Cellular profile of bronchoalveolar lavage fluid in Turkish miners

O Kayacan, S Beder, D Karnak

Pneumoconiosis is still a health problem in Turkey and has a relatively high incidence. Retired underground miners were investigated to document alveolitis, and to observe the difference in the cellular profiles of bronchoalveolar lavage (BAL) fluid with or without pneumoconiosis. Twenty nine retired male miners and 17 controls, eight non-smokers (four male, four female) and nine smokers (six male, three female), without any dust exposure were evaluated. According to the International Labor Office 1980 classification system, the miners were allocated to three subgroups: eight without pneumoconiosis, 11 with simple pneumoconiosis, and 10 with progressive massive fibrosis (PMF). Spirometric tests and arterial blood gases analysis were done and fiberoptic bronchoscopy and BAL were performed in all subjects. The study and the control subjects were comparable in respect to age, smoking habits, except the non-smoker controls, and the duration of dust exposure, except the controls. The amount of recovered BAL fluid was lower in all miners compared with the non-smoker controls (p<0.05). The amount of recovered BAL fluid and the total cell count correlated significantly (r = 0.48, p<0.01). The percentage of lymphocytes in the BAL fluid of miners without pneumoconiosis and with PMF (p<0.05) and that of non-smoker controls.

Alveolitis was not a representative feature of Turkish subjects with an occupational history of underground mining, and BAL fluid cellular profile did not seem to be different in miners with or without pneumoconiosis.

SUBJECTS AND METHODS

Study and control subjects
Twenty nine retired miners, all males, were evaluated. Twenty six were coal workers, two were antimony miners, and one was a lead-zinc miner. The subjects in the study group were allocated to three subgroups: eight without pneumoconiosis, 11 with simple pneumoconiosis, and 10 with PMF. Eight non-smokers (four males, four females) and nine smokers (six males, three females) without any dust exposure served as controls.

Gender, age, smoking habits of the study subjects and controls and the duration of dust exposure of the miners were registered. Posteroanterior chest radiographs were taken, spirometric tests and arterial blood gases analysis were done, and fiberoptic bronchoscopy and BAL were performed in all subjects. Control subjects had undergone fiberoptic bronchoscopy for haemoptysis or cough work-up. They have been followed up thereafter for one year and no disease was detected. Informed oral consent was obtained from all study subjects. This study has been approved by the local ethical committee.

Chest radiographs
Posteroanterior chest radiographs were evaluated according to the International Labor Office 1980 classification system by a consensus of three trained readers. Briefly, there are two major types of parenchymal opacities: large (>10 mm) and small.

Abbreviations: BAL, bronchoalveolar lavage; FEV\textsubscript{1}, forced expiratory volume in the first second; FVC, forced vital capacity; PMF, progressive massive fibrosis
Small opacities are classified on the basis of shape and size. Rounded and irregular opacities are categorised according to size: p (s) <1.5 mm, q (t) 1.5–3, and r (u) 3–10 mm. The profusion of small opacities is rated on a 12 category scale ranging from 0/0 to 3/3. Large opacities are classified on the basis of diameter: A, 1–5 cm; B, >5 cm or less than the right upper lobe area; and C, greater than the right upper lobe area. Miners with only small opacities were classified as having simple pneumoconiosis and those with large opacities besides the small opacities were classified as having progressive massive fibrosis (PMF).1

**Spirometric tests**

Spirometric evaluation of the study subjects was done on a Vitalograph alpha spirometer. Forced vital capacity (FVC), forced expiratory volume in the first second (FEV1), and FEV1/FVC ratio were measured.

**Arterial blood gas analysis**

Arterial blood samples were analysed by ABL300. Partial pressures of oxygen and carbon dioxide and oxygen saturation were measured.

**Fibreoptic bronchoscopy and BAL procedure**

After one night fasting, subjects were premedicated with atropine 1/4 mg subcutaneously and topically anaesthetised. The bronchoscope was inserted transorally and after having examined the tracheobronchial tree thoroughly, it was wedged into the right middle lobe. Five aliquots of 20 ml of 0.9% saline solution at room temperature was instilled and immediately retrieved with a hand-held syringe. The dwell time was assessed as zero.

