Non-fibrous inorganic particles in bronchoalveolar lavage fluid of pottery workers

M Falchi, L Paoletti, S Mariotta, S Giosue, L Guidi, L Biondo, P Scavalli, A Bisetti

Abstract

Aim—To study the actual exposure of pottery workers to silica particles, as their risk of silicosis is potentially high because of the presence of inhalable crystalline silica particles in the workplace.

Methods—Nine pottery workers underwent bronchoalveolar lavage. The recovered fluid was analysed for cytopathological and mineralogical content by analytical transmission electron microscopy. The data were compared with those obtained from a control group composed of seven patients with sarcoidosis and six patients with haemoptysis.

Results—Cytological results showed a similar profile in exposed workers and controls, whereas in patients with sarcoidosis a lymphocytic alveolitis was found. Microanalysis of the particulate identified the presence of silicates, CRSs, and metals. Pottery workers had higher numbers of total particles and CRSs, and had a higher silicate/metal ratio. In five workers, the presence of zirconium silicate was also detected. Patients with sarcoidosis had the lowest number of particles, and an inverted silicate/metal ratio.

Conclusion—Microanalysis by transmission electron microscopy can provide useful information to assess occupational exposure to dusts.

Keywords: occupational exposure; bronchoalveolar lavage fluid; silicosis; transmission electron microscopy

Inhaled silica particles injure the lung tissue. This involves alveolar macrophages and, through an immunological mechanism, it eventually causes silicosis.

Bronchoalveolar lavage (BAL) is an easy procedure that can provide useful information about the actual exposure to dusts in the workplace by analysing recovered BAL fluid.

The risk of developing silicosis is well known among pottery workers because of the presence of inhalable crystalline silica particles in working environments. Therefore, industrialised countries have adopted measures to prevent risk and compensate the workers. The Italian ceramics industry adopted (on the basis of recommendations from the American Conference of Governmental Industrial Hygienists (ACGIH)) the limit of 0-05 mg/m³ for airborne inhalable crystalline silica particles in working environments, above which the payment of an insurance premium is provided for exposed workers. The crystalline silica particles are one of the principal components in ceramic and enamel mixtures. On average they constitute 35%-38% of the vitreous china mixture and 22%-26% of enamels.

Civita Castellana is one of the largest Italian industrial areas involved in the production of vitreous china, fire clay, and pottery articles (about 30% of the total Italian production). In this area the concentration of crystalline silica particles in working environments has been controlled since 1980. Nevertheless, some cases of silicosis are still diagnosed in the people employed in these plants.

To study the exposures of pottery workers in actual working conditions, we compared the mineralogical analysis, by analytical transmission electron microscopy, of the particles found in BAL fluid of nine workers employed in pottery plants in Civita Castellana for more than 10 years, with the mineralogical analysis of the BAL fluid of a control group composed of seven patients with sarcoidosis and six with haemoptysis, none of whom had ever been occupationally exposed to dusts.

Materials and methods

STUDY POPULATION

The exposed workers were nine male workers (mean (SD) age 51-1 (6-9); three smokers, four ex-smokers, and two non-smokers) employed in the ceramics industry for at least 10 years. They were recruited during a routine check up. Information on smoking and work history was recorded for each subject. Patients were defined as ex-smokers when they had stopped smoking for at least two years. Lung function tests were obtained, and chest x ray films were classified according to International Labour Organisation (ILO) 1980 criteria.

The control group had never been occupationally exposed to dusts. They were: seven housewives (mean (SD) age 50-8 (13), one smoker and six non-smokers) with pulmonary sarcoidosis stage II (all patients were at their first check up and had never been treated for sarcoidosis before; sarcoid granulomas were found by histological examination of the lymph nodes) and six patients with haemoptysis (five men and one woman, mean (SD) age 38-6 (9-1), three smokers, one ex-smoker, and two non-smokers), all with lung function test scores inside the normal range and without abnormalities on chest x ray film.
Table 1  Characteristics and mineralogical analysis of the nine pottery workers

