Hospital with approval from the ethics committee of Beijing Chao-Yang Hospital, Capital Medical University (No. 2018-Ke-119). Written informed consent was obtained from all participants.

Consent for publication

Not applicable.

Availability of data and materials

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

Competing interests

None declared.

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Authors' Contributions

NW was responsible for completing the analysis of data and writing. NW and CX performed all data collection. YF, CD, and RM were responsible for recruiting the patients, collecting plasma samples, and making enzyme-linked immunosorbent assay (ELISA). QY contributed as the primary investigator and was responsible for designing the study, recruiting the patients, and writing the manuscript. All authors have read and approved the final manuscript.

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Tables

Table1 Demographics of the study participants

	Silicosis	Asbestosis	HCS	P Values
N	231	163	118	
Male:Female	142:89	99:64	73:45	0.867
Age (years)	65.2±11.5	68.2±8.0	63.1±10.8	0.137
BMI (kg/m ²)	24.8±4.1	25.6±4.2	25.5±3.1	0.054
Smoking status				
Current smoker (n)	116	32	25	0.000
Commulative pack-years, median(IQR)	17.8[1.8-41.3]	15.0[1.5-50.0]	13.0[0.5-23]	0.006
Pulmonary function test				
FVC (predicted %)	68.7±42.4	71.5±21.6	83.7±16.4	0.000
FEV ₁ (predicted %)	56.8±40.1	68.3±21.8	81.6±11.3	0.000
FEV ₁ /FVC (%)	69.2±23.6	83.3±21.1	83.8±10.6	0.000
TLC (predicted %)	72.4±39.4	76.2±21.4	86.7±9.2	0.002
DL _{CO} SB (predicted %)	73.7±24.4	63.8±20.7	80.3±8.7	0.010
CPI	24.2±17.8	35.9±17.4	9.7±2.1	0.006
PaO ₂ [mmHg,M[P25-P75] (room air,at rest)	83.3[74.0-90.7]	82.2[73.1-88.7]	90.2[72.0-94.6]	0.236
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

Data are presented as n, percentage, or mean± SD.

BMI, body mass index; HCs, healthy controls; FVC, forced vital capacity; FEV₁, forced expiratory volume in first second; DL_{CO}SB, diffusing capacity of the lung for carbon monoxide; CPI, composite physiologic index; PaO₂, arterial partial pressure of oxygen; NA, not available.

P values were computed by chi-square test for sex and smoking status, one-way analysis of variance for age, BMI, FVC, FEV₁, FEV₁/FVC ratio, DL_{CO}SB, CPI and PaO₂. t test was used to estimate stages of pneumoconiosis.

Table 2 Plasma concentrations of CCL18, CXCL13, OPN and periostin in the patients with various stages of silicosis

	StageI	StageII	StageIII	PValues
N	68	61	102	
CCL18ng/ml	67.0±22.9	67.0±21.7	80.8±29.7	0.023
CXCL13pg/ml	80.6±66.5	79.8±55.9	99.2±70.2	0.209
OPNng/ml	72.2±48.8	88.1±43.9	145.0±76.7	0.000
Periostinng/ml	15.2±9.7	13.2±7.9	16.2±4.6	0.148

Data are presented as mean±SD.

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.

Table 3 Cut-off values and the identifying ability of CCL18, OPN and CXCL13 by ROC curve analysis

	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

CCL18,CC-chemokine ligand 18; CXCL13,CXC motif chemokine 13; OPN,osteopontin.

Figures

Figure 1

Comparisons of plasma concentrations of CC-chemokine ligand 18 (CCL18), CXC motif chemokine 13 (CXCL13), osteopontin (OPN), periostin and fibulin-3 among the study groups using the Kruskal–Wallis test. * P<0.05, ** <0.01

Figure 2

Correlations between CCL18, CXCL13 and OPN and pulmonary function parameters in the patients with silicosis.

Figure 3

(a) Receiver operating characteristic (ROC) curve analysis to differentiate patients with silicosis from HCs. (b) ROC curve analysis to differentiate patients with asbestosis from HCs