Plasma metabolic profiling of the patients with silicosis and asbestosis: a case-control study

Mi Zhou
Beijing Chaoyang Hospital
Changjiang Xue
Beijing Chaoyang Hospital
Yali Fan
Beijing Chaoyang Hospital
Na Wu
Beijing Chaoyang Hospital
Jing Ma
Beijing Chaoyang Hospital
Qiao Ye (yeqiao_chaoyang@sina.com)
Beijing Chaoyang Hospital, Capital Medical University https://orcid.org/0000-0002-0932-0487

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Abstract

Background Silicosis and asbestosis are types of pneumoconiosis with distinct clinical characteristics and underlying metabolisms, induced by inhalation of silica and asbestos, respectively. However, metabolic profiling involved in the diseases is still unknown. The plasma metabolic profiling was compared among silicosis, asbestosis and healthy controls.

Methods In a case-control study, plasma was collected from 30 patients with silicosis, 30 patients with asbestosis and 20 healthy controls. Metabolic profiling was carried out using liquid chromatography mass spectrometry. Metabolic networks and the biological relevances of the identified metabolic derangements were identified using Kyoto Encyclopedia of Genes and Genomes database.

Results Compared with the healthy controls, 37and 39 metabolites were detected in the plasma of patients with silicosis and asbestosis, respectively, of which 22 metabolites were present in both silicosis and asbestosis. These metabolites were mainly lipid, amino acid and carnitine. The plasma metabolites in silicosis included 23 lipids, 6 amino acids and 8 carnitines, of which 13 lipids, 4 amino acids and 7 carnitines were up-regulated, respectively. Similarly, 11 out of 13 lipids, 11 out of 14 amino acids, and 12 out of 12 differentially expressed carnitines were up-regulated in asbestosis patients. The expression levels of 22 metabolites differed significantly between the patients with silicosis and asbestosis. Three major common pathways were identified among the three groups, including arginine and proline metabolism, glycine, serine and threonine metabolism, and alanine, aspartateand glutamate metabolism.The KEGG database identified arginine and proline metabolism as the most closely related metabolic pathway.

Conclusions Metabolic profiling of silicosis and asbestosis identified lipids, aminoacids and carnitines that were common to both groups, but the types and quantities of the up-regulated substances differed between the groups. Pathways inducing lung inflammation and fibrosis are common in both diseases, while pathways related to oxidative stress and tumorigenic are different between silicosis and asbestosis.

Background

Pneumoconiosis is a group of heterogeneous diseases characterized by diffuse lung tissue fibrosis caused by long-term inhalation of productive dust and its retention in the lungs. Such exposure usually occurs in occupational situations, and pneumoconiosis is thus generally considered as an occupational lung disease [1]. Silicosis is one of the most common subtypes of pneumoconiosis in China, in line with the country’s rapidly growing industrialization [2]. In addition, China currently sustains a high level of asbestos production and use, resulting in a long-term asbestos-related disease burden[3]. Despite the classification in pneumoconiosis that silicosis and asbestosis are induced by inhalation of various inorganic dusts, the clinical features and underlying mechanisms have differences and similarities. Silicosis is caused by the inhalation of crystalline silica dust, marked by inflammation and fibrosis in the

form of nodular lesions, predominantly in the upper lobes of the lungs [1].It is characterized by dyspnea on exertion, cough, chest pain and cyanosis, and may be misdiagnosed as lung cancer or tuberculosis. Asbestosis is a chronic, progressive, diffuse and irreversible pulmonary fibrosis, with pleural plaque formation and hypertrophy caused by the long-term inhalation of asbestos fibers, which severely impairs the patient's lung function and significantly increases the incidences of lung and pleural malignancies [4]. Compared with silica, asbestos fibers are thinner, more penetrating and more easily distributed throughout the lungs because of their specific physical properties. Chest images showed that diffuse reticular are predominantly on the bilateral lower lobes, mimicking idiopathic pulmonary fibrosis (IPF)[4, 5]. Asbestos exposure can also cause various malignant tumors such as bronchial lung cancer and pleural mesothelioma[6], and asbestosis patients have a higher risk of lung cancer[7].

Silica and asbestos are complex natural minerals with distinct physical and chemical characteristics, and the mechanisms of pulmonary interstitial fibrosis caused by crystalline silica and asbestos fibers have been extensively studied. Animal experiments showed that interleukin-6 (IL-6), IL-1β, tumor necrosis factor-α, and other inflammatory mediators in lung tissue and bronchial lavage fluid were elevated in the silica or asbestos-induced mouse models [8–11], and changes in these inflammatory cytokines have also been found in clinical and in vitro studies [12–15]. Crystalline silica and asbestos fibers entering the airways are engulfed by macrophages, resulting in phagocyte necrosis and consequent release of the internalized silica and asbestos particles, which are then engulfed by other macrophages. This repeated process of phagocytosis, necrosis, and re-phagocytosis induces the inflammation and activation of the reactive oxygen species system, associated with pulmonary fibrosis and cancer [16–18]. Among oligomerization domain-like receptors, activation of the NLRP3 inflammasome appears to be a pathogenic pathway following exposure to either crystalline silica or asbestos fibers, leading to pulmonary fibrosis and lung cancer [19]. Studies of crystalline silica and asbestos fibers suggested that the up-regulation of inflammatory cytokines after dust inhalation was related to the Fas/FasL pathways, which were in turn thought to be related to fibrosis [13, 20]. Previous studies also suggested that asbestos pathogenesis may involve endoplasmic reticulum stress [21].