<table>
<thead>
<tr>
<th>Case</th>
<th>Smoking</th>
<th>Age (y)</th>
<th>Duration of work (y)</th>
<th>Duty</th>
<th>x-ray film*</th>
<th>Lung function</th>
<th>All particles/ml BAL fluid</th>
<th>Crystalline silica particles</th>
<th>Size/μm</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>no</td>
<td>39</td>
<td>22</td>
<td>Furnaces</td>
<td>p&lt;1/1-0</td>
<td>Normal</td>
<td>29923</td>
<td>9236</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>ex</td>
<td>43</td>
<td>22</td>
<td>Furnaces</td>
<td>p&lt;1/1-0</td>
<td>Obstructive</td>
<td>57000</td>
<td>12828</td>
<td>1260</td>
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<tr>
<td>3</td>
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<td>56</td>
<td>11</td>
<td>Furnaces</td>
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<td>Normal</td>
<td>130000</td>
<td>27300</td>
<td>15000</td>
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<tr>
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<td>yes</td>
<td>46</td>
<td>16</td>
<td>Furnaces</td>
<td>p&lt;1/1-0</td>
<td>Normal</td>
<td>185827</td>
<td>70809</td>
<td>36100</td>
</tr>
<tr>
<td>5</td>
<td>ex</td>
<td>59</td>
<td>22</td>
<td>Furnaces</td>
<td>p&lt;2/1-1</td>
<td>Normal</td>
<td>192762</td>
<td>9570</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>ex</td>
<td>58</td>
<td>20</td>
<td>Furnaces</td>
<td>p&lt;1/1-2</td>
<td>Normal</td>
<td>19996</td>
<td>19560</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>ex</td>
<td>53</td>
<td>16</td>
<td>Blunting</td>
<td>p&lt;1/1-0</td>
<td>Normal</td>
<td>43440</td>
<td>13028</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>yes</td>
<td>52</td>
<td>19</td>
<td>Enamelling</td>
<td>p&lt;1/1-1</td>
<td>Normal</td>
<td>97051</td>
<td>21926</td>
<td>33069</td>
</tr>
<tr>
<td>9</td>
<td>yes</td>
<td>54</td>
<td>15</td>
<td>Decorator</td>
<td>p&lt;1/1-1</td>
<td>Normal</td>
<td>54183</td>
<td>12442</td>
<td>829</td>
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* Chest x-ray film according to ILO classification.

All patients underwent fibroptic bronchoscopy and BAL as part of the diagnostic procedure. Written informed consent was obtained from all subjects.

FIBROPTIC BRONCHOSCOPY AND BAL

Fibroptic bronchoscopy was carried out 30 minutes after intramuscular administration of atropine (0.5 mg) and clorpromazine (50 mg). Local anaesthesia was with 0.4% oxibuprocalaine. The BAL was performed in the middle lobe. The fibroscope was wedged in the selected subsegmental bronchus, and five 20 ml boluses of sterile saline solution heated to 37°C were injected and immediately withdrawn by mechanical suction.

BAL FLUID PROCESSING AND ANALYSIS

The first aliquot of the BAL fluid was discarded, the other four aliquots were pooled. The fluid was filtered through gauze and the recovered amount recorded. Total and differential cell counts were performed with a Burker haematocytometer and cytocentrifuge preparations were stained with May-Grünwald-Giemsa. At least 300 cells were identified by microscopic examination (× 1000). Twenty five millilitres of BAL fluid were used for mineral particle analysis by transmission electron microscope. The BAL fluid samples were put in polyethylene containers with 10 ml 5% ultrapure sodium hypochlorite (Aldrich-Chemie) and, if necessary, 10 ml 30% hydrogen peroxide. The containers were kept at 30°C for between 30 minutes and one hour, until complete oxidation of the organic fraction. The resulting solution was diluted with deionised water and then filtered through cellulose nitrate filters (25 mm diameter) with a pore size of 0.45 μm (Sartorius AG, Goettingen-Germany). The filter surfaces were lightly smoothed by a plasma etching before use. Then the filters were dried, covered with a thin carbon film, cut, mounted on standard 200 mesh copper grids for transmission electron microscope, and exposed to acetone fumes until the filter dissolved. All the reagents and the solutions used for sample preparation were prefiltered to avoid contamination with particles. Also, two blank samples were processed in the same manner as BAL samples in different phases of the study and analysed; no mineral particles were found in the electron micrographs.

The mineral particulate on carbon films was analysed by a transmission electron microscope Philips EM430 equipped with an EDAX energy dispersive spectrometer for microanalysis of x-ray films. For each subject 25 to 40 grid apertures (an aperture effective area being about 8400 μm²) were examined in at least five different grids; an accelerating voltage of 250 kV and a magnification of × 10 000 were used. The particles detected were identified by morphology, chemical composition (elements with atomic number > 11), and crystalline structure. The detected particles were grouped as three types—namely, (a) silicates (particles containing silica), (b) crystalline silica particles (particles which showed an EDAX spectrum composed only of a Si peak and with a diffraction pattern indicative

Table 2  Characteristics and mineralogical analysis of the patients with sarcoidosis

