Biopersistence of Nonfibrous Mineral Particles in the Respiratory Tracts of Subjects following Occupational Exposure

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Transmission electron microscopy analysis (TEMA) was used to analyze the bronchoalveolar lavage fluid (BALF) of 262 subjects occupationally exposed (OE) to nonfibrous mineral particles (NFMP) and 42 controls not occupationally exposed to mineral dusts. OE subjects were divided into three groups according to the lapse of time since last exposure: ≤1 year (E1), >1 year and ≤10 years (E2), ≥10 years (E3). The total BALF mineral particle concentration was significantly higher in OE patients than in controls and was closely related to the time lapse since last exposure to NFMP (median values for OE, 7.7x10^5 particles/ml; E1, 9x10^5 particles/ml; E2, 5x10^5 particles/ml; E3, 4.3x10^5 particles/ml). No statistical difference was observed for age and smoking habits between OE and control subjects. Concentrations of crystalline silica and metals (exogenous iron, aluminum, metallic alloys and other metals) were significantly higher in OE subjects than in controls, and even though these mineral concentrations decreased with increasing time since last occupational exposure, they still remained higher in the E3 group than in controls. Crystalline silica and metals were thus identified as biopersistent NFMP in the human lung using BALF ATEM method. This method is a useful tool in assessing occupational exposure to NFMP, even when a long period has elapsed since last exposure, and may be used in studying etiology of some respiratory diseases. — Environ Health Perspect 102(Suppl 5):269-275 (1994)

Key words: biopersistence, bronchoalveolar lavage, nonfibrous mineral particles, crystalline silica, metals, occupational exposure, transmission electron microscopy analysis

Introduction

Many respiratory diseases result from accumulation of fibrous or nonfibrous mineral particles (NFMP) in the lung (1). Although analysis of lung samples provides the most direct index for studying the accumulation of mineral dusts in the lung, biopsy or autopsy tissue is rarely available. Bronchoalveolar lavage provides a safe noninvasive tool for analysis of alveolar cytological, immunological, and mineralogical parameters (2-6).

Chemical and mineralogical analysis of the BALF provides a good estimate of lung dust load and may be very helpful in establishing etiological diagnosis of occupational lung disorders. Many studies performed on fibrous particles in humans have demonstrated that the concentration of asbestos bodies in BALF is correlated with the parenchymal concentration (7). Characterization and quantification of asbestos bodies (using optical microscopy) or of fibers (using electron microscopy) in BALF or lung tissue is very useful in interstitial diseases, pulmonary malignancies, and mesothelioma (7-11). These markers provide a good estimate of previous exposure to asbestos even long after exposure has ceased, as asbestos fibers remain in the lungs for a long period.

In contrast, few studies have been performed on NFMP in BALF, perhaps due to the lack of specific markers such as asbestos bodies and to the complexity of the methods required—transmission electron microscopy (TEM) or scanning electron microscopy (SEM), each with associated analytical techniques. Moreover, some NFMP are found in almost all subjects (12,13). Some studies have focused on mineralogical analysis of particles in macrophages or in BALF of pneumoconiotic patients (14,15), of subjects with various occupational exposure (13,16-19) or of those not occupationally exposed (16,19,20). These studies provided mineralogical profiles suggestive of a given occupational exposure. Good agreement has been observed between the particle types in BALF and lung samples of subjects without recent significant occupational exposure to NFMP (20). Mineralogical determination of trace elements has been made in alveolar macrophages or lavage fluids of controls (21) or of workers exposed in various industrial settings (22,23). There have been few studies of the relationship between time since last exposure and the mineralogical profile measured in BALF (14,19).

This current study was undertaken to determine the biopersistence of NFMP in human lung. Analysis of BALF using TEM was performed in active and retired workers occupationally exposed to NFMP. The objective was to determine whether the mineralogical profile recovered from these workers was influenced by time since cessation of exposure and whether the analysis of NFMP in BALF could be used for the assessment of past occupational exposure which could be related to respiratory diseases.