Asbestosis and silicosis have similar fibrotic processes but different pathological appearances and prognoses. Metabolic profiling could identify clinically distinct silicosis and asbestosis subgroups and thus help to identify potential therapeutic targets. Metabolomics is a sensitive method for detecting subtle alterations in biological pathways by quantitative profiling of metabolites in biofluids, cells and tissues. Recent studies showed that the occurrence of IPF, cystic fibrosis and other interstitial lung diseases were related to metabolic abnormalities [22–25]. However, despite the different chemical properties of silica and asbestos fibers, the metabolic differences between silicosis and asbestosis remain unknown. In the current study, we explored the similarities and differences in circulating metabolites in patients with silicosis and asbestosis and compared them with healthy controls by liquid chromatography-mass spectrometry (LC-MS)-based metabolomics analysis.

Materials And Methods

Study populations

This was a case–control study conducted in individuals with silicosis or asbestosis and healthy controls using a metabolomics approach. Thirty patients with silicosis and thirty with asbestosis were recruited from Department of Occupational Medicine and Toxicology, Beijing Chao-Yang Hospital, China, between January and June, 2018. All patients were diagnosed according to the diagnostic criteria for pneumoconiosis based on the 2011 International Labor Office classification [26]. Patients with interstitial lung diseases other than pneumoconiosis, including pulmonary infection, autoimmune disease, liver disease, intestinal disease, hyperlipidemia, uncontrollable diabetes and malignancy were excluded. Twenty healthy volunteers matched for age, sex and smoking status were recruited from the healthy examination center of Beijing Chao-Yang Hospital during the same period. The screening process for the enrolled patients is shown in Fig. 1. Participants were classified as non-smokers or smokers, of which smokers included current smokers or people who had quit smoking <12 months ago. Cigarette smoking was indicated by pack-years. To avoid bias caused by emphysema, the composite physiologic index (CPI) was selected as a predictor of severity of pulmonary fibrosis[27]. All patients were asked to complete a standardized questionnaire to collect information about their occupational history. All jobs throughout the individuals' working life were taken into account. Thirty patients with silicosis were local residents who had been exposed to silica dust through processing of excavation and digging (n=16, 53.3%), polishing and buffing (n=8, 26.7%), abrasive blasting and sand blasting (n=3, 10%) and handling raw materials (n=3, 10%) in factories operating from the 1960s to 1990s. Thirty patients with asbestosis were local residents who had been exposed to chrysotile dust or fibers in asbestos products during the manufacture of asbestos textiles (n=27, 90%) or asbestos-based products (n=3, 10%) during heat insulation or boiler maintenance work. The asbestos-product plants were in operation from the 1950s to 1970s. Our hospital was the center for occupational diseases located 20–30 km from these plants. 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.

This study 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.

Samples collection

A volume of 5 mL of venous blood was collected from each participant after overnight fasting and was centrifuged at 2,750 rpm at room temperature for 10 min within 40 min after collection. The plasma samples were then stored at −80℃ until analysis.

Plasma samples preparation and metabolomics analysis

The frozen plasma samples were thawed at 4℃ and 100μL aliquots and were added to 300μL methanol or acetonitrile. Plasma (100μL) was added to labeled 1.5-mL centrifuge tubes with the required markers at known concentrations. The samples were separated by reversed-phase chromatography and

hydrophilic chromatography, respectively, as described previously [28]. Briefly, for reversed-phase chromatography, plasma samples were thawed, and chloroform/methanol was added. The samples were then ultrasonicated. Water was added to the mixture followed by centrifugation, and the lower chloroform layer was concentrated and dried. Isopropyl alcohol/acetonitrile was then added, and the sample was dissolved by ultrasonication. The solution was centrifuged, and the supernatant was transferred for analysis. For hydrophilic chromatography, acetonitrile was added to the thawed plasma samples. The mixture was fully oscillated, and the upper layer was taken for analysis. The sample quality was tested at the beginning of the test and after every seven samples to monitor the process and ensure the reliability of the data.

The samples were subjected to mass spectrometry using a hybrid quadrupole or bitrap mass spectrometer (Q Exactive, Thermo Fisher Scientific, Beijing, China) equipped with a HESI-II probe (Thermo Fisher Scientific, Beijing, China). The liquid quality system was controlled by Xcalibur 2.2 SP1.48 software. Data acquisition and quantitative treatment of targeted metabolites were controlled by the same software. Relevant metabolic pathways and networks were identified using MetaboAnalyst 4.0 software as a web-based metabolomics data processing tool and the Kyoto Encyclopedia of Genes and Genomes (KEGG), a self-sufficient and integrated resource consisting of genomic, chemical and network information.

Statistical analysis

The clinical data were expressed as numbers, percentages and mean ± standard deviation. Differences in measured data among groups were analyzed by analysis of variance (ANOVA) and least significant difference *t*-test. Measured data that did not conform to a normal distribution were analyzed by Kruskal–Wallis or Mann–Whitney *U* test. The calculators were analyzed by χ2 test. Statistical analyses were carried out using SPSS 23.0 for Windows (IBM, Armonk, NY, USA), and $P < 0.05$ was considered statistically significant.

To confirm certain metabolites as selective and sensitive biomarkers of pathogenesis in the respective groups, differences in metabolites between the two groups were analyzed by orthogonal partial least squares discriminant analysis (OPLS-DA) and clear separation between the groups was demonstrated by OPLS-DA score scatter plots. Significantly different metabolites between groups were filtered out based on variable importance for projection (VIP) values (VIP > 1) and *t*-test results ($P < 0.05$).