The recovered BAL fluid and the total cell count were measured. The amount of normal saline instilled did not differ between the groups. However, the amount of recovered BAL fluid was lower in miners with or without pneumoconiosis compared with the non-smoker controls. The percentage of alveolar macrophages were higher in miners compared with the non-smoker controls.

**RESULTS**

The results of the study subjects are shown in table 1.

The amount of normal saline instilled did not differ between the groups. The per cent of total cells and absolute number of cells per ml of recovered BAL fluid.

**Statistics**

The statistical analysis was carried out on a PC by the SPSS program (version 8.0). All data were expressed as the mean (SD), and comparison between the groups was done by multiple variance analysis. For the correlation analysis Pearson’s test was applied. Any p value less than 0.05 was considered significant.

**Table 1** Characteristics of the study subjects

<table>
<thead>
<tr>
<th>Gender (M:F)</th>
<th>Non-smoker controls (n=8)</th>
<th>Smoker controls (n=9)</th>
<th>Miners without pneumoconiosis (n=8)</th>
<th>Simple pneumoconiosis (n=11)</th>
<th>Progressive massive fibrosis (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>46.4 (13.3)</td>
<td>43.3 (7.6)</td>
<td>52.8 (8.9)</td>
<td>54.1 (8.9)</td>
<td>50.3 (12.8)</td>
</tr>
<tr>
<td>Smoking habits (pack-years)</td>
<td>21.1 (9.2)</td>
<td>–</td>
<td>22.1 (15.0)</td>
<td>11.3 (16.3)</td>
<td>32.4 (46.0)</td>
</tr>
<tr>
<td>Type of work</td>
<td>–</td>
<td>Coal worker</td>
<td>Coal worker</td>
<td>Coal worker</td>
<td>7 coal workers, 2 antimony miners, 1 lead-zinc miner</td>
</tr>
<tr>
<td>Duration of exposure (years)</td>
<td>–</td>
<td>–</td>
<td>11.9 (8.8)</td>
<td>15.3 (8.1)</td>
<td>23.0 (6.8)</td>
</tr>
</tbody>
</table>

*Statistics*

The statistical analysis was carried out on a PC by the SPSS program (version 8.0). All data were expressed as the mean (SD), and comparison between the groups was done by multiple variance analysis. For the correlation analysis Pearson’s test was applied. Any p value less than 0.05 was considered significant.

**Table 2** Spirometric test and arterial blood gases analysis results

<table>
<thead>
<tr>
<th></th>
<th>FVC (L)</th>
<th>FEV1 (L)</th>
<th>FEV1/FVC</th>
<th>PaCO2</th>
<th>PaO2</th>
<th>SaO2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-smoker controls (n=8)</td>
<td>101.1 (12.7)</td>
<td>105.8 (14.5)</td>
<td>89.4 (4.7)</td>
<td>84.3 (7.2)</td>
<td>35.2 (4.5)</td>
<td>96.9 (1.2)</td>
</tr>
<tr>
<td>Smoker controls (n=9)</td>
<td>102.0 (16.5)</td>
<td>100.9 (15.7)</td>
<td>87.4 (12.8)</td>
<td>80.5 (11.2)</td>
<td>33.2 (3.2)</td>
<td>95.9 (2.0)</td>
</tr>
<tr>
<td>Miners without pneumoconiosis (n=8)</td>
<td>77.1 (17.5)</td>
<td>70.8 (20.7)*</td>
<td>82.3 (14.9)</td>
<td>72.3 (8.7)</td>
<td>37.0 (3.1)</td>
<td>94.7 (1.0)</td>
</tr>
<tr>
<td>Simple pneumoconiosis (n=11)</td>
<td>84.6 (13.0)</td>
<td>86.8 (14.4)</td>
<td>88.9 (12.6)</td>
<td>77.3 (5.4)</td>
<td>36.0 (1.5)</td>
<td>95.4 (1.2)</td>
</tr>
<tr>
<td>Progressive massive fibrosis (n=10)</td>
<td>101.1 (12.7)</td>
<td>73.7 (23.3)**</td>
<td>83.1 (14.5)</td>
<td>71.0 (11.8)</td>
<td>36.7 (3.1)**</td>
<td>93 (2.4)</td>
</tr>
</tbody>
</table>

*p<0.01 versus non-smoker and smoker controls; **p<0.001 versus non-smoker and smoker controls; ***p<0.001 versus non-smoker controls.