Methods

Study Population

The exposed group consisted in 262 subjects for which a mineralogical analysis of NFMP in BALF was requested in our...
Table 1. Characteristics of study subjects.

| Characteristic          | Controls | E1 + E2 + E3 | p  | E1   | E2   | E3   | p  |
|-------------------------|----------|--------------|----|------|------|------|----|
| Number of subjects      | 42       | 262          |    | 186  | 38   | 38   |   |
| Gender: male/female     | 24/18    | 250/12       |    | 177/9| 37/1 | 36/2 |   |
| Male/female ratio       | 1.3      | 20.8         |    | 19.7 | 37   | 16   |   |
| Age                      | 48.9     | 50.1         | NS | 45.4 | 57.1 | 65.5 | ***|
| (16.8)                  | (13.3)   | (10.5)       | (12.2) | (10.8) |    |      |   |
| Smoking, %              |          |              |    |      |      |      |    |
| Undetermined            | 7.1      | 13           |    | 14   | 13.2 | 7.9  |   |
| Never                   | 40.5     | 23.7         |    | 21.5 | 28.9 | 26.9 | NS |
| Ever                    | 52.4     | 63.3         |    | 64.5 | 57.9 | 63.2 |   |
| Pack-years for smokers  | 30.8     | 24.7         | NS | 23.4 | 29.5 | 26.9 | NS |
| (24.9)                  | (14.5)   | (13.9)       | (18.7) | (12.7) |    |      |   |

NFMP, nonfibrous mineral particles. NS, not significant. *Level of significance when E1 + E2 + E3 group is compared with controls. †Level of significance when E1, E2, D3 groups are simultaneously compared. ‡Data shown as mean (standard deviation). *p < 0.05. †p < 0.01. ‡p < 0.001.

Table 2. Total particle concentrations in BALF (× 10^5/mI). *

| Characteristic          | Controls | E1 + E2 + E3 | p  | E1   | E2   | E3   | p  |
|-------------------------|----------|--------------|----|------|------|------|----|
| Mean                    | 4.4      | 18.4         |    | 20.9 | 14.7 | 9.0  |   |
| (SD)                    | (11.0)   | (35.2)       |    | (39.6) | (22.4) | (22.4) |   |
| Median                  | 2        | 7.7          | ***| 9    | 5    | 4.3  |   |
| (5–95% percentiles)     | (0.7–10.4)| (0.49–57)    |   | (0.52–80.5)| (0.4–48) | (0.96–30) |   |

NFMP, nonfibrous mineral particles. *Numbers in brackets indicate the number of subjects for each group of latency. They differed from Table 1 because relevant count was impossible in some BALF. This was mainly explained by presence of aggregated particles. †Level of significance when E1 + E2 + E3 group is compared with controls. ‡Level of significance when E1, E2, and E3 groups are compared simultaneously. *p < 0.05. †p < 0.01. ‡p < 0.001.

Laboratory between 1981 and 1992. This analysis was performed on samples collected in more than 30 hospitals. For each patient, information on tobacco smoking and work history including dates of beginning and end of each occupation, were recorded. All patients had at least one job in which they were exposed to NFMP, either mentioned on the questionnaire or as deduced from the job title. Occupationally exposed (OE) patients were divided into three groups according to the time lapse between the end of the last exposure to NFMP and the date of BALF. Group E1 was subjects for whom the time since last exposure was ≤1 year; for group E2 the period was >1 year but <10 years, and for group 3, ≥10 years.

The controls were 42 subjects without occupational exposure to NFMP, recruited during the same period.