Results

Demographics of the participants

The demographics of the participants are shown in Table 1. Patients with silicosis were significantly younger and mainly male compared with patients with asbestosis (both $P<0.05$). The latency period of asbestosis was significantly longer than that of silicosis($P<0.001$). Forced vital capacity (FVC) %pred and diffusing capacity for carbonmonoxide of the lungs (DLCO) %pred were both significantly lower in the

asbestosis compared with the silicosis group (both $P<0.05$). Arterial partial pressure of oxygen (PaO2) at rest in room air and CPI also differed significantly between the silicosis and asbestosis groups(both $P<0.05$).

Chest HRCT scans showed multiple well-defined nodules and/or progressive massive fibrosis predominantly in the upper lobes in the silicosis group, compared with lower lobe reticular abnormalities or interstitial pneumonia pattern with pleural thickening in the asbestosis group.

Different metabolomic profiles of patients with silicosis and asbestosis and healthy controls

Samples were analyzed by reversed-phase and hydrophilic chromatography and the results were subjected to principal component analysis (PCA) to verify the presence of the three groups. The PCA score plot had considerable outliers and indicated distinctseparation among the groups (Fig. 2A). Regions distant from the origin represented metabolites that differed among the groups, while regions near the origin of the plot represented metabolites that were similar in all groups.

OPLS-DA was applied to the data to maximize the separation among the groups and to identify metabolites able to discriminate between groups. The performance statistics of the OPLS-DA model indicated a good separation between controls and patients with silicosis (R2Y = 0.953, Q2 = 0.904) and between patients with asbestosis and healthy controls (R2Y = 0.985, Q2 = 0.939) (Fig. 2B, 2C). We also compared patients with silicosis and asbestosis and the OPLS-DA score plot demonstrated clear separation between the silicosis and asbestosis groups (R2Y = 0.952, Q2 = 0.632) (Fig. 2D). Similar clear separation was indicated by the hydrophilic chromatography results.

Identification of metabolites

Metabolites in silicosis/asbestosis patients compared with healthy controls

We explored metabolites potentially able to discriminate between patients with silicosis or asbestosis and healthy controls, which is a case-control study. To the best of our knowledge, this represents the first comparative study to assess the metabonomics of pneumoconiosis. Some metabolites differed between patients with silicosis or asbestosis and healthy controls (Fig. 3A, 3B). Compared with the healthy controls, 37 and 39 metabolites were detected in the plasma of patients with silicosis and asbestosis, respectively, of which 22 were present in both the silicosis and asbestosis groups. These metabolites were mainly lipids, amino acids and carnitines. The differential metabolites in silicosis patients included 23lipids, six amino acids and eight carnitines, of which 13, four and seven, respectively, were up-regulated. Similarly, 13 lipids, 14 amino acids and 12 carnitines were differentially detected in the asbestosis group, of which 11, 11, and 12 were up-regulated.

Comparison of metabolites between patients with silicosis and asbestosis

We further clarified the differences in plasma metabolites between patients with silicosis and asbestosis. Twenty-two metabolites were significantly differentially detected between the two groups (Fig. 3C).

Plasma levels of 11 lipids, 8 amino acids and 3e carnitines were higher in asbestosis compared with silicosis patients.

Metabolic pathways in silicosis and asbestosis groups

To identify the metabolic networks and the biological relevance of the identified metabolic derangements in the silicosis and asbestosis groups, we analyzed the results using MetaboAnalyst 4.0 software and the KEGG database. The pathways identified in the silicosis and asbestosis groups were not identical (Fig. 4A, 4B). Among three groups, the three major common pathways were arginine and proline metabolism, glycine, serine and threonine metabolism and alanine, aspartate and glutamate metabolism. The KEGG database identified arginine and proline metabolism as the most closely related metabolic pathway (Fig. 4C).

Metabolites in silicosis and asbestosis subgroups according to their severity

To further analyze the levels of plasma metabolites under different severity of the disease, the silicosis group were divided into simple silicosis group (SIL-1) and complicated silicosis group (SIL-2) identified from the clinical, radiological and functional data[26]. Similarly, the asbestosis group was classified into ASB-1 (DLCO ≥ 60% pred) and ASB-2 (DLCO < 60% pred) according to the diffusing capacity of lung function. We then compared the plasma metabolites of the silicosis and asbestosis subgroups according to the severity of the disease with those of the healthy controls, separately (Fig. 5A, 5B). We also present heat maps of plasma metabolites of silicosis and asbestosis subgroups compared with healthy controls (Fig.S1A, B).

Discussion

The current physiological and HRCT results revealed differences in clinical characteristics between silicosis and asbestosis. Asbestosis had a longer latency than silicosis, suggesting that asbestos dust might have a slower pathogenic effect than silica dust. Pulmonary function values, including FVC and DLCO, were lower in the asbestosis group, and PaO2 and CPI also differed between the two groups. In terms of HRCT appearance, chest imaging showed small round shadows or progressive massive fibrosis in patients with silicosis, compared with irregular reticular abnormalities in the lower lobes with pleural thickening in patients with asbestosis.

