Pulmonary inflammation in asbestos-exposed subjects with borderline parenchymal changes on HRCT

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Summary
Many asbestos-exposed subjects have minor parenchymal changes on high resolution computed tomography (HRCT) that do not fulfill the diagnostic criteria for pulmonary fibrosis and asbestososis. We investigated if these borderline parenchymal changes in asbestos-exposed subjects are related to pulmonary inflammatory activity.

Exhaled nitric oxide was measured, exhaled breath condensate collected and HRCT scanned in 104 subjects with moderate to high occupational asbestos exposure. Forty-one healthy unexposed subjects served as a comparison group.

After excluding other pulmonary diseases, 35 asbestos-exposed subjects had normal parenchymal findings and 31 subjects had borderline parenchymal changes on HRCT. Lung function was poorer in the latter group, but there was no difference in the degree of asbestos exposure between these groups. As compared with the unexposed comparison group, asbestos-exposed subjects with borderline parenchymal changes had increased alveolar NO concentration (3.0 ± 0.2 vs. 2.3 ± 0.1 ppb, p = 0.008) and increased levels of leukotriene B4 (12.2 ± 1.1 vs. 3.3 ± 0.8 pg/ml, p < 0.001) and 8-isoprostane (9.4 ± 0.7 vs. 7.3 ± 0.6 pg/ml, p = 0.021) in breath condensate. Asbestos-exposed subjects with normal parenchymal findings had only increased breath condensate levels of leukotriene B4 (11.4 ± 0.9, p < 0.001).

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sporadic changes only, or (3) mild to extreme pulmonary fibrosis (i.e., asbestosis). As the current study focused on the borderline parenchymal changes, subjects with asbestosis (group 3) were excluded from the analysis. Inflammatory markers in exhaled air, breath condensate and serum were compared between the unexposed comparison group, asbestos-exposed subjects with normal parenchymal findings, and those with borderline parenchymal changes.

Of the 104 asbestos-exposed men recruited, 33 were excluded based on the above mentioned exclusion criteria. Thirty-five subjects had normal parenchymal findings on HRCT (fibrosis class 0), 31 subjects had borderline parenchymal changes (fibrosis classes 0.5–1.5), and 5 subjects had pulmonary fibrosis (fibrosis classes ≥ 2.0) regarded as asbestosis (Fig. 1).

**HRCT grading**

HRCT was scanned (Siemens Somatom Plus 4; Siemens Medical, Erlangen, Germany) with 1 mm slices taken at 3 cm intervals using imaging parameters of 130–140 kV and 100–111 mA. The HRCT images were scored using consensus reading by two experienced thoracic radiologists (RJ and TV) blinded to the medical information of the patients. Pulmonary fibrosis, emphysema, parietal pleural plaques, and pulmonary nodules were scored separately as described previously. The semiquantitative scoring of the HRCT findings indicating interstitial lung fibrosis (septal thickening, subpleural lines, parenchymal bands or honeycombing) in both lungs was made according to a scale of classes from 0 to 5. Fibrosis class 0 represents normal parenchymal finding, class 1 represents borderline parenchymal finding with minor sporadic changes only, and classes 2–5 represent mild to severe diffuse pulmonary fibrosis (Table 1). If the readers could not match the findings exactly with any given fibrosis class, five subclasses (0.5, 1.5, 2.5, 3.5, 4.5) were used. The fibrosis class 2 has been considered as a threshold for the diagnosis of asbestosis.

**Exhaled NO measurement**

Exhaled NO was measured with a Sievers NOA 280 analyser (Sievers Instruments, Boulder, Colorado, USA) at four exhalation flow rates of 50, 100, 200 and 300 ml/s without nose clips. The desired exhalation flow rates were achieved by letting the patients exhale through a mass flow meter connected to a computer-controlled adjustable flow restrictor. Three valid (flow rate within ± 2 ml/s from the target flow) NO measurements at each flow rate were collected. Alveolar NO concentration and bronchial NO flux were calculated according to Tsoukias and George as previously described by using exhalation flow rates of 100, 200 and 300 ml/s. In short, NO output (exhaled NO concentration / flow rate) was plotted against flow rate, and a linear regression was set. The slope and intercept of the regression line are approximates of alveolar NO concentration and bronchial NO flux, respectively. Except for one subject who was excluded from the analysis due to problems with NO measurement, linearity between NO output and flow rate at range 100–300 ml/s was good (r ≥ 0.97).
Exhaled breath condensate