FEV1, forced expiratory volume in the first second; FVC, forced vital capacity; PaCO2, partial pressure of carbon dioxide; PaO2, partial pressure of oxygen; SaO2, oxygen saturation.

*Statistics*

The statistical analysis was carried out on a PC by the SPSS program (version 8.0). All data were expressed as the mean (SD), and comparison between the groups was done by multiple variance analysis. For the correlation analysis Pearson’s test was applied. Any p value less than 0.05 was considered significant.

**RESULTS**

The study subjects were comparable in respect to age, smoking habits, and the duration of dust exposure. The characteristics of the study subjects are shown in table 1.

Eight non-smokers and nine smokers served as the controls and were comparable with the study subjects with respect to age; the smoking habits of the study subjects were also comparable to the smoker controls. The retired miners were comparably exposed to inorganic dust, while the controls were free from any dust exposure at the risk of pneumoconiosis.

The spirometric test results of the subjects can be seen in table 2. The FVC (p<0.01) and FEV1 (p<0.001) of the miners without pneumoconiosis were significantly lower compared with both control groups. The FEV1 of the subjects with PMF was significantly lower compared with non-smoker controls (p<0.001). FVC, FEV1, and FEV1/FVC were correspondingly related with the duration of exposure (r = −0.42, p<0.05; r = −0.56, p<0.01; r = −0.43, p<0.05, respectively).

The results of the arterial blood gases analysis documented a slight hypoxaemia without any statistical significance in miners (table 2). The partial pressures of carbon dioxide in all subjects were in normal range; however, that of PMF patients were significantly higher than the non-smoker controls, without any clinical relevance.

The amount of normal saline instilled did not differ between the groups. However, the amount of recovered BAL fluid was lower in miners with or without pneumoconiosis compared with the non-smoker controls (p<0.05) (table 3).

The amount of recovered BAL fluid and the total cell count correlated significantly (r = 0.48, p<0.01).

The total cell count of BAL fluid and the percentage of alveolar macrophages were higher in miners compared with
Coal mine dust exposure may result in several pathological processes, including simple pneumoconiosis and PMF, chronic bronchitis, emphysema, and dust-related airflow limitation. \(^*\) All miners except those with simple pneumoconiosis \((p<0.01)\) were found to have pneumoconiosis. In a survey of 12,300 coal workers, approximately 12% of the miners were found to have pneumoconiosis. \(^*\) Turkey. In a survey of 12,300 coal workers, approximately 12% of the miners were found to have pneumoconiosis. \(^*\) The results of in vitro, animal, and human investigations support four basic mechanisms in the etiology of coal workers’ pneumoconiosis and silicosis:

1. Direct cytotoxicity of coal dust or silica, resulting in lung cell damage, release of lipases and proteases, and eventual lung scarring.
2. Activation of oxidant production by pulmonary phagocytes, which overpowers the antioxidant defenses and leads to lipid peroxidation, protein nitrosation, cell injury, and lung scarring.
3. Activation of mediator release from alveolar macrophages and epithelial cells, which leads to recruitment of polymorphonuclear leukocytes and macrophages, resulting in the production of proinflammatory cytokines and reactive species and further lung injury and scarring.
4. Detection of growth factors from alveolar macrophages and epithelial cells, stimulating fibroblast proliferation and eventual scarring.