These differences in the clinical characteristics and underlying mechanisms of silicosis and asbestosis groups warranted further study. We therefore performed a pilot study using LC-MS-based metabolomics to discriminate between silicosis and asbestosis and thus improve the precision of health care. Compared with healthy controls, 37 and 39 metabolites were differentially detected in the plasma of patients with silicosis and asbestosis, respectively, of which 22 occurred in both the silicosis and asbestosis groups. Lipids, amino acids and carnitines were the main metabolites associated with silicosis and asbestosis, and most of the differentially expressed metabolites were up-regulated, especially in patients with asbestosis. We also found that plasma metabolites may differentially

expressed in silicosis and asbestosis patients according to the disease severity. The severity of silicosis or asbestosis varies, as do its plasma metabolites, some of which may be associated with disease severity.

Lipids are the components of cell membranes and are an essential human nutrient. In addition to their structural role in cell membranes, they are also involved in energy storage, signal transduction, enzyme activation, growth factors, antioxidants, signal recognition and immunity [29]. A previous metobonomics study found that the sphingolipid metabolic pathway was down-regulated; the arginine pathway was up-regulated, and glycolysis, mitochondrial beta-oxidation and the tricarboxylic acid cycle were disrupted in lung tissue from patients with IPF [30]. The energy consumption during lung structural remodeling may contribute to the pathogenesis of IPF. Excessive lipid metabolism in the present study indicated abnormal cellular energy metabolism inpatients with silicosis and asbestosis, suggesting similar metabonomic changes in non-IPF chronic lung fibrotic diseases. Pneumoconiosis involves lung inflammation caused by organic dust, and proteins, carbohydrates and especially lipids were elevatedin pulmonary edema fluid in acute respiratory distress syndrome, which is also an inflammatory condition [31]. Lysophosphatidic acid (LPA) is produced by activated platelets and fibroblasts and was increased in damaged skin and fibrotic lung in various fibrosis models [32]. The hydrolysis of lysophosphatidylcholine by lysophospholipase D/autotaxin represents one pathway for LPA synthesis. In the current study, lysophosphatidylcholine levels were significantly higher in patients with silicosis or asbestosis compared with healthy controls, and LPA levels were the highest in the asbestosis group. In addition, lipid mediators exert wide-ranging and sometimes opposing effects on multiple tissues. Compared with a healthy control group, bronchial lavage fluid levels of prostaglandin E2 were significantly increased in IPF patients, but there was no significant difference between the two groups in serum levels of prostaglandin E2 [33]. In the present study, patients with asbestosis had more severe pulmonary dysfunction than those with silicosis due to the extensive lung fibrosis. The percentage of lipid up-regulation was also higher in the asbestosis compared with the silicosis group (84.62% vs 56.52%, $P < 0.05$), which may indicate the different levels of protective lipid metabolites.

Amino acids are the basic components of proteins and are required as raw materials for protein synthesis. They also participate in the formation of enzymes, hormones and some vitamins. Ornithine can also be converted to proline and hydroxyproline for collagen formation in fibrosis [34]. Increased levels of creatine, putrescine, spermidine, 4-hydroxyproline and the proline-hydroxyproline dipeptide were found in fibrotic lung tissue from patients with IPF compared with normal lung tissue [30]. Compared with the healthy controls, plasma levels of amino acids, including ornithine, creatine and hydroxyproline, were largely increased in patients with silicosis and asbestosis. Silicosis and asbestosis were characterized by similar changes in amino acid metabolism to IPF, possibly related to the fibrotic process. As for lipid metabolism, the effects of asbestos fibers on amino acid metabolism were more obvious than those of silica. Glutamate synthase levels were previously shown to be elevated in lung tissue from patients with pleural mesothelioma compared with adjacent normal tissues [35]. Furthermore, L-type amino acid transporters were associated with tumors, and their expression was increased in tumor samples, while expression levels of amino acids differed among different tissues [36]. In the current study, plasma levels

of amino acids were up-regulated in both silicosis and asbestosis, especially in asbestosis. In addition to lung cancer, a prospective population-based study showed that asbestos exposure was also associated with other cancers, such as gastric and colon cancer [37]. A chest HRCT study of patients with asbestosis found that subpleural dots and lines were close to the chest wall and located < 5 mm from the inner chest wall, which may indicate that asbestos fibers, unlike silica, may also potentially cause pleural mesothelioma [38].

Carnitines are amino acid-like substances involved in the metabolism of fat into energy. Carnitines promote the transport and oxidation of fatty acids and the utilization of carbohydrates and amino acids, improve body tolerance, prevent lactic acid accumulation, delay aging and have antioxidant properties[39]. Carnitine levels were significantly decreased in lung tissue, and mitochondrial beta-oxidation was reduced in patients with IPF[30]. In contrast to IPF, plasma carnitine levels were largely up-regulated in the current patients with silicosis and asbestosis (except for one carnitine in the silicosis group), compared with the healthy controls. Further studies are needed to determine if the increase in plasma carnitine levels in patients with silicosis and asbestosis is related to lipid metabolism or to the disease itself.

The present study had several limitations. First, there may have been some selection bias. The enrolled population was from a single medical center and was therefore not fully representative of patients with silicosis and asbestosis. Furthermore, all the enrolled patients were of Chinese Han ethnicity, which may overlook any potential ethnic or geographical effects. This potential inclusion bias might affect the validity of the results. More males were enrolled the study, indicating that men were more prone to silicosis for occupational reasons. Males are at increased risk of exposure to silica dust because they are more likely to be involved in manual labor, such as excavation and digging, polishing and buffing, and more men were therefore enrolled in the present study. Second, the average age of patients with asbestosis was older than that of those with silicosis. However, we previously reported that asbestosis had a longer latency than silicosis, and this discrepancy may therefore be related to the different latencies [40]. Third, we did not include a group with exposure to dust without silicosis or asbestosis because the plants were no longer in operation. Finally, although the systemic metabolic profiling of silicosis and asbestosis is accessible and noninvasive, it may not fully represent the metabolic process of the lungs. Further research on metabolomics may include both plasma and lung tissue for analyzing.