Exhaled breath condensate was collected during 15 min of tidal breathing with Ecoscreen condenser (Ecoscreen, Jaeger, Hoechberg, Germany) while wearing nose clips. The samples were stored at −70 °C until assayed. LTB₄ and 8-isoprostan e concentrations in the condensates were measured by immunoassay with a detection limit of 2 pg/ml (Cayman Chemical Company, Ann Arbor, Michigan, USA). The coefficients of variation were 5.7% for MPO and 6.0% for LTB₄.

Serum samples

Venous blood was drawn as previously described. Serum levels of myeloperoxidase (MPO) were measured by immunoassay with a detection limit of 0.2 pg/ml (HyCult Biotechnology, Uden, The Netherlands) while wearing nose clips. The concentration of interleukin-6 (IL-6) was determined by enzyme immunoassay with a detection limit of 0.3 pg/ml (PeliPair ELISA, Sanquin, Amsterdam, the Netherlands). The coefficients of variation were 8.7% for MPO and 6.1% for IL-6.

Statistical analysis

All the inflammatory parameters were normally distributed (Kolmogorov–Smirnov test). Analysis of variance with LSD post-test or t-test were used to compare variables between all the three groups or between the asbestos-exposed groups, respectively. Chi²-test was used for binary variables. Pearson’s r was used to test for correlations between different inflammatory variables and lung function indices. The results are presented as mean ± SEM. A p-value < 0.05 was considered as statistically significant. SPSS 12.0.1 software (SPSS Inc., Chicago, Illinois, USA) was used for statistical analysis. Based on the SDs in different inflammatory markers in our previous studies, the current study was calculated to have a power of 90% to detect a difference of =0.5 ppb in alveolar NO concentration and differences of =3.0 pg/ml and =4.5 pg/ml in EBC levels of 8-isoprostan e and LTB₄, respectively.

Results

Subject characteristics for the three groups are presented in Table 2. Asbestos-exposed subjects with borderline parenchymal changes were on average slightly older than subjects in the other two groups. However, age was not correlated with any of the inflammatory markers in exhaled breath in any of the three groups. The proportion of ex-smokers was lower in the unexposed comparison group than in the asbestos-exposed subjects. There were no differences in asbestos exposure between the subjects with normal or borderline parenchymal findings on HRCT, but on average, subjects with borderline parenchymal changes on HRCT had poorer lung function and more extensive pleural plaques than subjects with normal parenchymal findings. The asbestos exposure index did not correlate with the levels of any inflammatory markers in exhaled breath or serum, or with the degree of parenchymal or pleural changes on HRCT scans.

Alveolar NO concentration was increased in asbestos-exposed subjects with borderline parenchymal changes on HRCT (3.0 ± 0.2 ppb) as compared with the unexposed


<table>
<thead>
<tr>
<th>Table 2</th>
<th>Subject characteristics.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Asbestos-exposed subjects</td>
</tr>
<tr>
<td></td>
<td>Normal parenchymal findings</td>
</tr>
<tr>
<td>N</td>
<td>35</td>
</tr>
<tr>
<td>Age (years)</td>
<td>62 ± 1</td>
</tr>
<tr>
<td>Ex-smokers/never-smokers</td>
<td>25/10ₐ</td>
</tr>
<tr>
<td>Pack-years among ex-smokers</td>
<td>15 ± 2</td>
</tr>
<tr>
<td>Asbestos exposure (years)</td>
<td>35 ± 2</td>
</tr>
<tr>
<td>Exposure: moderate/heavy</td>
<td>6/29</td>
</tr>
<tr>
<td>Exposure index</td>
<td>81 ± 9</td>
</tr>
<tr>
<td>FVC below normal, n (%)</td>
<td>8 (23%)</td>
</tr>
<tr>
<td>TLCO below normal, n (%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>FVC (% predicted)</td>
<td>92 ± 3ₐ</td>
</tr>
<tr>
<td>FEV₁ (% predicted)</td>
<td>92 ± 2ₐ</td>
</tr>
<tr>
<td>TLCO (% predicted)</td>
<td>109 ± 2ₐ</td>
</tr>
<tr>
<td>TLCO/VA (% predicted)</td>
<td>109 ± 3</td>
</tr>
<tr>
<td>Pleural calcifications on HRCT, n (%)</td>
<td>24 (69%)</td>
</tr>
<tr>
<td>Extent of pleural plaques (cm²)</td>
<td>286 ± 24</td>
</tr>
</tbody>
</table>