Particle Analysis

All chemicals employed for preparing the samples were filtered using Millipore membranes (0.45-μm pore size), and were taken in all procedures to avoid contamination. Recovered BALF (2–39 ml, median: 17 ml) was collected in flasks containing 10 ml of 10% formalin, then treated with sodium hypochlorite for 2 h at room temperature to digest all organic material. The mixture was then concentrated by filtration through a carbon-coated polycarbonate membrane (Nuclepore, 0.4 μm pore size). After drying for 5 min in an oven, the Nuclepore membrane was again coated with a carbon film and the collected particles were transferred onto copper TEM grids as previously described (24). The mineralogical analysis was performed using an analytical transmission and scanning electron microscope (TEM SCAN Jeol EX II) equipped with an energy dispersive X-ray spectrometer (TRACOR TN 5502). For each sample, particles were analyzed at magnification ×20,000 for morphology and elementary chemical composition in randomly selected fields (24 μm²). The numerical concentrations per ml of BALF of all types of NFMP, greater than 0.1 μm, were first determined by scanning a sufficient number of fields to obtain at least 200 particles. In a second step, relative percentages of different mineralogical species were determined by analysis of 50 to 100 particles in randomly selected fields. Each particle was identified by its morphological features, electron diffraction pattern, and microanalysis spectrum. Nineteen mineralogical species were identified: crystalline silica, amorphous silica, kaolin, mica, talc, chlorite, other phyllosilicates, feldspars, other silicates, calcite, exogenous iron, endogenous iron, aluminium, titanium, metallic alloys, other metals, fly ash, others, and undetermined. Exogenous and endogenous iron particles were differentiated by their diffraction patterns and phosphorus content, as the former showed crystalline features and no phosphorus, while the latter was noncrystalline and contained phosphorus. Other metals indicated any of the following: antimony, bismuth, bromine, cadmium, cerium, chromium, cobalt, copper, gold, lead, magnesium, manganese, molybdenum, nickel, palladium, tin, tungsten, zinc, or zirconium. "Others" referred to particles containing magnesium and calcium (probably dolomite), or sulfur and barium (probably barite). "Undetermined" referred to particles without any diffraction pattern, which were probably carbonaceous particles, since the microanalysis system only detected chemical elements with atomic number greater than 10. In the following results, the word "metals" includes exogenous iron, aluminium, metallic alloys, and other metal particles. Because the purpose was to study exogenous contaminants, the relative percentages of mineralogical species have been calculated excluding endogenous iron.

Statistical Methods

One-way analysis of variance was used to compare age and tobacco consumption between exposed subjects and controls. The test also analyzed these factors according to the period elapsed since exposure among exposed subjects. The relationships between total particle concentration, age, and smoking were studied in previously exposed workers and controls using the Spearman rank correlation test. Because the distribution of particle concentrations was neither normal nor logarithmic and variances were different between OE and control groups, nonparametric statistics (Wilcoxon rank sum test) were used to compare total and relative particle concentrations between E1+E2+
Table 3. Relative particle concentrations in BALF (percentage of total particles).a,b

| Particle type | Controls [42] | E1 + E2 + E3 [246] | E1 [178] | E2 [36] | E3 [32] |
|--------------|---------------|---------------------|----------|---------|---------|
|               | Mean (SD) | Median | Mean (SD) | Median | Mean (SD) | Median | Mean (SD) | Median | Mean (SD) | Median |
| Crystalline silica | 17.2 (14.7) | 25.8 | 22.9 (0-50) | ** | 26.4 | 23.9 | 25 (0-64) | 22 | 23.3 | 22 |
| Amorphous silica | 4.5 (5.4) | 3.6 | 0 (30-62) | *** | 4 | 0 | 2.9 (0-64) | 0 | 2.6 | 0 NS |
| Kaolin | 12.8 (8.6) | 12 | 7.7 | 4 (0-30) | *** | 7.3 | 4 | 6.8 (0-24) | 4 | 11.1 | 5.3 NS |
| Mica | 11.3 (9.4) | 9.5 | 8.4 | 4 (0-26) | ** | 6.9 | 3.1 | 12.1 (0-26) | 8 | 12.6 | 6 ** |
| Feldspars | 7 (3.0) | 6 | 4 | 0 (0-10) | NS | 2.6 | 0 | 2.4 (0-16) | 4 | 4.9 | 0 NS |
| Other silicates | 5.7 (4.5) | 6 | 4.7 | 2.7 (0-12.5) | NS | 4.4 | 2 | 4.9 (0-14) | 3.5 | 6.5 | 4 NS |
| Phyllosilicates | 13 (19.3) | 5.7 | 2 | * | 5.3 | 2 | 6.7 | 1 | 6.9 | 3 |
| Exogenous iron | 5.1 (6.9) | 2.2 | 8.4 | 7 (0-22) | NS | 6.4 | 4 | 5.8 (0-36) | 4 | 4.6 | 2 NS |
| Aluminum | 2.2 (4.3) | 0 | 6.6 | 2 (0-10.7) | * | 7 | 2 | 6.2 | 1 | 5.1 | 2 NS |
| Titanium | 4.9 (6.8) | 4 | 3 | 0 (0-17.6) | ** | 2.8 | 0 | 4.3 | 2 | 2.5 | 3 |
| Metallic alloys | 5.1 (8.3) | 2.7 | 6.1 | 4 (0-16) | NS | 6.4 | 4 | 5.2 | 2 | 4 | 3.7 NS |
| Other metals | 1.3 (2.3) | 0 | 2.3 | 0 (0-6-7) | NS | 2.5 | 0 | 1 | 0 | 2.8 | 0 NS |
| Fly ash | 1.5 (2.0) | 0.5 | 1.2 | 0 (0-8-4) | ** | 1.3 | 0 | 0.8 | 0 | 0.6 | 0 NS |
| Others | 2.5 (7.1) | 0 | 3 | 0 (0-14) | NS | 3.5 | 0 | 1.4 | 0 | 1.8 | 0 NS |