Conclusions

This study emphasized the use of metabolic plasma profiling for characterizing the metabolite signatures of patients with silicosis and asbestosis and identifying changes in metabolic pathways that might be involved in the pathogeneses of these diseases. The results of this study may further our understanding of the metabolic differences between silicosis and asbestosis as well as their disease severity. However, this was a pilot study, and further studies are warranted to explore the metabolic mechanisms of pneumoconiosis. We should expand the sample size to screen and verify the plasma metabolic markers reflecting the specific diagnosis and severity of the disease. Furthermore, the specific metabolic pathways

may provide useful targets for antifibrotic drugs in patients with progressive fibrotic interstitial lung disease such as pneumoconiosis.

Abbreviations

ANOVA: analysis of variance; ARDS: respiratory distress syndrome;ARDs: asbestos-related diseases; ASB: asbestosis patients; BALF: bronchoalveolar lavage fluid; BMI: body mass index; CF: cystic fibrosis; CON: healthy controls;CPI: composite physiologic index; DLCO: diffusing capacity of the lung for carbon monoxide; FEV_1: forced expiratory volume in the first second; FVC: forced vital capacity;HRCT: high resolution computerized tomography; IL: interleukin; ILDs: interstitial lung diseases; ILO: International Labor Organization; IPF: idiopathic pulmonary fibrosis;KEGG: Kyoto Encyclopedia of Genes and Genomes; LATs: L-type amino acid transporters; LC-MS: liquid chromatography mass spectrometry; LPA: lysophosphatidic acid; LPC: lysophosphatidylcholine; NLRs: nucleotide binding and oligomerization domain (NOD)-like receptors; OPLS-DA: orthogonal partial least squares discriminant analysis; PCA: principal component analysis; PGE: prostaglandin E; ROS: reactive oxygen species; SD: standard deviation; SIL: silicosis patients; PaO_2: arterial partial pressure of oxygen; TNF: tumor necrosis factor; UIP: usual interstitial pneumonia; VIP: variable importance for projection.

Declarations

Ethical statement This work was conducted at Beijing Chao-Yang Hospital with approval from the ethics committee of Beijing Chao-Yang Hospital, Capital Medical University. Informed consent was documented in writing.

Consent for publication Not applicable

Competing interests The authors have no conflicts of interest to declare.

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Author's contributions MZ was responsible for completing the analysis of data and writing. MZ and CJX performed all data collection. YLF, NW and JM were responsible for recruiting the patients, collecting plasma samples and making the metabolomics analysis. QY contributed as primary investigator and was responsible for designing the study, recruiting the patients and writing the manuscript.All authors have red and approved the final manuscript.

Data availability statement Data are available upon request.

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Tables

Figures

Table 1 Demographics of the study population

	SIL	ASB	CON	*P*-value	*P1*	*P2*	*P3*
Patients (n)	30	30	20	NA	NA	NA	NA
Age (yrs)	60.83±7.93	69.40±9.54	62.05±7.22	<0.001	<0.001	0.618	0.003
Male (n)	25	14	8	0.002	0.012	0.007	1.000
BMI	23.40±3.00	23.95±2.04	23.61±1.88	0.673	0.378	0.762	0.626
Smokers (n)	15	13	10	0.848	0.608	1.000	0.646
Current smokers (n)	4	3	6	0.151	0.690	0.153	0.074
Smoking (pack-yrs)	16.85±12.82	14.77±7.90	15.1±7.68	0.839	0.586	0.671	0.939
Duration of dust exposure (yrs)	13.02±9.77	14.03±12.16	NA	NA	0.722	NA	NA
Latency (yrs)	25.1±14.01	47.3±11.94	NA	NA	0.000	NA	NA
PaO_2 (mmHg)	85.8±11.51	80.24±8.94	NA	NA	0.041	NA	NA
FVC (%pred)	76.64±7.05	72.78±4.88	81.34±4.27	0.000	0.010	0.005	0.000
FEV_1 (%pred)	74.61±8.40	71.50±8.41	80.16±5.78	0.001	0.129	0.016	0.000
FEV_1/FVC (%)	74.82±4.94	72.50±6.11	80.67±5.38	0.000	0.108	0.000	0.000
DLCO (%pred)	75.87±14.47	66.85±13.99	83.90±3.22	0.000	0.010	0.068	0.000
CPI	26.44±10.12	33.28±9.10	NA	NA	0.008	NA	NA

Data are presented as n, percentage, mean ± SD

Abbreviations: SIL, silicosis group; ASB, asbestosis group; CON, healthy controls; BMI, body mass index; PaO_2, arterial partial pressure of oxygen; FVC, forced vital capacity; FEV_1, forced expiratory volume in first second; DLCO, diffusing capacity of the lung for carbon monoxide; CPI, composite physiologic index; NA, not available

P, compared among three groups; *P1*, SIL vs. ASB; *P2*, SIL vs. CON; *P3*, ASB vs. CON