FVC, forced vital capacity. FEV₁, forced expiratory volume in 1 s. TLCO, carbon monoxide transfer factor adjusted for haemoglobin. TLCO/VA, specific carbon monoxide transfer factor adjusted for haemoglobin. HRCT, high resolution computed tomography.

ₐ p < 0.05 as compared with the unexposed comparison group.
ₐₐ p < 0.05 as compared with asbestos-exposed subjects with normal parenchymal findings.
comparison group (2.3 ± 0.1 ppb, p = 0.009) or asbestos-exposed subjects with normal parenchymal findings (2.2 ± 0.2 ppb, p = 0.004). Asbestos-exposed subjects with normal parenchymal findings had a slightly lower bronchial NO flux (0.7 ± 0.1 nl/s, p = 0.033) as compared with the unexposed comparison group (1.0 ± 0.1 nl/s), whereas subjects with borderline parenchymal changes (0.8 ± 0.1 nl/s, p = 0.142) did not differ significantly from the comparison group. Subjects with borderline parenchymal changes were not different from the comparison group at any flow rate (Table 3).

8-isoprostane levels in exhaled breath condensate were increased in asbestos-exposed subjects with borderline parenchymal changes but not in subjects with normal parenchymal findings, as compared with unexposed subjects (Fig. 3). Condensate levels of LTB4 were increased both in asbestos-exposed subjects with borderline and normal parenchymal findings (Fig. 3, Table 3). Serum levels of IL-6 and MPO were increased only in asbestos-exposed subjects with borderline parenchymal changes (Table 3).

In the unexposed comparison group, bronchial NO flux was lower in ex-smokers than in never-smokers (0.8 ± 0.1 vs. 1.1 ± 0.1 nl/s, p = 0.019), but alveolar NO concentration or markers in EBC were not significantly different between ex-smokers and never-smokers. Among the asbestos-exposed subjects, there were no differences in any of the inflammatory markers in exhaled breath between ex-smokers and never-smokers.

In asbestos-exposed subjects, alveolar NO concentration correlated positively with 8-isoprostane and negatively with LTB4 levels in exhaled breath condensate (Fig. 4). Extent of pleural plaques on HRCT correlated positively with serum levels of IL-6 (r = 0.282, p = 0.024) and condensate levels of 8-isoprostane (r = 0.254, p = 0.043). There were no other significant correlations between inflammatory markers and indices of lung function either.

The correlations between different markers were similar if analysed separately in the subgroups of subjects with normal or borderline parenchymal findings on HRCT.

Discussion

In the present study, almost half of the subjects with moderate to heavy asbestos exposure had borderline parenchymal findings with minor sporadic changes on HRCT not fulfilling the diagnostic criteria for asbestosis. These subjects had also more extensive pleural plaques and poorer lung function than those with normal parenchymal findings, although there was no difference in the degree of asbestos exposure. Subjects with borderline parenchymal changes had increased alveolar NO concentration, increased levels of LTB4 and 8-isoprostane in breath condensate, and increased levels of IL-6 and MPO in serum, while only condensate levels of LTB4 were increased in asbestos-exposed subjects with normal parenchymal findings.

