

PD-1/PD-Ls Pathway Down-regulation in Peripheral Cells Correlates with Asbestosis Severity

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Research article

Keywords: asbestosis, silicosis, lung fibrosis, PD-1/PD-Ls, lung function, T cell activation

Posted Date: October 30th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-99019/v1>

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Abstract

Background: Asbestosis and silicosis are characterized by diffuse or nodular interstitial lung fibrosis resulting from exposure to asbestos or silica dust, respectively. This study was designed to detect programmed cell death protein (PD-1)/programmed death-ligands (PD-Ls) expression in patients with asbestosis and silicosis and to explore the possible clinical significance of PD-1/PD-Ls expression in the two diseases.

Methods: Thirty patients with asbestosis, 23 patients with silicosis and 25 healthy controls were consecutively recruited for and provided informed consent to participate in the study. Clinical data were collected from patient clinical charts. PD-1/PD-Ls expression in peripheral blood (PB) was detected by flow cytometry.

Results: PD-1 expression on CD4⁺ or CD8⁺ peripheral T cells was significantly lower in patients with asbestosis and silicosis than in healthy controls. Similarly, PD-L1 and PD-L2 expression on CD14⁺ monocytes was significantly lower in patients with asbestosis and silicosis than in healthy controls. In addition, there was no significant difference in PD-1, PD-L1 or PD-L2 expression between the asbestosis and silicosis groups. Moreover, the proportions of PD-1⁺ CD4⁺T cells and PD-1⁺ CD8⁺T cells in patients with asbestosis were positively correlated with the percentage of forced vital capacity (FVC) predicted.

Conclusion: Lower PD-1 expression on CD4⁺T or CD8⁺ T cells in PB was positively correlated with parameters representing disease severity, implying that pulmonary fibrosis development was positively correlated with PD-1/PD-Ls pathway down-regulation.

Background

Interstitial lung disease (ILD), diffuse parenchymal lung disease, is a group of diseases which involves in lung interstitium and alveolar cavity, resulting in dysfunction of alveolar-capillary [1]. Pulmonary fibrosis is one of the advanced histopathological features of various ILDs. Pneumoconiosis, an ILD associated with occupational environments, is a heterogeneous group of diseases caused by inorganic mineral dust. The occupational dust exposures induce lung inflammation cascades and structural damage that can lead dust-related lung disorders including pneumoconiosis as well as chronic obstructive pulmonary disease (COPD). In our previous study, occupational dust exposure as well as heavy smoking is associated with an increased risk of combined COPD and pneumoconiosis, especially patients with silicosis and coal workers' pneumoconiosis [2].

Asbestosis and silicosis are two types of pneumoconiosis. Asbestosis is characterized by diffuse interstitial pulmonary fibrosis caused by long-term asbestos exposure. The most common clinical symptom of asbestosis is progressive dyspnoea on exertion. The disease is associated with restrictive lung impairment and decreased diffusing capacity [3, 4]. Chest high-resolution CT (HRCT) shows the presence of usual interstitial pneumonia (UIP) or nonspecific interstitial pneumonia (NSIP) of any

classification mainly involving the small airways [1]. Silicosis is a nodular fibrotic lung disease caused by the inhalation of free crystalline silicon dioxide or silica and is also one of the most important occupational diseases worldwide [5]. In our recent study, we reported an outbreak of accelerated silicosis caused by artificial stone dust. The artificial stone-associated silicosis is characterized by a shorter latency, rapid radiological progression and accelerated loss of lung function, which is different from natural stone-associated silicosis [6].

Asbestosis and silicosis are currently incurable and may be progressive even after dust exposure cessation [7]. The pathogenesis of asbestosis and silicosis remains unclear. Therefore, studies aiming to deepen the understanding of the mechanisms underlying the development of asbestosis and silicosis and to identify potential targets for the treatment of the diseases will provide a foundation for the development of new therapies.

PD-1, also known as CD279, is a member of the B7/CD28 immunoglobulin superfamily. Two PD-1 ligands, PD-L1 (B7-H1 or CD274) and PD-L2 (B7-H2 or CD273) exist [8]. In antigen presentation, PD-1 combines with PD-L1 or PDL2 and then inhibits T cell and B cell activation and reduces cytokine production and killing ability; thus, PD-1 functions as negative regulator in antigen presentation [9].

There are no studies showing whether the PD-1/PD-Ls signalling pathways play a role in cellular immunology in the pulmonary fibrotic diseases caused by asbestos or silica. We explored PD-1/PD-Ls pathway expression in asbestosis or silicosis and elucidated the relationship between pathway expression and clinical indexes. Moreover, we demonstrated the immunomodulatory effects of the PD-1/PD-Ls pathway in human pulmonary fibrosis to deepen the understanding of the mechanism underlying the development of pulmonary fibrosis and to provide clues for the identification of new drug targets for the treatment of pulmonary fibrosis.

Methods

Patients and control subjects

Thirty patients with asbestosis and 23 patients with silicosis were consecutively recruited from the Department of Occupational Medicine and Toxicology, Beijing Chao-Yang Hospital . Clinical data pertaining to several parameters, including age, sex, current and past medical history, occupational history and pulmonary function values, were collected from patient clinical charts.

Asbestosis was diagnosed in the following patients: (1) patients with a definite occupational history of exposure to chrysotile dust through asbestos product manufacturing and a prolonged latency; (2) patients whose lungs exhibited honeycombing and septal and interlobular fissure thickening, as well as diffuse pleural thickening and/or pleural plaques, on chest HRCT; and (3) patients in whom other known causes of ILD were excluded [10]. Silicosis was diagnosed in the following patients: (1) patients with a definite occupational history of exposure to silica dust through stone sculpture manufacturing and a prolonged latency; (2) patients whose lungs featured multiple small nodules along the lymphatic

distribution, as well as nodules that even fused into masses, on chest HRCT; and (3) patients in whom tuberculosis and malignancy were excluded [11]. Twenty-five subjects who underwent routine health examinations and showed no evidence of disease were enrolled in the study as healthy controls.

All of the patients were in a stable clinical state and showed no clinical, radiographic, or electrocardiographic signs of heart failure, acute pulmonary infection, or pulmonary thromboembolism. None of the patients were receiving treatment with corticosteroids and/or immunosuppressants. The study was conducted in accordance with the ethical standards of Beijing Chao-Yang Hospital, Capital Medical University. All the participants provided written informed consent before enrolling in the study.

Computed tomography scans

HRCT was performed with 1-mm sections and 1-s scan times. The apex-base scans, which included both lungs in the field of view, were performed at 10-mm intervals. The CT images were reviewed independently by two experienced thoracic radiologists, and the CT patterns were obtained and recorded by two observers. Patients who showed evidence of coexisting emphysema (>5 % of total lung volume) on HRCT were not included in the study.