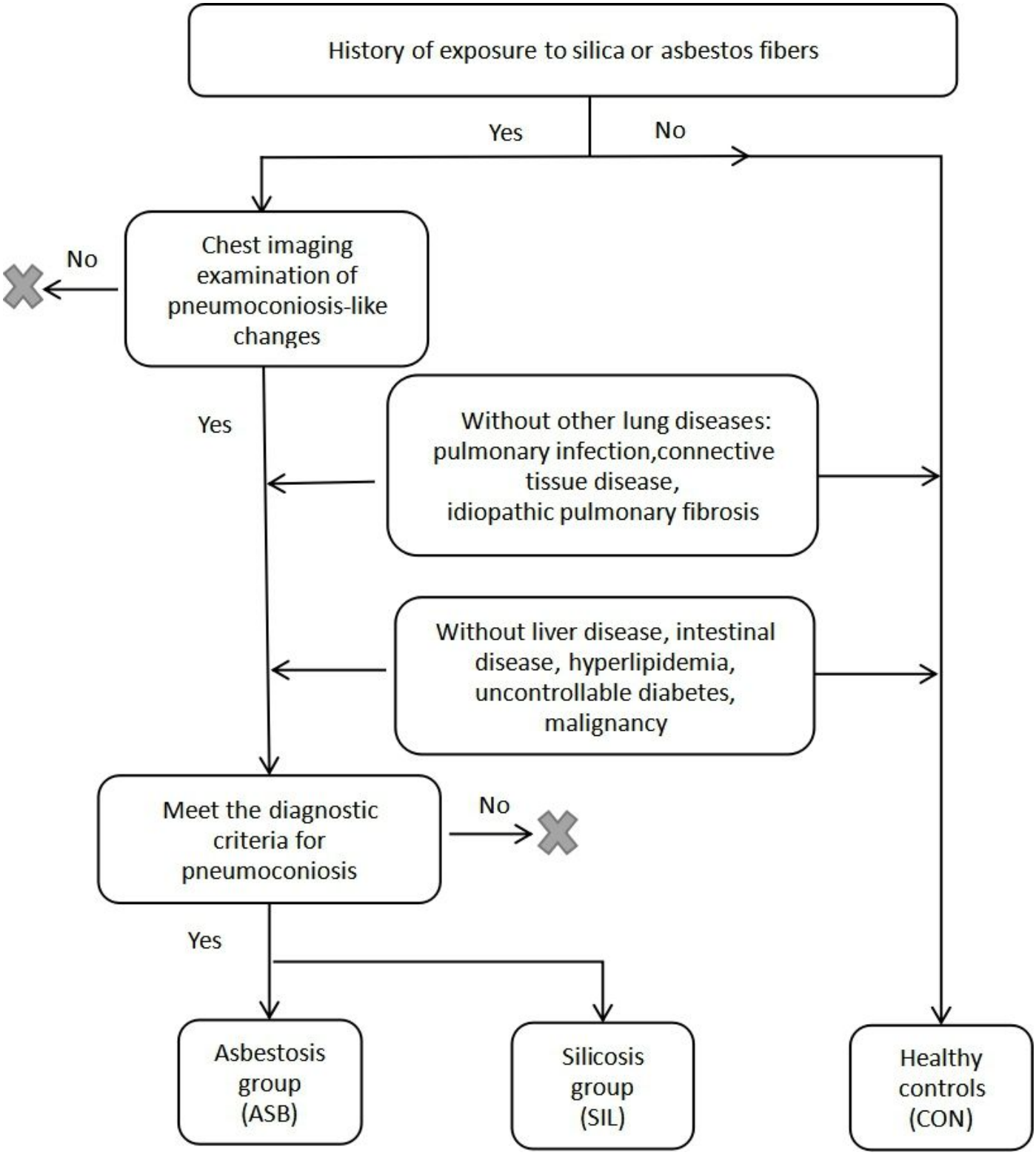

Figure 1

Flow chart of the screening process of the population in this study

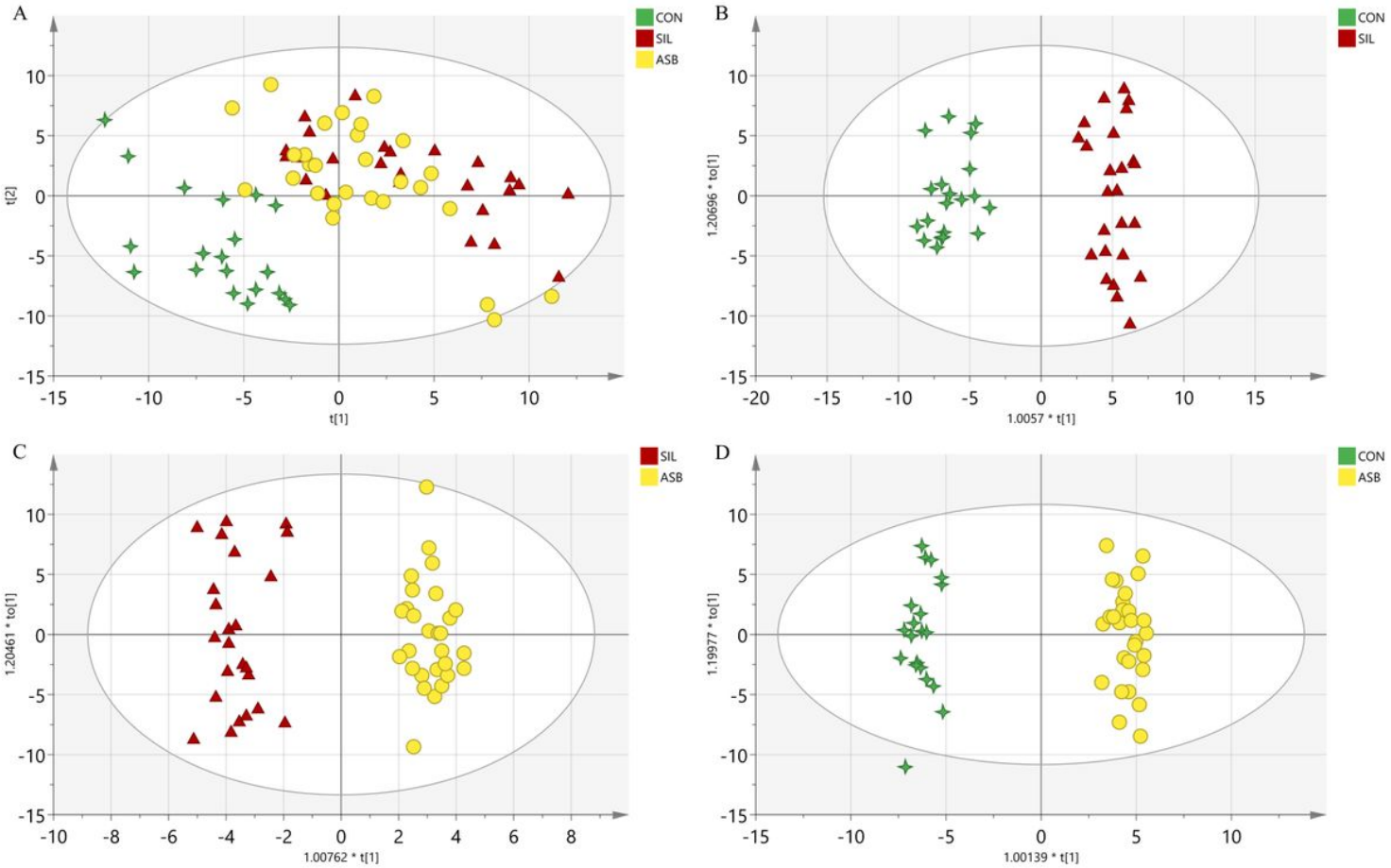

Figure 2

Analysis of plasma samples by chromatography. (A) PCA score plot (R2X = 0.53, Q2 = 0.310) obtained from healthy controls (CON), silicosis group (SIL) and asbestosis group (ASB), (B) OPLS-DA score plot (R2Y = 0.953, Q2=0.904) obtained