One previous study reports increased exhaled NO concentration measured at a single flow rate in subjects with asbestosis or pleural plaques. We have recently demonstrated that the increased NO output in asbestosis is peripheral in origin, which is in line with the parenchymal inflammation and fibrosis in these subjects. In the present study, alveolar NO concentration was normal in asbestos-exposed subjects with normal parenchymal findings on HRCT, and increased in those with borderline parenchymal changes, but was even higher in subjects with diffuse fibrosis (asbestosis) in our previous study (3.2 ± 0.4 ppb). It seems, thus, that alveolar NO is related to the degree of pulmonary fibrosis rather than to asbestos exposure per se. As we excluded subjects with obstructive lung function or any emphysema detectable on HRCT, we believe that the increased alveolar NO concentration in asbestos-exposed subjects with borderline parenchymal changes is related to asbestos induced process and not to other pulmonary diseases. This is also supported by the ability of asbestos to induce the expression of inducible NO synthase (iNOS) in human pneumocytes in vitro.
Alveolar NO has been found to correlate with impaired lung function in diseases like idiopathic pulmonary fibrosis and COPD.\textsuperscript{10,11} However, there was no significant correlation in the present study between alveolar NO and lung function. If we had included also subjects with severe pulmonary fibrosis (i.e. asbestosis) and thereby widened the range in disease severity a correlation might have been found.

Bronchial NO flux was decreased in asbestos-exposed subjects with normal parenchymal findings, and to a lesser extent also in those with borderline parenchymal changes on HRCT. This might be related to the higher proportion of ex-smokers in the asbestos-exposed subjects, as decreased bronchial NO flux is reported in healthy ex-smokers as compared with never-smokers.\textsuperscript{22} The decreased bronchial NO flux in asbestos-exposed subjects with normal parenchymal findings was reflected also as decreased exhaled NO concentrations at flow rates of 50 and 100 ml/s, in which the relative proportion of NO from bronchial sources is bigger than in the higher flow rates. In contrast, the increase in peripheral NO output in subjects with borderline parenchymal changes was not reflected as increased NO concentrations at any single flow rate. This demonstrates the superiority of the multiple flow rate method in assessing the changes in peripheral NO output.

Condensate levels of LTB\textsubscript{4} were increased in both groups with asbestos exposure, but 8-isoprostane levels were increased only in subjects with borderline parenchymal

<table>
<thead>
<tr>
<th>Asbestos-exposed subjects</th>
<th>Borderline parenchymal changes n = 31</th>
<th>Unexposed comparison group n = 41</th>
</tr>
</thead>
<tbody>
<tr>
<td>FENO\textsubscript{0.05} (ppb)</td>
<td>17.3 ± 1.2\textsuperscript{a}</td>
<td>20.0 ± 2.0</td>
</tr>
<tr>
<td>FENO\textsubscript{0.1} (ppb)</td>
<td>9.8 ± 0.7\textsuperscript{a}</td>
<td>11.2 ± 1.0</td>
</tr>
<tr>
<td>FENO\textsubscript{0.2} (ppb)</td>
<td>5.9 ± 0.4</td>
<td>6.9 ± 0.6</td>
</tr>
<tr>
<td>FENO\textsubscript{0.3} (ppb)</td>
<td>4.7 ± 0.3</td>
<td>5.7 ± 0.5</td>
</tr>
<tr>
<td>CA\textsubscript{NO} (ppb)</td>
<td>2.2 ± 0.2</td>
<td>3.0 ± 0.2\textsuperscript{b}</td>
</tr>
<tr>
<td>J\textsuperscript{awNO} (nl/s)</td>
<td>0.7 ± 0.1\textsuperscript{a}</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>EBC 8-isoprostane (pg/ml)</td>
<td>8.3 ± 0.6</td>
<td>9.5 ± 0.8\textsuperscript{a}</td>
</tr>
<tr>
<td>EBC LTB\textsubscript{4} (pg/ml)</td>
<td>11.4 ± 0.9\textsuperscript{c}</td>
<td>12.3 ± 1.1\textsuperscript{c}</td>
</tr>
<tr>
<td>S-IL-6 (pg/ml)</td>
<td>3.5 ± 0.3</td>
<td>4.5 ± 0.6\textsuperscript{a}</td>
</tr>
<tr>
<td>S-MPO (pg/ml)</td>
<td>141 ± 7</td>
<td>158 ± 6\textsuperscript{a}</td>
</tr>
</tbody>
</table>

FENO\textsubscript{0.05}, Exhaled NO concentration at exhalation flow rate of 50 ml/s.
CA\textsubscript{NO}, alveolar NO concentration.
J\textsuperscript{awNO}, bronchial NO flux.
EBC, exhaled breath condensate.
\textsuperscript{a}p < 0.05 as compared with the unexposed comparison group.
\textsuperscript{b}p < 0.01 as compared with the unexposed comparison group.
\textsuperscript{c}p < 0.001 as compared with the unexposed comparison group.