NFMP, nonfibrous mineral particles. NS, not significant; SD, standard deviation. a Numbers in brackets indicate the number of subjects for each group of subjects. b Data on undetermined particles are not presented because of their very low frequency in most subjects. c Values in brackets are for 5 and 95% percentiles. d Level of significance when controls are compared with the E1 + E2 + E3 group (Wilcoxon rank sum test). e Level of significance when E1, E2, and E3 groups are compared simultaneously (Kruskal-Wallis test). f p < 0.01. g **p < 0.001.

Results

Study Population

Distribution of subjects between the groups E1, E2, and E3; and by age, sex, and smoking histories, are presented in Table 1. The sex ratio was very different between controls and exposed subjects, since almost all NFMP-exposed subjects were male. No statistical differences were found between E1 + E2 + E3 group versus control group according to smoking habits nor to cumulative smoking expressed as pack-years for each smoker. No significant difference was observed among E1, E2, and E3 for smoking. As expected, age increased significantly with lapse of time since exposure, E3 subjects being the oldest.

Total Particle Concentrations in BALF

The sex ratio was different in controls and exposed groups, but since no significant difference was observed in the total particle concentrations of male versus female controls, all controls were retained in the following analysis.

Total particle concentration of NFMP was statistically higher in exposed workers than in controls (Table 2). Median total particle concentrations exhibited significant decreases as a function of increasing time lapse since last exposure in NFMP-exposed subjects. No significant effect of smoking was observed on total particle concentrations in BALF among controls and exposed subjects (data not shown). A significant decrease of total particle concentrations with increasing age was observed in controls (r = −0.31, p < 0.05).

Elementary Chemical Particle Analysis

The relative and absolute particle concentrations for 17 main particle types are given in Tables 3 and 4. NFMP-exposed subjects exhibited significantly higher relative particle concentrations than controls for crystalline silica and aluminum and significantly decreased relative particle concentrations for amorphous silica, kaolin, mica, chlorite, calcite, titanium, and fly ash.
Table 4. Absolute particle concentrations in BALF (x 10^4/ml).10