Pulmonary function tests

Pulmonary function tests were performed according to American Thoracic Society guidelines [12]. We recorded the arterial partial pressure of oxygen (PaO₂), FVC, forced expiratory volume in the first second (FEV₁), FEV₁/FVC ratio, total lung capacity (TLC), and diffusing capacity of the lung for carbon monoxide (DLCO) (single-breath method, with the values corrected for the current haemoglobin level) of each subject.

Cell collection

PB samples were collected from each subject, placed in ethylenediaminetetraacetic acid-treated tubes and processed to measure PB mononuclear cell (PBMC) counts for subsequent flow cytometry setup procedures. The blood samples were layered onto Ficoll-Paque Plus (Amersham Biosciences, Amersham, Bucks, UK) and centrifuged (400 g for 20 min at 21°C), and then PBMCs were harvested. The cells were washed once in divalent cation-free Hanks balanced salt solution at 300 g for 5 min at 4°C. The PBMCs were subsequently resuspended, and the number of viable cells was counted.

Flow cytometry

Freshly obtained human PBMC samples were stained with anti-hCD274-FITC, anti-hCD279-PE, anti-hCD4-APC, anti-hCD8-PerCP, anti-hCD14-FITC, anti-hCD274-PE, anti-hCD45-PerCP and matched isotypic controls and were incubated in a dark room for 30 min at 4 °C. Anti-hCD8-FITC, anti-hCD4-APC, anti-hCD28-PE, anti-hCD69-PerCP, anti-hHLA-DR-PerCP, and anti-hCD38-PE antibodies were used for the surface marker staining of effector T cells. All antibodies were purchased from BD Biosciences (San Jose, CA, USA). Data

acquisition and analysis were performed with Canto II Software (BD Biosciences, San Jose, CA, USA). Approximately 10^5 cells were acquired for subsequent data analyses.

Statistical analysis

Values are presented as the mean \pm standard deviation (S.D.) or as medians and interquartile ranges (IQRs) when appropriate. Group comparisons were performed by analysis of variance, Student's *t* test, Wilcoxon's rank-sum test, or chi-square testing as appropriate, and correlations were assessed by Pearson's correlation test or Spearman's rank correlation coefficient. $P < 0.05$ was considered statistically significant. Statistical analysis was performed with SPSS for Windows V17.0 (Chicago, IL, USA) and GraphPad Prism 5 (San Diego, CA, USA).

Results

Demographic characteristics of the study population

Thirty patients with asbestosis, 23 patients with silicosis and 25 healthy controls were evaluated in this study (Table 1). All the participants enrolled in the study were not current smokers. Patients with silicosis and healthy controls were significantly younger than patients with asbestosis ($p < 0.01$); however, there was no significant difference in age between the silicosis and healthy control groups. The PaO_2 was significantly lower in the asbestosis group than in the silicosis and control groups ($p < 0.05$). Pulmonary function testing indicated that restrictive ventilation and/or impaired gas exchange were present in the asbestosis group, while normal lung function or mild airflow limitation, as well as a mildly decreased DLCO, were present in the silicosis group.

Table 1
Demographics of asbestosis, silicosis and healthy controls

	asbestosis	silicosis	controls	p value*
Subjects	30	23	25	-
Age, years	71.3 ± 8.7	52.9 ± 12.0	46.8 ± 5.4	0.000
Female/male, n	17/13	14/9	7/18	0.040
Smoker/non-smoker, n	12/18	4/19	13/12	0.043
PaO ₂ , mmHg	77.63 ± 11.73	79.94 ± 14.12	87.92 ± 2.69	0.041
FVC, %pred	61.18 ± 23.30	88.87 ± 22.36	89.16 ± 2.54	0.000
FEV ₁ , %pred	59.74 ± 24.15	70.47 ± 27.59	89.80 ± 8.45	0.000
FEV ₁ /FVC,%	75.81 ± 10.90	67.07 ± 16.55	87.24 ± 2.82	0.000
TLC, %pred	74.07 ± 20.87	83.02 ± 31.25	90.52 ± 3.02	0.290
DLco, %pred	55.86 ± 25.93	76.47 ± 17.96	85.84 ± 3.24	0.000
Data are presented as Means ± S.D..*: p value denotes statistical differences among three groups;				

PD-1 expression on circulating CD4⁺ or CD8⁺ T cells was decreased in patients with asbestosis or silicosis

As shown in Fig. 1, there were no significant differences in circulating CD4⁺ or CD8⁺ T cell fractions between the asbestosis and silicosis and healthy control groups ($p > 0.05$). We first investigated PD-1 (CD279) expression in PB in the different groups, as shown in Fig. 2a and 2b. PD-1 expression on CD4⁺T cells in PB was significantly lower in the asbestosis (mean 7.7530%) and silicosis (mean 6.6760%) groups than in the healthy control group (mean 11.7900%, $p < 0.01$, respectively) (Fig. 2c). Similarly, PD-1 expression on CD8⁺T cells in PB was also significantly decreased in the asbestosis (mean 9.5560%) and silicosis groups (mean 9.1320%) compared to the healthy control group (mean 14.6700%, $p < 0.05$, respectively) (Fig. 2d); however, there was no significant difference in PD-1 expression between the asbestosis and silicosis groups (Fig. 2c-d).

PD-L1 expression on circulating CD4⁺ T cells was decreased in patients with asbestosis or silicosis

As shown in Fig. 3, PD-L1 (CD274) was detected mainly on circulating CD4⁺T cells rather than on CD8⁺T cells. PD-L1 expression on circulating CD4⁺T cells was significantly decreased in the asbestosis (mean 0.2115%) and silicosis (mean 0.3100%) groups compared to the healthy control group (mean 0.7050%, p

< 0.05 each) (Fig. 3c). No significant difference in PD-L1 expression on circulating CD8⁺ T cells was noted among three groups (Fig. 3d).

PD-L1 and PD-L2 expression on circulating CD14⁺ monocytes was decreased in patients with asbestosis or silicosis

In addition to detecting PD-1/PD-Ls expression on circulating T cells, we also detected PD-L1 (CD274) and PD-L2 (CD273) expression on CD14⁺ monocytes in PB. As shown in Fig. 4a-b, the percentages of circulating PD-L1⁺ CD14⁺ monocytes were significantly decreased in the asbestosis (mean 0.2115%) and silicosis (mean 0.3100%) groups compared to the control group (mean 0.7050%, $p < 0.01$, respectively) (Fig. 4c). Similarly, the percentage of circulating PD-L2⁺ CD14⁺ monocytes was lower in the asbestosis (mean 0.5408%) and silicosis (mean 0.5251%) groups than in the healthy control group (mean 1.161%, $p < 0.01$, respectively) (Fig. 4d). No significant difference in PD-Ls expression on monocytes was noted between the asbestosis and silicosis group (Fig. 4c-d).