Data obtained from exposed workers lend support to these mechanisms. \(^*\)

Pneumoconiosis is still an important health problem in Turkey. In a survey of 12,300 coal workers, approximately 12% of the miners were found to have pneumoconiosis. \(^*\) The present study is the first to evaluate the cellular profile in miners in Turkey.

The yield of cells in BAL fluid is significantly dependent on the condition of the prealveolar airway. \(^*\) Recovery of fluid may markedly be attenuated in subjects with airflow obstruction. \(^*\) Occupational dust exposure leads to airflow obstruction and focal emphysema adjacent to the coal macule. The instilled BAL fluid may be trapped in the alveolar space in such subjects.

## Table 3

<table>
<thead>
<tr>
<th>Amount of given saline for BAL (ml)</th>
<th>Recovered BAL fluid (% of given)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-smoker controls ((n=8))</td>
<td>100 (16.7)</td>
</tr>
<tr>
<td>Smoker controls ((n=9))</td>
<td>105.5 (9)</td>
</tr>
<tr>
<td>Miners without pneumoconiosis ((n=8))</td>
<td>102.2 (16.7)</td>
</tr>
<tr>
<td>Simple pneumoconiosis ((n=11))</td>
<td>100 (0)</td>
</tr>
<tr>
<td>Progressive massive fibrosis ((n=10))</td>
<td>102.0 (6.3)</td>
</tr>
</tbody>
</table>

\(^*\)p<0.05 when compared with non-smoker controls.

## Table 4

<table>
<thead>
<tr>
<th>Total cell count ((x10^3))</th>
<th>Alveolar macrophages (%)</th>
<th>Lymphocytes (%)</th>
<th>Neutrophils (%)</th>
<th>Eosinophils (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-smoker controls ((n=8))</td>
<td>5.6 (3.4)</td>
<td>80.4 (10.0)</td>
<td>12.0 (10.3)</td>
<td>6.8 (3.9)</td>
</tr>
<tr>
<td>Smoker controls ((n=9))</td>
<td>23.9 (31.7)</td>
<td>85.2 (8.8)</td>
<td>8.3 (5.5)</td>
<td>6.3 (6.6)</td>
</tr>
<tr>
<td>Miners without pneumoconiosis ((n=8))</td>
<td>8.1 (7.5)</td>
<td>89.2 (20.7)</td>
<td>3.5 (5.5)</td>
<td>6.8 (14.5)</td>
</tr>
<tr>
<td>Simple pneumoconiosis ((n=11))</td>
<td>8.1 (7.6)</td>
<td>94.9 (3.5)</td>
<td>1.8 (1.3)</td>
<td>3.0 (3.0)</td>
</tr>
<tr>
<td>Progressive massive fibrosis ((n=10))</td>
<td>8.4 (7.4)</td>
<td>85.7 (24.4)</td>
<td>3.1 (3.0)</td>
<td>11.2 (25.8)</td>
</tr>
</tbody>
</table>

\(^*\)p<0.05 versus non-smoker controls.

## Table 5

<table>
<thead>
<tr>
<th>Total cell count ((x10^3/ml))</th>
<th>Alveolar macrophages</th>
<th>Lymphocytes</th>
<th>Neutrophils</th>
<th>Eosinophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-smoker controls ((n=8))</td>
<td>14.7 (7.9)</td>
<td>11.4 (5.6)</td>
<td>2.1 (3.0)</td>
<td>1.3 (0.7)</td>
</tr>
<tr>
<td>Smoker controls ((n=9))</td>
<td>33.5 (14.5)</td>
<td>24.1 (15.6)</td>
<td>2.5 (1.4)</td>
<td>1.8 (2.6)</td>
</tr>
<tr>
<td>Miners without pneumoconiosis ((n=8))</td>
<td>19.1 (16.2)</td>
<td>18.5 (16.0)</td>
<td>0.3 (0.3)*</td>
<td>0.2 (0.2)</td>
</tr>
<tr>
<td>Simple pneumoconiosis ((n=11))</td>
<td>23.0 (18.6)</td>
<td>22.5 (17.1)</td>
<td>0.4 (0.3)*</td>
<td>0.8 (1.1)</td>
</tr>
<tr>
<td>Progressive massive fibrosis ((n=10))</td>
<td>21.6 (17.9)</td>
<td>16.2 (13.9)</td>
<td>0.7 (0.9)</td>
<td>4.8 (13.8)</td>
</tr>
</tbody>
</table>