| Particle type            | Controls [41] | E1 + E2 + E3 [205] | NFMP-exposed subjects |
|--------------------------|---------------|---------------------|------------------------|
|                          | Mean (SD)     | Median (SD)         | Mean (SD)              | Median (SD) |
| Crystalline silica       | 75.1 (30.7)   | 471.4 (172.2)       | 531.5 (196.5)          | 365.1 (107.8) | 231.1 (106.6) | NS          |
| Amorphous silica         | 11.7 (3.2)    | 68.5 (NS)           | 80.3 (NS)              | 33.4 (NS)    | 36.2 (NS)     | NS          |
| Kaolin                   | 46.3 (19.2)   | 177.4 (24.4)        | 197.5 (25.6)           | 73.5 (21.6)  | 75.6 (18)     | NS          |
| Mica                     | 57 (15.2)     | 177.2 (24)          | 164.6 (14.9)           | 218.1 (31.2) | 206 (70.1)    | NS          |
| Talc                     | 6 (0.2)       | 42.2 (0)            | 39.3 (0)               | 13.5 (0)     | 94.7 (0)      | NS          |
| Chlorite                 | 3.2 (0)       | 25.4 (0)            | 31.9 (0)               | 9.2 (0)      | 4 (0)         | NS          |
| Phyllosilicates          | 1.9 (0)       | 12 (0)              | 9.5 (0)                | 27.1 (0)     | 10 (0)        | NS          |
| Feldspars                | 7.2 (2.8)     | 41 (4)              | 37.1 (5)               | 63.7 (22.3)  | 87.8 (42.4)   | NS          |
| Other silicates          | 12.6 (4.4)    | 70.8 (14)           | 79.3 (14)              | 48.7 (7.4)   | 43.9 (12.8)   | NS          |
| Calcite                  | 18.3 (9.5)    | 34.2 (14)           | 31.3 (13.5)            | 42.6 (0)     | 42.3 (16.4)   | NS          |
| Exogenous iron           | 11.9 (0)      | 324.3 (20)          | 343.3 (23)             | 467.9 (7.6)  | 35.3 (18.8)   | NS          |
| Aluminum                 | 5.6 (0)       | 87.2 (0)            | 102.4 (6.6)            | 56.1 (2.6)   | 28.4 (9.4)    | NS          |
| Titanium                 | 13.7 (7.1)    | 55.2 (0)            | 65.9 (0)               | 25.3 (2.4)   | 22 (12)       | NS          |
| Metallic alloys          | 16.7 (4.5)    | 384.9 (14)          | 444.5 (17.6)           | 77.6 (0)     | 51.8 (41.2)   | NS          |
| Other metals             | 3.3 (0)       | 52 (0)              | 63 (0)                 | 13.2 (0)     | 29.2 (0)      | NS          |
| Fly ash                  | 7.3 (1.5)     | 24.6 (0)            | 31.1 (0)               | 5.5 (0)      | 6.4 (0)       | NS          |
| Others                   | 5.6 (0)       | 118.7 (0)           | 154.2 (0)              | 16.3 (0)     | 17.7 (0)      | NS          |

Comparison of the three exposed groups revealed significant differences only for mica and titanium.

Analysis of absolute particle concentrations revealed significant increases in crystalline silica, feldspar, exogenous iron, aluminum, metallic alloys, and fly ash in NFMP-exposed subjects compared to controls. In contrast, only absolute particle concentrations for mica were significantly different among E1, E2, and E3 groups.

Biopersistence of NFMP in BALF

The E3 subjects are the most pertinent group for the study of biopersistence because they represent the group with the longest duration since last occupational exposure to NFMP. Compared with controls, the BALF for E3 subjects had significantly higher concentrations of crystalline silica, aluminum and metallic alloys (Table 5).

The distribution of concentrations of crystalline silica and metals (exogenous iron + aluminum + metallic alloys + other metals) as a function of duration since cessation of exposure to NFMP is given in Figure 1. Both mineral phases exhibited a decrease in concentration with increasing duration, with median values for the E3 group remaining significantly higher than those for controls.

Discussion

The total particle concentration was higher in OE subjects than in controls, primarily due to the high proportion of recently exposed workers (E1) in the OE subjects. Analysis of relative and absolute NFMP concentrations in BALF revealed significant differences between OE subjects and controls. The increase of a given mineral as a possible result of occupational exposure automatically reduces the relative percentages of other minerals without any significance in terms of biological response. Therefore, only increased mineralogical species in OE workers compared to controls were considered pertinent in terms of pulmonary dust load, especially when the increased absolute concentrations of the minerals.

Crystalline silica, aluminum and metals (i.e., exogenous iron + aluminum, m + metallic alloys + other metals) exhibited increased retention in the BALF of OE subjects in terms of relative percentage as well as absolute concentrations. Some individual metals (exogenous iron and metallic alloys) exhibited increased retention in the BALF of OE subjects only in terms of absolute concentration. Although relative particle concentrations of amorphous silica, kaolin, mica, chlorite, calcite, and titanium were
Table 5. Biopersistence of NFMP in the human lung: comparison of E3 and control groups.