T cell activation status varies in patients with asbestosis and silicosis

We also explored the variations in effector T cell activation status in patients with asbestosis and silicosis. CD28 (a costimulatory molecule), HLA-DR (a more general marker of T cell activation), CD38 (a marker of cell activation) and CD69 (an early marker transiently expressed on activated T cells) expression on circulating CD4⁺ and CD8⁺ T cells was evaluated (Fig. 5).

The percentages of circulating CD28⁺CD8⁺T cells were significantly lower in patients with asbestosis and silicosis than in healthy controls ($p < 0.05$) (Fig. 5b), and the percentages of circulating HLA-DR⁺CD8⁺T cells were significantly higher in patients with asbestosis and silicosis than in healthy controls ($p < 0.05$) (Fig. 5d). No significant differences in the percentages of CD28⁺CD4⁺T cells and HLA-DR⁺CD4⁺T cells were noted between the asbestosis and silicosis groups ($p > 0.05$, Fig. 5a, c).

The percentage of CD69⁺CD8⁺T cells in PB was significantly increased in the asbestosis group compared to the healthy control group ($p < 0.05$), and the percentage CD69⁺CD8⁺T cells tended to be non-significantly higher in the silicosis group than in the healthy control group ($p > 0.05$) (Fig. 5f). The percentages of CD38⁺CD4⁺T cells and CD38⁺CD8⁺T cells in PB were not different between the asbestosis and silicosis groups and the healthy control group ($p > 0.05$) (Fig. 5g, h). Moreover, the percentage of PD-1⁺CD8⁺T cells was positively correlated with the percentage of CD28⁺CD8⁺T cells in PB in the asbestosis ($r = 0.4640$, $p = 0.0194$) and silicosis groups ($r = 0.5100$, $p = 0.0320$) (Fig. 6).

Down-regulation of the percentages of PD-1⁺ CD4⁺ and PD-1⁺CD8⁺ T cells was correlated with lung function parameters signifying disease severity

Pulmonary functional parameters, such as FVC, have been used as the predictors of the severity of chronic fibrotic lung diseases, such as idiopathic pulmonary fibrosis (IPF) [13]. The percentage of FVC

predicted was a valid and robust predictor of clinical endpoints in patients with IPF. The proportions of PD-1⁺ CD4⁺T cells and PD-1⁺CD8⁺T cells were positively correlated with the percentage of FVC predicted in the asbestosis group ($r = 0.5910$, $p = 0.0076$; $r = 0.5070$, $p = 0.0267$, respectively) (Fig. 7).

Discussion

Our study demonstrates that PD-1 expression on CD4⁺T or CD8⁺ T cells in PB was significantly lower in patients with asbestosis and silicosis than in healthy controls. Moreover, the proportions of CD4⁺PD-1⁺ T cells and CD8⁺PD-1⁺T cells in the asbestosis group were positively correlated with the percentage of FVC predicted. CD28 expression on CD8⁺T cells in PB was significantly decreased, while CD69 and HLA-DR expression on CD8⁺T cells in PB was significantly increased in the asbestosis group compared to the healthy control group. In addition, the proportions of CD8⁺PD-1⁺T cells were positively correlated with the proportions of CD8⁺CD28⁺T cells in PB in the asbestosis and silicosis groups.

Asbestosis and silicosis are ILDs of occupational origin caused by the inhalation of asbestos and small silica crystals and are characterized by chronic diffuse aseptic lung tissue inflammation. Inorganic dusts, such as silica and asbestos, deposit in the distal airways, where mucociliary clearance is unable to affect them. Macrophages residing in the small airways are believed to ingest the silica crystals and asbestos, thereby stimulating the generation of an inflammatory response consisting of cytokines [14]. During this period, lymphocytes and other cells become activated and begin secreting profibrotic cytokines and growth factors, such as transforming growth factor (TGF)- β 1, IL-13 and platelet derived growth factor (PDGF) [15]. The monocyte/macrophage, T lymphocytes and associated cytokines play an important role in the development of human fibrotic lung diseases and animal models of pulmonary fibrosis [16]. The generation of an effective immune response involves antigen-specific T cell expansion and effector function differentiation [17]. T cell activation requires two signals. The first signal involves the activation of the T cell receptor via the recognition of antigens presented by the major histocompatibility complex on antigen presenting cells (APCs), and the second signal involves the ligation of co-stimulatory and co-inhibitory molecules expressed on APCs and T cells belonging to the B7 and tumour necrosis factor families. The PD-1/PD-Ls pathway in the B7/CD28 family, which consists of the PD-1 receptor and its ligands, PD-L1 and PD-L2, plays a critical role in regulating T cell activation and autoimmune tolerance [18, 19]. PD-1 was first isolated from a murine T cell hybridoma undergoing programmed cell death by Ishida and colleagues in 1992 [20]. PD-1 is expressed on T cells, Tregs, B cells, activated monocytes, dendritic cells (DCs), natural killer (NK) cells and natural killer T (NKT) cells [8]. Moreover, PD-1 is an immunoglobulin (Ig) superfamily member featuring an N-terminal IgV-like domain, a transmembrane domain, and a cytoplasmic domain with an immunoreceptor tyrosine-based inhibitory motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM) [8, 18]. The binding of PD-1 by PD-L1 or PD-L2 induces the blockade of B and T cell proliferation, inhibits the secretion of cytokines, and influences T cell survival [9, 21]. The PD-1/PD-L axis represents a relevant negative feedback loop for maintaining immune homeostasis, but it is also of crucial importance for restricting tumor immunity and controlling inflammatory response to injury in the normal lung tissues. Numerous findings indicate this pathway

exerts an influence on granulomatous diseases [22, 23], chronic infections [24–26], tumours [27], and autoimmune disease [28, 29]. PD-1/PD-Ls modulation or dysfunction may play important roles in the development of these diseases.

In our study, PD-1/PD-Ls pathway expression in PB cells was down-regulated both in asbestosis and silicosis. Although patients with asbestosis were older than those with silicosis and healthy controls ($p < 0.01$), age did not affect our final results. A previous study showed that PD-1 mRNA and protein expression levels increased with ageing [30]. Therefore, the cause of the lower PD-1 expression in asbestosis noted in our study was the disease itself rather than age. The inhibitory ability of the PD-1/PD-Ls pathway is decreased in asbestosis, which promotes T cell activation, resulting in increased cytokine secretion. More importantly, we showed that the proportions of CD4⁺PD-1⁺ T cells and CD8⁺PD-1⁺ T cells in asbestosis were positively correlated with the percentage of FVC predicted. Thus, we speculated that enhancements of T cell activation and cytokine secretion may aggravate the inflammatory response, ultimately leading to more severe pulmonary fibrosis.