\(^*\)p<0.05 versus non-smoker controls.
with airflow obstruction and emphysema. We observed airflow obstruction in all miners, except those with simple pneumoconiosis, compared with the smoker and/or the non-smoker controls. The amount of recovered BAL fluid was significantly lower in all miners with or without pneumoconiosis, compared with the non-smoker controls. The amount of recovered BAL fluid was significantly lower in all miners with or without pneumoconiosis, compared with the non-smoker controls. The amount of recovered BAL fluid was significantly lower in all miners with or without pneumoconiosis, compared with the non-smoker controls.

The results of BAL in coal workers’ pneumoconiosis are controversial. More inflammatory cells were detected in recovered BAL fluid in subjects exposed to inorganic dust who had asbestosis, coal workers’ pneumoconiosis, or silicosis compared with normal controls, although the proportion of large cell fluid was not significantly different. Alveolar macrophages dominated these inflammatory cells. Although the proportions of neutrophils were increased in pneumoconiosis, the neutrophils represented a small proportion of the total inflammatory cell populations recovered. An increased cellularity was observed with a prominent increase in macrophages and some increments in lymphocytes and neutrophils in workers exposed to quartz with or without pneumoconiosis.

Increased cellularity in the BAL fluid in patients with simple pneumoconiosis was detected, but there was no change in white cell count. On the other hand, a striking increase in lymphocyte count was found in three subjects with rapidly developing silicosis and coexistent disorders like connective tissue disorder, sarcoidosis, extrinsic allergic alveolitis, radiation lung, or diffuse interstitial fibrosis. The authors also observed a diminished phagocytic and bacterial activity of the alveolar macrophages on Staphylococcus aureus after 24 hours’ survival.

The lymphocyte count was increased in the BAL fluid of a heterogeneous group of patients exposed to organic or inorganic dusts. These lymphocytes were activated T lymphocytes and the increment was pronounced in CD8+ T cells. The authors postulated that the monocyte-macrophage system and lymphocytes were activated whether the offending agent was organic or inorganic dust. A raised cell count with relative lymphocytosis, increased permeability, and enhanced interleukin-6 and decreased interferon-γ in the BAL fluid of subjects with idiopathic pulmonary fibrosis and patients with simple pneumoconiosis was demonstrated.

These research data state that a raised cellularity and lymphocytic and/or neutrophilic alveolitis in BAL fluid is frequently found in subjects with pneumoconiosis. We detected an increased cellularity in BAL fluid in miners compared with the non-smoker controls, however, the difference was not significant. In contrast to previous data, we found a significant decrement in lymphocytes in miners with or without pneumoconiosis. As mentioned above, airflow obstruction may account for the lower recovery of BAL fluid and the depletion of lymphocytes in the present study subjects.

In previous research on BAL fluid cellularity there are no definite data on whether the miners with alveolitis were concurrently exposed to inorganic dust. The subjects in the present study had all been retired for many years before the study, but the time elapsed was not registered. The cessation of exposure for a long enough period of time may account for the lack of alveolitis.

Although miners are exposed to similar dust with a different concentration, some never develop pneumoconiosis. However, some develop simple pneumoconiosis with nodular or linear opacities seen on chest radiographs or they further develop PMF characterised by conglomerates besides nodular or linear opacities detected by radiographs. Many cytokines have been investigated to try and discover why some people develop pneumoconiosis and some do not; genetic factors might also be involved in its development.

CONCLUSION

Alveolitis was not a representative feature in Turkish subjects with an occupational history of underground mining, and the BAL fluid cellular profile did not seem to be different in miners with or without pneumoconiosis. Some other factors like genetic predisposition and/or cytokines may be involved in the pathogenesis of pneumoconiosis.

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REFERENCES