| Particle type         | Relative particle concentrations (E3: n=32; controls: n=42) | Absolute particle concentrations (E3: n=24; controls: n=41) |
|-----------------------|------------------------------------------------------------|-----------------------------------------------------------|
| All particles         | NS                                                        | NS                                                       |
| Crystalline silica    | NS                                                        | *↑                                                       |
| Amorphous silica      | *↑                                                        | NS                                                       |
| Kaolin                | NS                                                        | NS                                                       |
| Mica                  | NS                                                        | **↑                                                      |
| Talc                  | NS                                                        | NS                                                       |
| Chlorite              | NS                                                        | NS                                                       |
| Other phyllosilicates | NS                                                        | NS                                                       |
| Feldspars             | NS                                                        | NS                                                       |
| Other silicates       | NS                                                        | *↑                                                       |
| Calcite               | NS                                                        | NS                                                       |
| Exogenous iron        | NS                                                        | NS                                                       |
| Aluminum              | NS*                                                       | NS                                                       |
| Titanium              | *↑                                                        | NS                                                       |
| Metallic alloys       | NS                                                        | *↑                                                       |
| Other metals          | NS                                                        | NS                                                       |
| Fly ash               | **↓                                                       | NS                                                       |
| Others                | NS                                                        | NS                                                       |

*Level of significance of the comparison of E3 and control subjects is as follows: "p < 0.05; "p < 0.01; "p < 0.001; NS, not significant. Arrow indicates way of variation of the result of E3 when compared to controls: ↑: increase; ↓: decrease. *p = 0.05. *p = 0.08

Figure 1. Evolution of absolute particle concentrations in BALF according to time since last job exposing to NFMP. Results are expressed as median of each group. "Metals" refers to exogenous iron + aluminum + metallic alloys + other metals. "p < 0.05. "p < 0.001 when compared with control subjects. NFMP, nonfibrous mineral particles.

Significantly increased, no difference was observed between OE and control groups in terms of absolute concentration. This indicated that the lung dust burden was not modified for these minerals in OE subjects. Absolute concentrations offeldspars and fly ash should be interpreted with caution since mean and median concentrations varied inversely. That inverse variation may be due to the dispersion of the individual values of the dust content in BALF, so that the mean value might have been strongly influenced by outliers, which did not influence the median concentrations.

Comparison of the mineralogical species in the BALF of E3 and control subjects suggests that crystalline silica and metals remained significantly elevated in the BALF of subjects more than 10 years after cessation of exposure when compared to controls. The absolute median concentration of mica was significantly higher in E3 subjects than in controls, and showed an increasing tendency when comparing E1, E2, and E3 groups (p < 0.05) although there was no statistical difference between E1+E2+E3 group and controls. No such trend was observed for "other silicates", although their absolute median concentration was also increased in E3 when compared to controls. These results suggest that mica and "other silicates" should not be considered as biopersistent minerals.

In previous studies of the mineralogical species encountered in the BALF of NFMP-exposed subjects, the populations evaluated and the methods used were different, making difficult any comparison with the present study. In only very few was TEM coupled with an energy dispersive X-ray analysis system used to study individual particles, as in the present study (12-15,18,20). This method provides better spatial resolution power than SEM. One author, also using TEM, expressed his results in terms of positive mineralogical species in alveolar macrophages (17). Others have determined trace elements in alveolar cells using energy dispersive X-ray fluorescence (21,23,25) and expressed results in ponderal concentration per 10^5 cells. Some authors used SEM fitted with X-ray spectrometry and polarized light microscopy to determine the mineralogical load of alveolar macrophages (16,19). All these factors make comparison difficult.

Another factor that may influence the mineralogical results and hence the possibility of comparing data, arises because our BALF samples were provided by many hospitals; it was not possible to ensure that the methodology used for sampling was identical.

Preparation of BALF samples may also greatly influence mineralogical analysis, especially when fragmentation of conglomerates occurs in highly concentrated samples. Review of methodological pitfalls linked to specimen preparation of BALF or lung tissue has been published for SEM and polarized light microscopy methods (26), and for comparison of SEM and TEM methods in lung tissue (27). The present technique avoids any centrifugation or sonication of the BALF samples, and the use of sodium hypochlorite as a tissue digestant is effective and does not seem to react with natural mineral dusts (28).