Various studies have clarified the crucial roles of PD-1/PD-Ls pathway in IPF patients. IPF is characterized by excessive accumulation of extracellular matrix in the interstitial and alveolar spaces leading to scarring and the destruction of the normal pulmonary epithelium [31]. IPF, a like cancer disease, should be considered as a neoproliferative disorder of the lung [32]. In a study, RNA sequencing pointed to PD-1 as significantly downregulated gene in human IPF lung tissue obtained by surgical biopsy [33]. However, a significant increase in expression of PD-L1 in the subset of invasive human lung fibroblasts isolated from explant lung tissues in the recently study and a increase in soluble PD-L1 serum levels was observed in IPF patients compared with healthy controls in the other polit study [34, 35]. Interestingly, in another study PD-L1 expression in peripheral blood was not increased in IPF patients compared to healthy control but PD-1 expression was increased significantly on T lymphocytes of IPF patients both in peripheral blood and lung tissue [36]. In our study, PD-1 and PD-L1 expression in asbestosis and silicosis has a different trend compared with IPF. There may be differences due to the following reasons: asbestosis and silicosis have different pathogenesis with IPF, which are ILDs of occupational origin caused by the inhalation of asbestos and small silica crystals. Macrophages, key regulators of fibrosis, residing in the small airways ingest the silica crystals and asbestos, producing numerous pro-fibrotic soluble mediators, chemokines, and matrix metalloproteases, thereby controlling extracellular matrix (ECM) deposition. The monocytes and macrophages function as antigen-presenting cells sending costimulatory and coinhibitory signals to T cells, thus with ability to promote Th2 responses which induce and activate TGF- β 1 in macrophages through an IL-13 and matrix metalloproteinase (MMP)9-dependent mechanism [37, 38]. Although, in our study, PD-L1 expression on macrophages were not tested, PD-L1 and PD-L2 expression on monocytes were decreased. Binding of PD-L1 and PD-L2 on monocytes to PD-1 on T cells creating an inhibitory signal was decreased, which increasing the activity of T cells, then may promote Th2 responses, resulting in fibrotic process. Based on numerous findings, macrophages seem to get distinct roles exhibiting a predominant phenotype dependent on different stimuli or a microenvironment. M1 or M2 phenotypes are considered more as a dynamic spectrum of

activation potentially [39, 40]. When tissues are damaged in the early phase, macrophages often display an inflammatory M1-like phenotype, that is characterized by the production of numerous growth factors and soluble mediators that stimulate local and recruited tissue fibroblasts to differentiate into myofibroblasts that facilitate synthesis of ECM components [39–41]. If the tissue-damaging factor persists, activated M1 cells can further exacerbate the inflammatory response by different mechanisms, leading to profound tissue damage. Thereafter, the monocytes and/or macrophages become the dominant population, exhibiting a mostly anti-inflammatory phenotype, that foster healing process and fibrosis through the secretion of MMPs (MMP12), tissue inhibitor of metalloproteinase (TIMP)-1, growth factors and cytokines such as TGF- β 1. In the final phase, macrophages take on a regulatory/suppressive phenotype, which have all been shown to restore homeostasis while suppressing fibrotic process, partly by blocking T cell proliferation and collagen synthesis by activated myofibroblasts. Thus, monocytes and macrophages of different phenotypes play unique and critical roles at each stage. Therefore, we speculate that the PD-1/PD-Ls pathway acting on monocytes/macrophages of the different phase may produce different results.

Heterogeneity in the stimuli, genetic predisposition and signaling mechanisms that promote profibrotic cell phenotypes may contribute to different clinical courses observed in patients with asbestosis and silicosis, as well as IPF. Thus, PD-1/PD-Ls pathway down-regulation might be a specific phenotype, or at least a particular development phase in asbestosis and silicosis.

Variations in effector T cell activation status were detected in patients with asbestosis and silicosis. These results showed that activated T cells are expressed in patients with asbestosis and silicosis and that these cells exhibit decreased CD28⁺ expression and increased HLA-DR⁺ expression. CD28, a marker of early T cell activation, is the second T cell activation signal, while HLA-DR is a marker of late T cell activation [42, 43]. CD28 expression is down-regulated in chronic T cell activation [44]. These findings indicated that T cell activation is induced by chronic inflammation in patients with asbestosis and silicosis. Interestingly, the percentage of PD-1⁺CD8⁺T cells was positively correlated with the percentage of CD28⁺CD8⁺T cells in PB in asbestosis and silicosis. This result may verify the finding that the lower the expression of PD-1 on T cells, the greater the activation of T cells and the greater the depletion of CD28. These data are demonstrative of the immunomodulatory effect of PD-1/PD-Ls in asbestosis and silicosis.

Our study did have some inherent limitations. We detected PD-1/PD-Ls pathway expression in PB in patients with asbestosis and silicosis but did not detect PD-1/PD-Ls pathway expression in the lung. In addition, we found no evidence to verify the causal relationship between declines in lung function and PD-1 expression. In spite of these limitations, ours was the first study to explore the PD-1/PD-Ls pathway in peripheral cells in human asbestosis and silicosis. We performed a comprehensive analysis of PD-1/PD-Ls pathway expression in peripheral cells in human asbestosis and silicosis. Our findings may facilitate the development of therapies for pulmonary fibrosis.

Conclusion

In conclusion, our study provided further evidence showing that the PD-1/PD-Ls pathway plays a role in the pathogenesis of asbestosis and silicosis. We first demonstrated that PD-1/PD-Ls pathway expression in PB cells was down-regulated in asbestosis and silicosis. Importantly, lower PD-1 expression on CD4⁺T or CD8⁺ T cells in PB was positively correlated with parameters representing disease severity, implying that a causal link exists between pulmonary fibrosis development and PD-1/PD-Ls pathway down-regulation. Our study may have identified the PD-1/PD-Ls pathway as a therapeutic target.

Abbreviations

PD-1: programmed cell death protein 1; PD-Ls: programmed cell death protein-ligands; PB: peripheral blood; FVC: forced vital capacity; ILD: interstitial lung disease; COPD: chronic obstructive pulmonary disease; HRCT: chest high-resolution CT; UIP: usual interstitial pneumonia; NSIP: nonspecific interstitial pneumonia; PaO₂:the arterial partial pressure of oxygen; FEV₁: forced expiratory volume in the first second; TLC: total lung capacity; DLCO: diffusing capacity of the lung for carbon monoxide; PBMC: peripheral blood mononuclear cell; IPF: idiopathic pulmonary fibrosis; TGF-β₁: transforming growth factor-β₁; PDGF: platelet derived growth factor; APC: antigen presenting cells; DCs: dendritic cells; NK: natural killer; ITIM: immunoreceptor tyrosine-based inhibitory motif; ITSM: immunoreceptor tyrosine-based switch motif; ECM: extracellular matrix; MMP: matrix metalloproteinase; TIMP: tissue inhibitor of metalloproteinase.

Declarations

Acknowledgments:

We thank all the participants who agreed to enrol in the study and are grateful to the staff of the Lung Function Lab of Beijing Chao-yang Hospital for their assistance.