from CON and SIL, (C) OPLS-DA score plot (R2Y = 0.985, Q2 = 0.939) obtained from CON and ASB, (D) OPLS-DA score plot (R2Y = 0.952, Q2 = 0.632) obtained from SIL and ASB

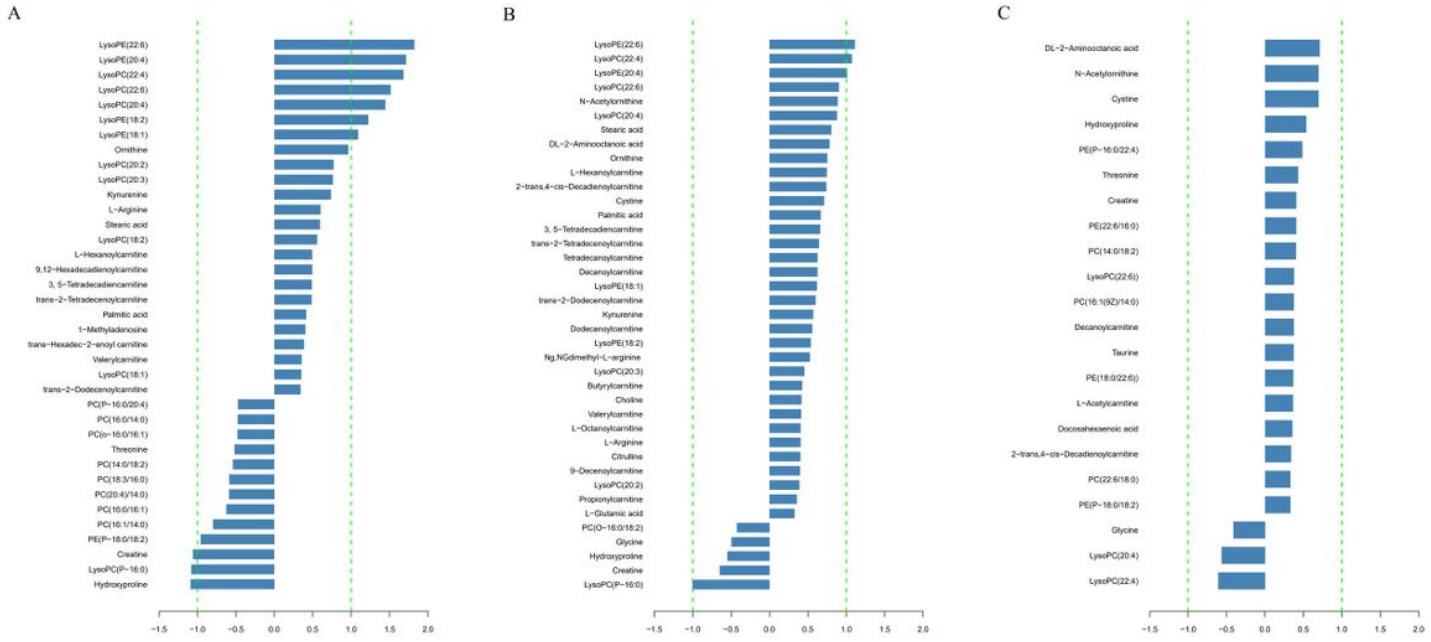

Figure 3

Plasma metabolites in silicosis, asbestosis and healthy controls. (A) silicosis group (SIL) vs healthy controls (CON), (B) asbestosis group (ASB) vs CON, (C) ASB vs SIL