The size of particles analyzed may create artificial differences since with TEM it is possible to count all particles >0.1 μm (13-15,18,20), while with polarized light microscopy, only particles >1 μm can be counted.

The site of sampling could also be a source of discrepancy, since topographic
variations in the distribution of asbestos fibers in the lung have been described (29,30). No such variations were described for NFMP in lung tissue (29,31), except in a study of bituminous coal miners, lungs where higher concentrations of particles in the upper left lobe were reported. However, the right lung was not studied and the degree of significance was not indicated (32). No study has assessed such topographic variations in the recovery of BALF particles.

Population characteristics also are important. One study reported significantly higher mean particle concentrations in lung tissue of males than in females (33), but to our knowledge no study has detected possible sex differences in BALF dust load. No significant difference was found between male and female controls in the present study, and the number of OE females was too low to allow any specific analysis.

No effect of smoking on total particle concentrations in BALF, of either controls or OE subjects, was observed in our study. Few studies have focused on the influence of smoking on the mineralogical content of BALF. While no significant effect of smoking was observed on the mineral content of alveolar macrophages using polarized light microscopy (19), kaolin and mica species have been significantly linked to smoking status (17), and tobacco has been shown to contain various mineral particles such as crystalline silica, feldspars, iron and titanium oxide particles, and phyllosilicates (kaolinite and others) (34). Some authors have reported significant effects of smoking on total NFMP retention and relative mineral species observed in human lung (31,35) while others did not (33,36). In one study the effect of smoking was shown to be limited to upper lobes, a region generally not affected by BALF sampling (31).

Calcium-containing particles have been proposed as smoke tracers (37). A slight but significant decrease of BALF concentration with age was noted in the control group, although one might expect a progressive accumulation of mineral dust with increasing age. This accorded with another study (36), and reinforces the finding of an increase both of total particle concentration and in the concentration of some mineralogical species in the BALF of E3 workers, since they were older than the controls. By contrast, other authors have reported an increase in average exogenous mineral particle, or trace elements concentrations with increasing age in lung tissue (33,38).

The relative percentage of different mineralogical species in OE subjects seemed globally to accord with results of another similar study of 51 subjects with various occupational exposure (18). The total particle concentration was of the same order of magnitude; but since the results were expressed differently, further comparison of mineralogical species was not possible. The results for controls and absolute particle concentrations in BALF were closely related to those of a study of 10 autopsy cases without known recent exposure to NFMP (20). Total particle concentrations of exposed subjects were in the range of those observed in another study in which BALF was performed on 77 workers with various occupations (13).

Biopersistence of specific mineralogical species in the respiratory tract of exposed subjects after termination of employment has been reported many years after cessation of work (15,18,22,23); an excess was found in the percentage of dusty positive alveolar macrophages in BALF of 12 retired granite workers when compared to active workers or controls (19). The originality of the present work was to use BALF to study all types of NFMP for biopersistence in the lung. A previous study did not find any significant differences in any of the particles analyzed, except for fly ash (excess in BALF, p < 0.05), nor was there any significant correlation between the absolute number of particles in BALF and lavaged lung tissue (20), as was previously observed for asbestos bodies (7). However, this study was performed on a small series of subjects without previously known occupational exposure to NFMP.

It is usually accepted that mineralogical analysis of BALF provides a useful proof of exposure when a significant increase of a mineral dust is observed, but a causal relationship with any given pathology generally cannot be proved from such analysis. Moreover, this method is not appropriate for soluble compounds (especially cobalt).

Since BALF sampling and mineralogical analysis procedures can influence the results obtained, methods should be standardized to allow formulation of guidelines concerning the significance of particle concentrations in BALF with respect to occupational exposure (13,18). Such standardization will permit interpretation of results even in cases where great individual variation was observed between subjects with closely related exposure to ubiquitous mineral species. Efforts still need to be made to study the relationship between the NFMP mineralogical profile in BALF and lung dust burden, both in unexposed and previously occupationally exposed workers.

Despite the qualifications mentioned here, we consider BALF mineralogical analysis a useful tool for physicians in assessing the etiology of respiratory diseases, even when exposure has ceased long before BALF was performed.

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