Authors' contributions:

MHQ: performed the collection of the data, the collection and processing of the samples, and the writing of the manuscript. QY: was a primary investigator and was responsible for designing the study, recruiting the patients and writing the manuscript. MHQ and YQC: performed the laboratory-based assays. MHQ: was a primary investigator and was responsible for designing the study, analysing the data and writing the manuscript. All authors read and approved the final manuscript.

Funding:

This work was supported by the National Natural Science Foundation of China (Grant No. 81070046) and the Beijing Natural Science Foundation (Grant No. 7142058).

Availability of data and materials:

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

Ethics approval and consent to participate:

Ethics approval for the study was obtained from the Ethics Committee of Beijing Chao-Yang Hospital, Capital Medical University. All subjects provided written informed consent before enrolling in the study.

Consent for publication:

Not applicable.

Competing interests:

The authors declare that they have no conflicts of interest.

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Figures

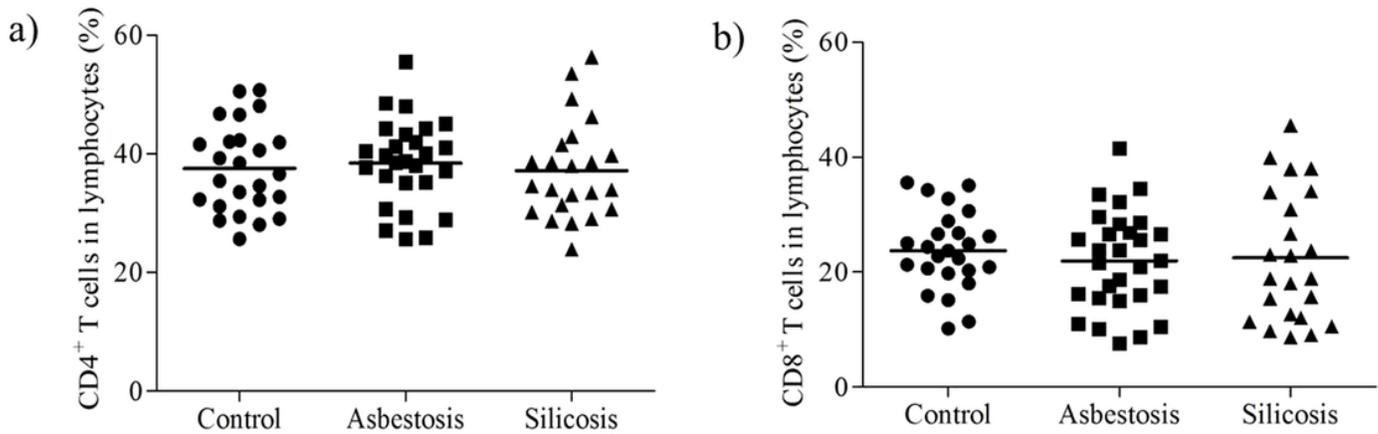


Figure 1

Percentages of CD4+ or CD8+T cells among lymphocytes in PB. The percentages of CD4+ or CD8+T cells among lymphocytes in PB of healthy controls and patients with asbestosis or silicosis (a-b).

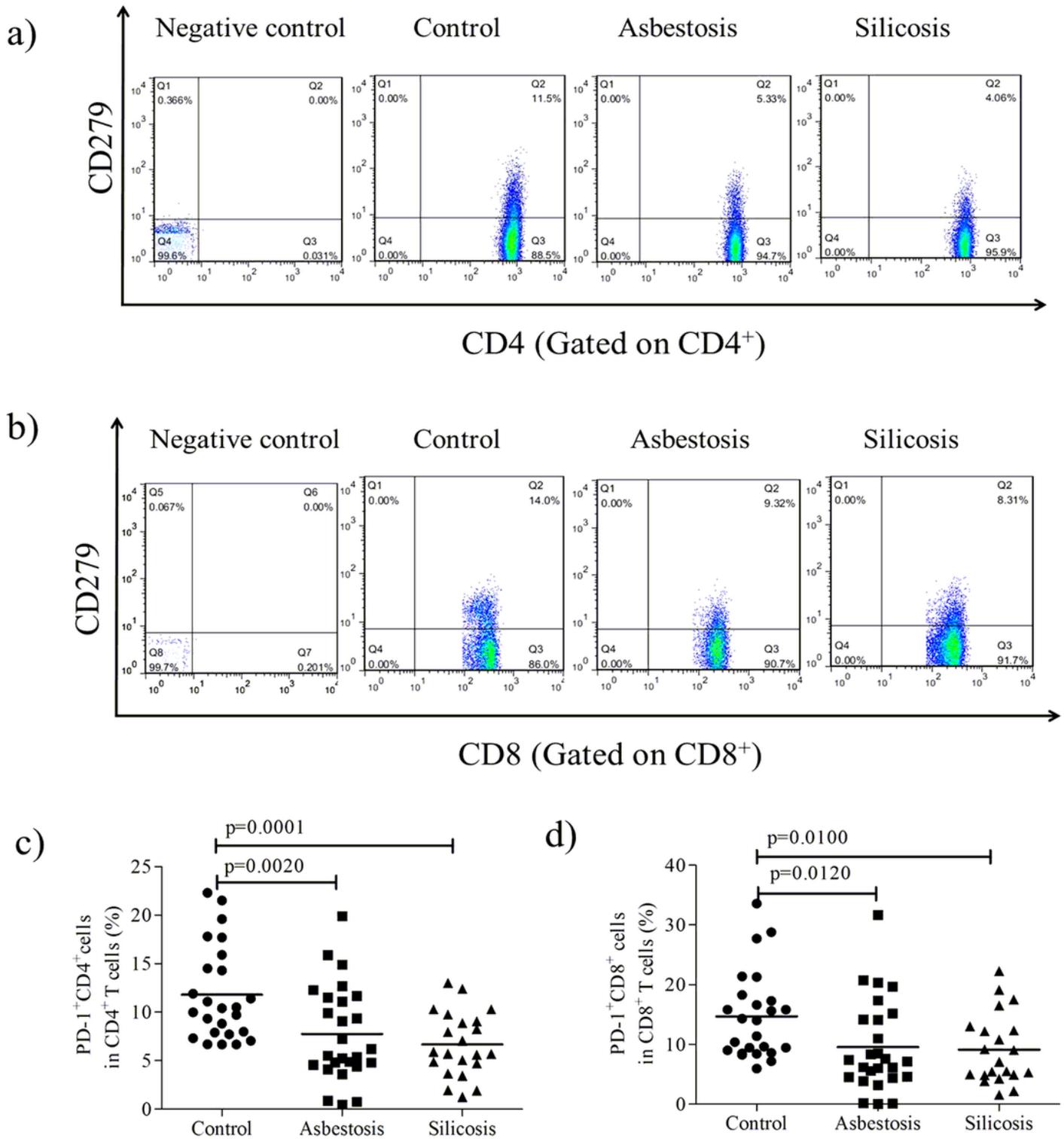


Figure 2

Analysis of PD-1(CD279) expression on CD4+T or CD8+T cells in PB. Representative flow cytometry analysis of the percentages of PD-1+CD4+ or PD-1+CD8+T cells in PB (a-b). The percentages of PD-1+CD4+ or PD-1+CD8+T cells in PB of healthy controls and patients with asbestosis or silicosis (c-d). A p value < 0.05 was considered statistically significant.