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age</th>
<th>Smoking</th>
<th>Job</th>
<th>All particles/ml BAL fluid</th>
<th>Crystalline silica particles</th>
<th>Silicates (%)</th>
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<tr>
<td>1</td>
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<td>Housewife</td>
<td>6409</td>
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<td>2</td>
<td>F</td>
<td>55</td>
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<td>13190</td>
<td>4770</td>
<td>36</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>45</td>
<td>no</td>
<td>Housewife</td>
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<td>5339</td>
<td>36</td>
</tr>
<tr>
<td>4</td>
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<td>40</td>
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<tr>
<td>6</td>
<td>F</td>
<td>55</td>
<td>no</td>
<td>Housewife</td>
<td>20326</td>
<td>6911</td>
<td>34</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>39</td>
<td>no</td>
<td>Housewife</td>
<td>38650</td>
<td>3632</td>
<td>30</td>
</tr>
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</table>

Table 3  Characteristics and mineralogical analysis of the patients with haemoptysis

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age</th>
<th>Smoking</th>
<th>Job</th>
<th>All particles/ml BAL fluid</th>
<th>Crystalline silica particles</th>
<th>Silicates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>36</td>
<td>yes</td>
<td>Employee</td>
<td>18202</td>
<td>2844</td>
<td>69</td>
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<tr>
<td>2</td>
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<td>yes</td>
<td>Worker</td>
<td>29048</td>
<td>40968</td>
<td>62</td>
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<tr>
<td>3</td>
<td>M</td>
<td>34</td>
<td>ex</td>
<td>Worker</td>
<td>50461</td>
<td>4266</td>
<td>47</td>
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<tr>
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<td>M</td>
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<td>6320</td>
<td>50</td>
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<td>no</td>
<td>Nurse</td>
<td>14647</td>
<td>1185</td>
<td>63</td>
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</tbody>
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of a crystalline structure; amorphous silica particles were pooled in the silicates group), (c) metal particles and particles containing different elements from silicon.

### STATISTICAL ANALYSIS

Statistical analysis was performed by Mann-Whitney U test in the evaluation of cytological and mineralogical data. The $\chi^2$ test was used to analyse the silicate and metal patterns, and the frequency of metal compounds. A P value $< 0.05$ was considered to be significant.

### Results

Tables 1 to 3 show the characteristics of the exposed and control subjects. In all pottery workers chest x ray films showed small opacities classified in categories 1 and 2 of the ILO classification, typical of pneumoconiosis. Six subjects worked on the furnaces, one in blunting, one in enamelling, and one in decoration. All of them had normal lung function tests except for one, who presented an obstructive pattern. The number of particles/ml (pp/ml) varied from 29923 to 199936, and crystalline silica particles/ml ranged from 9236 to 70890. A distinctive characteristic of five exposed subjects was the presence of zirconium silicate particles.

Table 4 shows the results of cytological and mineralogical analysis of BAL fluid. The percentage of BAL fluid recovered from pottery workers was significantly lower ($P < 0.05$) than in patients with haemoptysis, although the total cell count/ml did not show significant differences between groups.

Significant differences between groups were found in particle concentrations in BAL fluid. Occupational exposure of pottery workers is reflected by the average particle concentration which is about one order of magnitude greater than in the control group (patients with sarcoidosis ($P < 0.001$) and patients with haemoptysis ($P = 0.05$)).

As expected, the highest concentration of crystalline silica particles was obtained from pottery workers, and the relative difference from the other groups was significant ($P < 0.001$).

Differences among groups were also evident when comparing the average composition of the mineral particulate recovered in BAL fluid. Patients with haemoptysis showed an average frequency of metal, silicates, and crystalline silica particles comparable with those of non-exposed subjects as measured in previous studies. Patients with sarcoidosis showed a higher percentage of metal particles than did patients with haemoptysis. The exposed groups showed an inverted ratio of silicates/metal particles and an average frequency of 23.6% of crystalline silica particles.

In the metal particle group, the nine most frequent elements identified were: Al, Ca, Fe, FeCrNi alloys, Ni, Pb, Sb, Tl, Zn, and other particles containing Fe. Table 5 shows the relative frequency of elements detected in each group, and the percentage of subjects in whom the element was found. Particles containing Ti were detected in all subjects, Fe in all except one, and Ca in all except two. Aluminium and Zn were present in more than 50% of BAL fluids, and FeCrNi alloys, Sb, Ni, and Pb were rare. The differences in the frequency of elements in the groups were affected by the total amount of metal particles detected in each group. For instance, the high percentage of Ti detected in patients with sarcoidosis can be ascribed to the low number of particles detected in all, rather than to a specific type of exposure. Nevertheless, the frequency pattern of pottery workers resembles that of patients with haemoptysis but differs from that of patients with sarcoidosis.