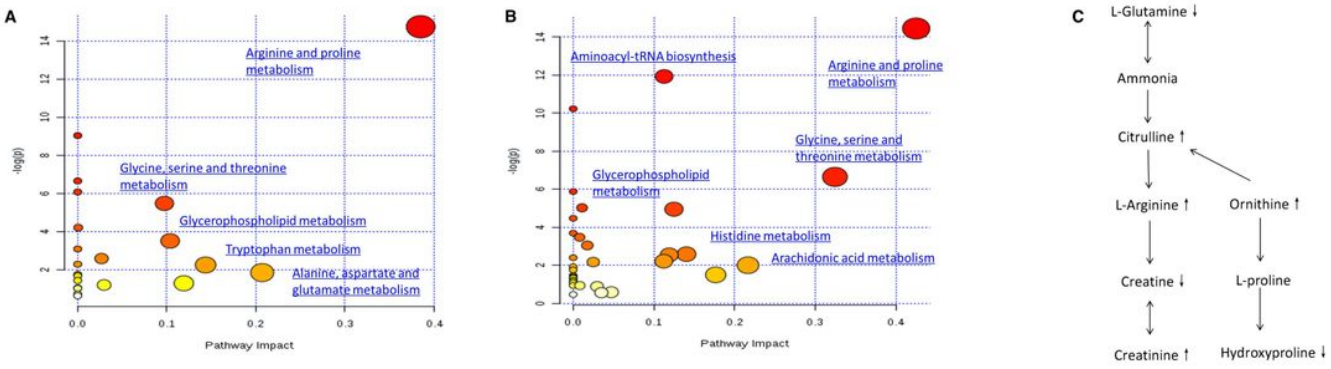

Figure 4

Plasma metabolic pathways in (A) silicosis group (SIL) and (B) asbestosis group (ASB). Metabolic view displays the matched pathways as circles, the color and size of each circle are based on P-value and pathway impact value, respectively. One of the major identical pathways in SIL and ASB: arginine and proline metabolism showed in (C). ↑: increased groups, ↓: decreased in groups

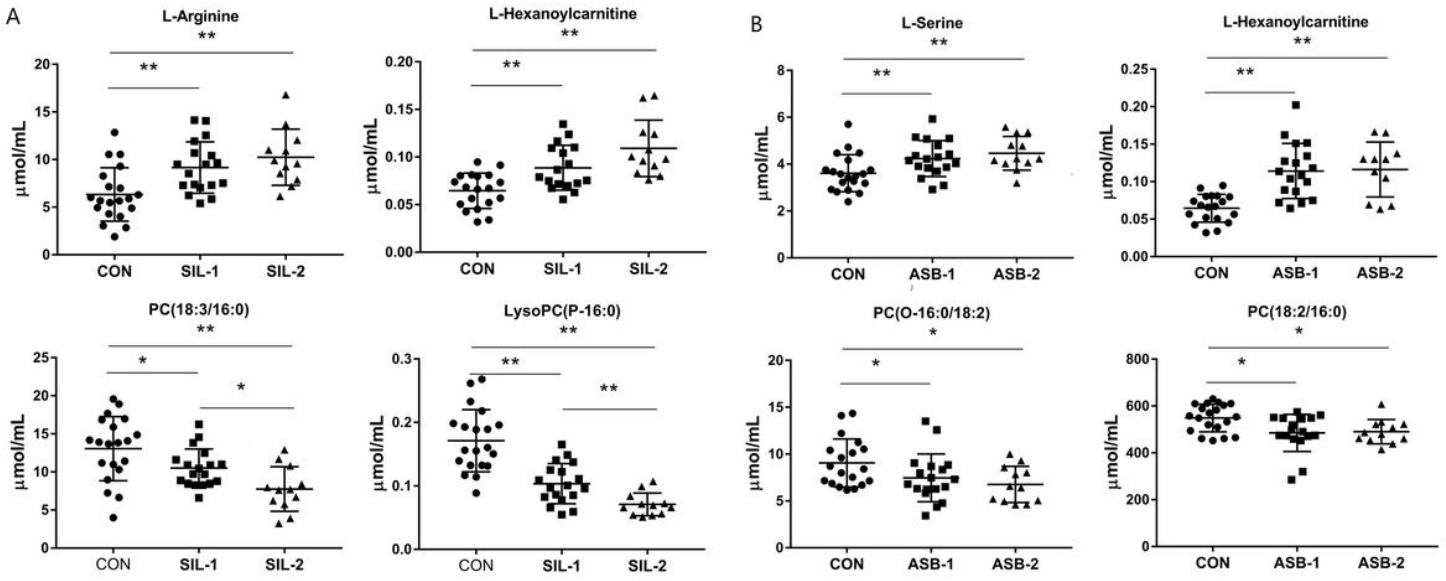

Figure 5

Representative plasma metabolites in the silicosis and asbestosis subgroups according to the severity of disease compared with those in healthy controls (CON). (A) simple silicosis (SIL-1) (n=18) and complicated silicosis (SIL-2) (n=12) vs CON (n=20); (B) asbestosis with DLCO≥60% pred (ASB-1) (n=19) and asbestosis with DLCO<60% pred (ASB-1) (n=11) vs CON (n=20)

Supplementary Files

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- Supplimentaryfile.docx