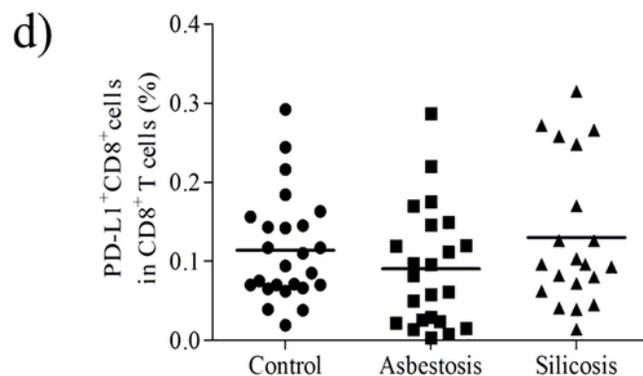
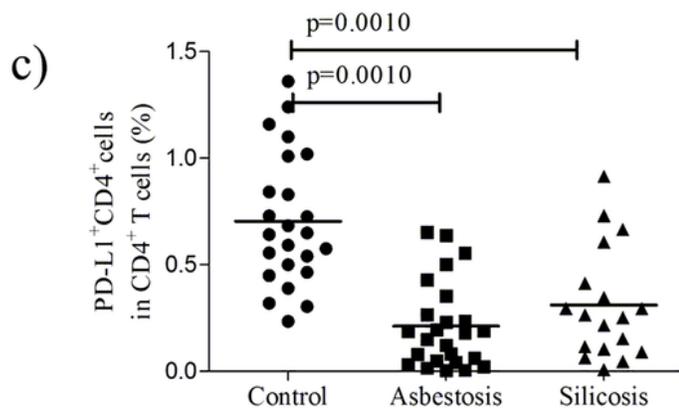
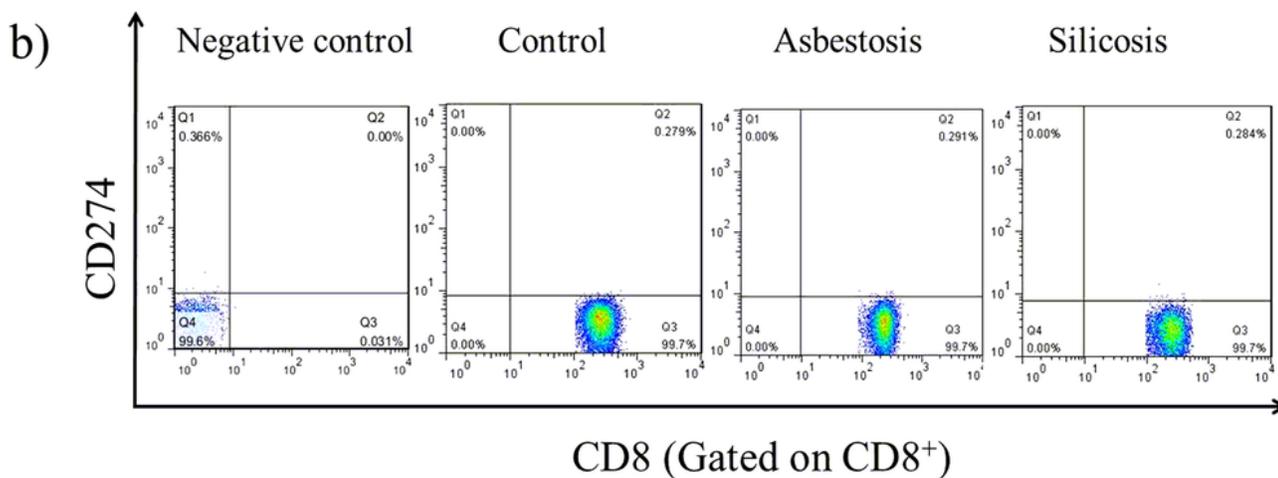
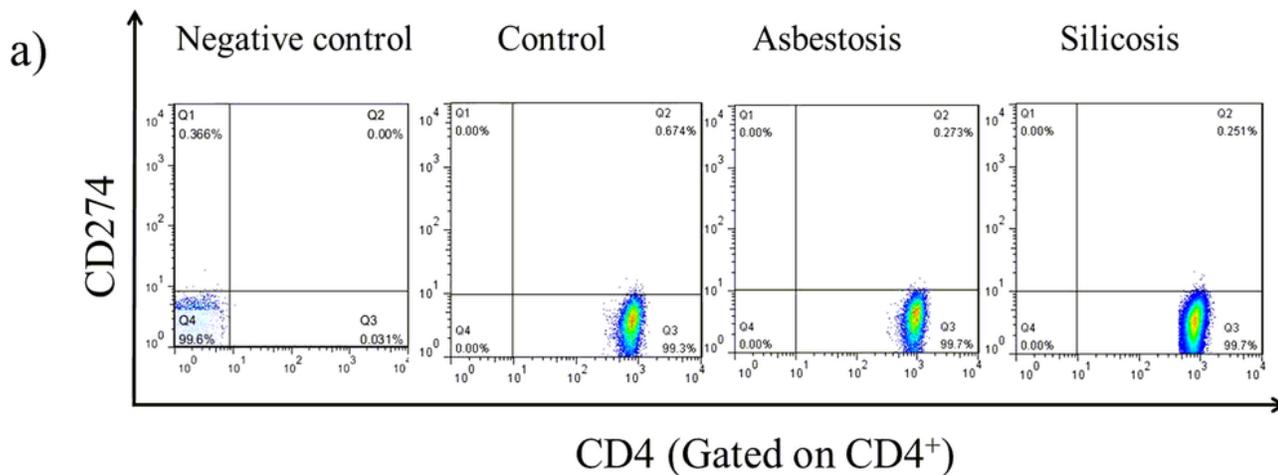


Figure 3

Analysis of PD-L1 (CD274) expression on CD4⁺T or CD8⁺T cells in PB. Representative flow cytometry analysis of the percentages of PD-L1⁺CD4⁺ or PD-L1⁺CD8⁺T cells in PB (a-b). The percentages of PD-L1⁺CD4⁺ or PD-L1⁺CD8⁺T cells in PB of healthy controls and patients with asbestosis or silicosis (c-d). A p value < 0.05 was considered statistically significant.