Figure 3  
Levels of 8-isoprostane and leukotriene B\textsubscript{4} (LTB\textsubscript{4}) in exhaled breath condensate (EBC) in asbestos-exposed subjects with normal (N, n = 35) or borderline (BL, n = 31) parenchymal findings on HRCT, and in the unexposed comparison group (n = 41)(mean ± SEM).

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LTB₄ is a potent chemotactic factor for neutrophils,¹⁴ and occupational asbestos exposure has been shown to provoke LTB₄ secretion in alveolar macrophages in vivo.²³ 8-isoprostane is produced by lipid peroxidation and can be used as a marker of oxidative injury in many diseases.¹⁵ These findings together with our present results suggest that LTB₄ level in EBC reflects the inflammatory response to asbestos, while EBC level of 8-isoprostane is related to the degree of oxidative stress, tissue damage and fibrosis. As also alveolar NO concentration was increased only in subjects with borderline changes on HRCT, it can be understood that 8-isoprostane correlated positively with alveolar NO. The negative correlation between condensate levels of LTB₄ and alveolar NO concentration further supports the hypothesis that LTB₄ is related to inflammatory state while 8-isoprostane and alveolar NO reflect tissue damage and fibrosis.

Asbestos exposure stimulates alveolar macrophages to produce IL-6, which is involved in recruitment of inflammatory cells and in fibroblast proliferation.¹ Increased serum levels of IL-6 have previously been shown in asbestos-exposed subjects with or without pulmonary fibrosis.¹⁶,²⁴ MPO is a major constituent of neutrophils and it generates hypochlorous acid and reactive nitrogen species. Asbestos exposure induces pulmonary MPO production that plays a role in the tissue damage and cancer risk.²⁵,²⁶ We found increased serum levels of IL-6 and MPO only in those asbestos-exposed subjects who had borderline parenchymal changes on HRCT. This suggests either that these subjects had more pronounced inflammatory response to asbestos than those with normal parenchymal findings, or that serum levels of IL-6 and MPO are related to the development of minor parenchymal changes on HRCT. It might be that individual differences in immune responses to asbestos determine the production of inflammatory mediators, and thereby the susceptibility to develop asbestos related diseases.

Interestingly, there was no correlation between the degree of asbestos exposure and the extent of pleural and parenchymal changes seen on HRCT. This might also be related to individual differences in immune responses to asbestos, which determines the susceptibility to develop fibrotic changes after asbestos exposure. However, the assessment of asbestos exposure was based on retrospective interview and this may cause some inaccuracy in the exposure indices.

Minor fibrotic changes on HRCT have been found even in asbestos-exposed subjects with normal lung function and chest X-ray.³,²⁷ When comparing HRCT findings with histological signs of asbestosis, it has been shown that parenchymal changes on HRCT should be bilateral or multifocal to suggest diffuse fibrosis. Unilateral or focal changes on HRCT are not considered sufficient for the diagnosis of diffuse fibrosis (asbestosis), although histological evidence of asbestosis like changes have been found in some cases with normal or nearly normal HRCT.⁷ The borderline parenchymal changes on HRCT might be an early sign of future diffuse fibrosis. If this is the case, the non-invasive markers of inflammation and oxidative stress used in the current study might be useful in predicting the individual risk of disease progression.

In conclusion, borderline parenchymal changes on HRCT in asbestos-exposed subjects are associated with pulmonary inflammation. The profile of inflammatory markers in asbestos-exposed subjects with borderline parenchymal changes resembles that found in patients with frank asbestosis in our previous study,¹⁶ while subjects with normal parenchymal finding but similar asbestos exposure have nearly normal levels of inflammatory markers. Such borderline parenchymal changes are therefore likely a mild or early form of the same pathological process that leads to asbestosis. A follow-up study is needed to assess if the markers of inflammation could be used to predict the risk of developing diffuse pulmonary fibrosis and asbestosis among subjects with borderline parenchymal changes.

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Conflict of interest statement

The authors have no conflicts of interest related to the study to be disclosed.

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