### Discussion

The aim of this study was to evaluate the actual exposure of a group of pottery workers to silica particles. The mineralogical analysis of BAL fluid is used as an advaut for the measurement of occupational exposure in patients with interstitial lung diseases. In the past, silica particles were identified by polarised light microscopy. Nowadays, scanning and transmission electron microscopy have considerably improved diagnosis by the identification and semiquantitative microanalysis of silica and other minerals in BAL fluid and biotic specimens. Many studies
have investigated the relations between exposure, retained dose, and pathological alteration in subjects exposed to different kinds of dust.\(^3\) 17-20

Christman et al\(^2\) found in the macrophages of granite workers a notable increase in particles with a spectrum of x ray film energies consistent with quartz, and a variety of aluminum, magnesium, and calcium silicate particles. There was a connection between the number of particles and the time of exposure: the pattern was obviously different from healthy controls. Funahashi et al\(^21\) studied the content of silica particles in the lungs of exposed and unexposed subjects by energy dispersive x ray film analysis and came to the conclusion that the lungs with abnormalities on x ray film consistent with silicosis had an amount of silica, expressed as Si/S (silicon/sulphur) ratio, higher than unexposed subjects without abnormal x ray films. Also Lusuardi et al\(^3\) found a higher Si/S ratio in exposed subjects than in controls and patients with interstitial lung diseases. Bernstein et al\(^2\) found a high burden of working substances in the BAL fluid of dental technicians, and came to the conclusion that results from analysis of BAL fluid reflect actual exposure in the workplace.

Our data confirm that it is possible to define the occupational exposure of subjects by the analysis of BAL fluid. The measurement of total particles in BAL fluid seems to be the best indicator of occupational exposure. The mineralogical pattern can give further information on the type of mineral particle pollution to which patients have been exposed. Gaudichet et al\(^22\) asserted that, in the case of ubiquitous particles, it is necessary to support the evidence of occupational exposure with a quantitative microanalysis of BAL fluid.

The mineralogical particulate pattern in BAL fluid from our unexposed subjects reflects the composition of aerosol particulate found in an urban atmosphere and BAL fluid particulate concentrations seem to be confined within the range 0.1-1 x 10\(^3\) pp/ml. Cytological examination showed a similar pattern in normal and exposed subjects, although patients with sarcoidosis showed classic lymphocytic alveolitis. The lowest mineral particle concentrations found in the group with sarcoidosis could be related not only to differences in cytological pattern but also to the fact that this group was made up of housewives, all non-smokers except one (six to seven cigarettes/day); all of them lived in modern urban flats with central heating and gas kitchens and were probably exposed to few breathable mineral particulates.

In non-occupationally exposed people different variables—that is, smoke, work history, passive smoking, residence, hobbies, etc.—interact to determine the mineral particulate burden deep in the lungs, whereas quality and quantity of mineral particles in BAL fluid of pottery workers is determined principally by occupational exposure. Silicosis is the result of a chronic exposure (20-40 years) to a airborne breathable concentration of crystalline silica particles (three to fivefold higher than the threshold limit value)\(^23\) and it has been shown that crystalline silica particles cause an immune response in the alveolar spaces, involving macromolecules and lymphocytes, that results in pulmonary fibrosis.\(^1\) 24-27 The presence of significant quantities of crystalline silica particles found in BAL fluid of pottery workers, may be related to the slight pathological alterations indicative of early stages of pneumoconioses.

The presence of zirconium particles in exposed patients deserves some consideration. Zirconium sand, mainly produced in Australia, is used as an opacifier, and typically accounts for 4%-12% of the enamelling mixture. Zirconium silicates are not present in Italian geological formations and, their commercial or industrial uses are mainly confined to foundries and the ceramics industry. Therefore the presence of zirconium silicate particles in BAL fluid could be used as a marker for the definition of occupational exposure in these specific activities. Lipppo et al\(^28\) have recently published a case report of hypersensitivity pneumonitis in a ceramic tile worker, attributed to exposure to zirconium silicate, but the data available now are insufficient to hypothesise a causal relation between exposure to zirconium and pulmonary fibrotic reaction. Further investigations should be conducted to explore the possibility of an additive or synergic action of crystalline silica particles and zirconium silicate particles in the development of the fibrogenic response of lung tissues.

5. Christman JW, Emerson RJ, Hemenway DR, Graham WCB, Davis GS. Effects of work exposure, retirement
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