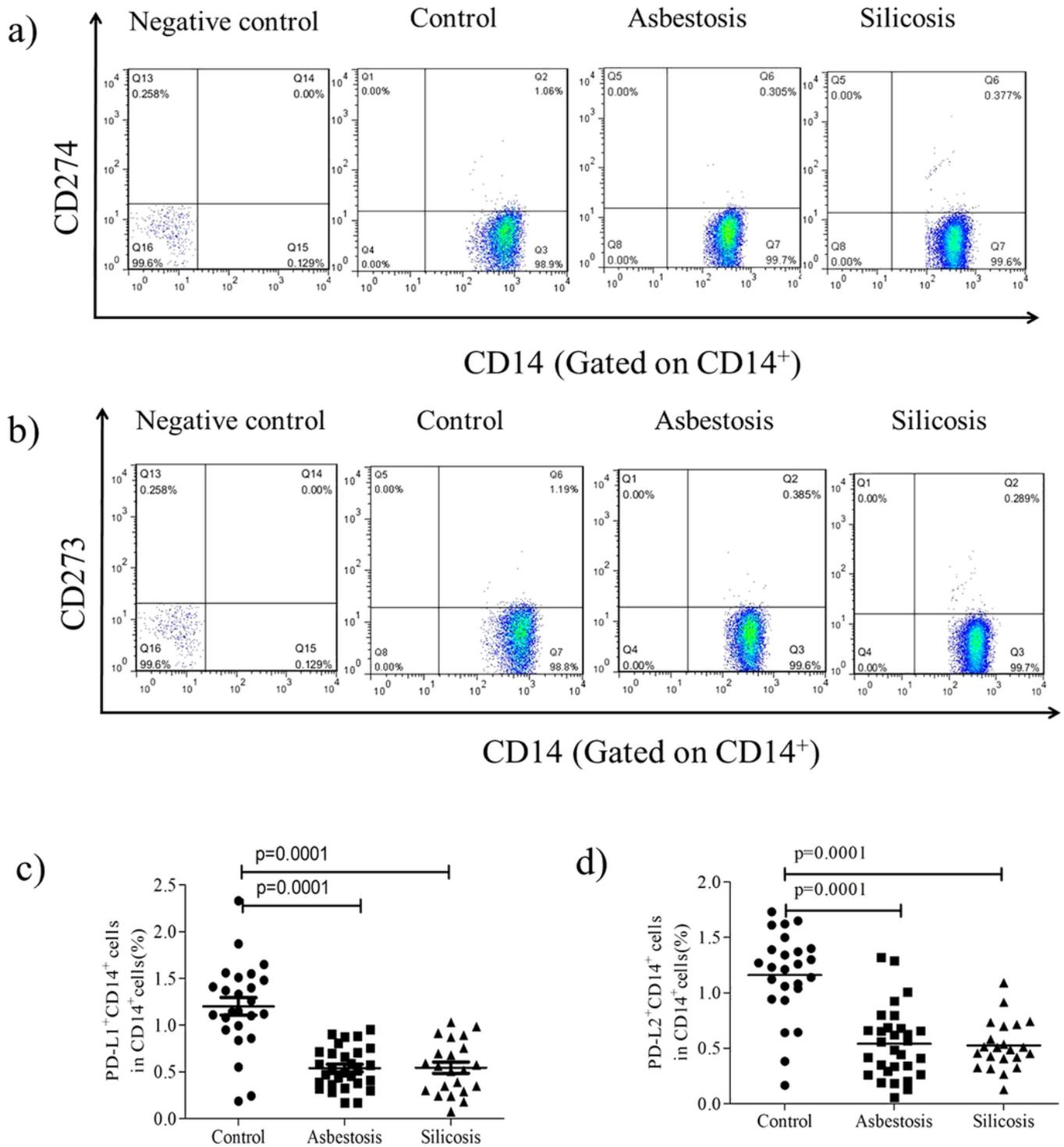


Figure 4

Analysis of PD-L1 (CD274) or PD-L2 (CD273) expression on CD14⁺ cells in PB. Representative flow cytometry analysis of the percentages of PD-L1⁺CD14⁺ or PD-L2⁺CD14⁺ cells in PB (a-b). The percentages of PD-L1⁺CD14⁺ or PD-L2⁺CD14⁺ cells in PB of healthy controls and patients with asbestosis or silicosis (c-d). A p value < 0.05 was considered statistically significant.

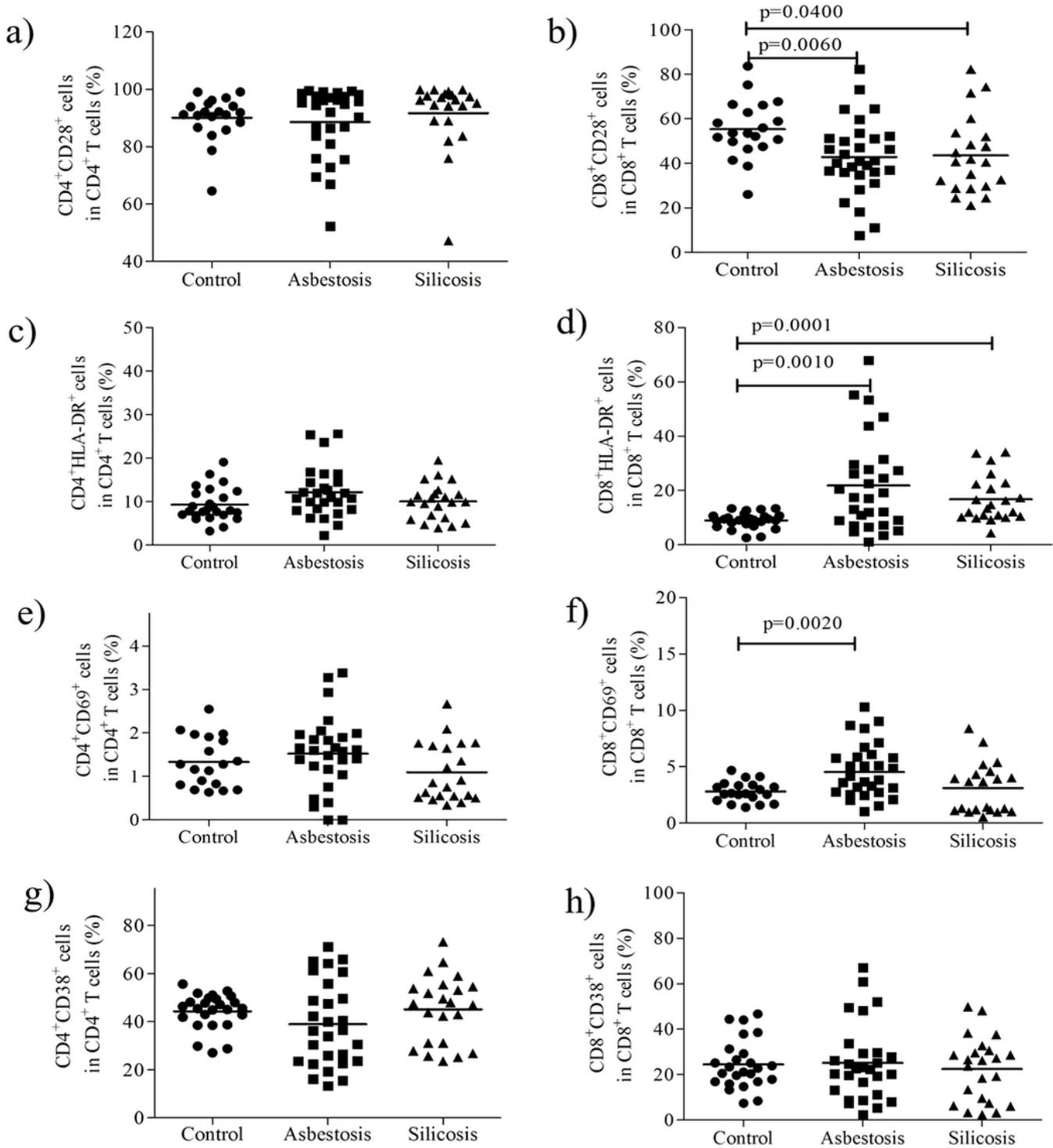


Figure 5

The effector circulating T cells activation status in PB. The percentage of CD28⁺ CD4⁺ or CD8⁺T cells among the three groups(a-b); the percentage of HLA-DR⁺ CD4⁺ or CD8⁺T cells among the three groups(c-d); the percentage of CD38⁺ CD4⁺ or CD8⁺T cells among the three groups(e-f); the percentage of CD69⁺ CD4⁺ or CD8⁺T cells among the three groups(g-h). A p value < 0.05 was considered statistically significant.

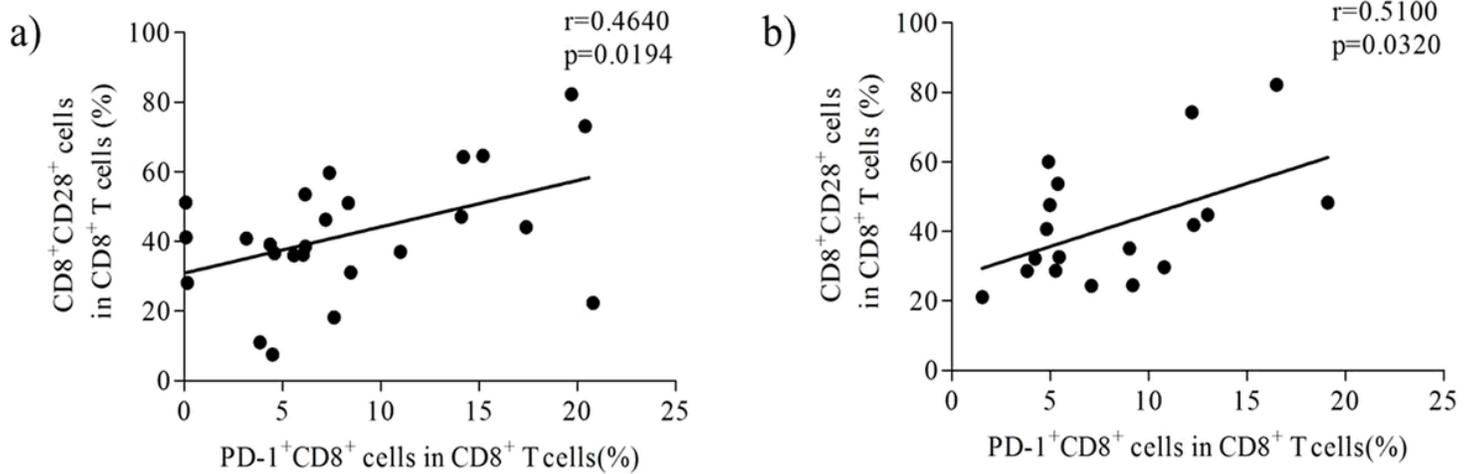


Figure 6

Correlations of PD-1⁺CD8⁺T cells fractions with CD28⁺CD8⁺T cells fractions in patients with asbestosis or silicosis. The PD-1⁺CD8⁺T cells fraction positively correlated with CD28⁺CD8⁺T cells fraction in PB of asbestosis ($r=0.4640$, $p=0.0194$) (a) or silicosis ($r=0.5100$, $p=0.0320$) (b).

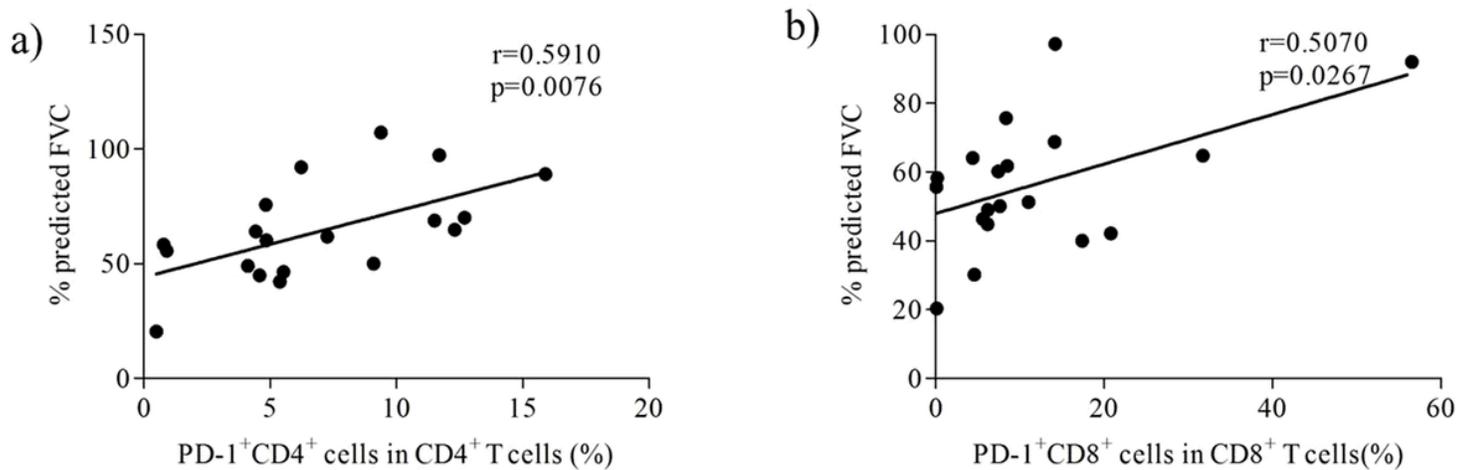


Figure 7

Correlations of PD-1⁺CD4⁺T cells or PD-1⁺CD8⁺T cells fractions with FVC% predicted in patients with asbestosis. The significantly positive correlation of PD-1⁺CD4⁺T cells fractions ($r=0.5910$, $p=0.0076$) (a) or PD-1⁺CD8⁺T cells fractions ($r=0.5070$, $p=0.0267$) (b) respectively with lung function value forced vital capacity (FVC) predicted in PB of asbestosis.