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THE RISK ASSESMENT IN OCCUPATIONAL EXPOSURE TO ASBESTOS DUSTS THROUGH SPUTUM CYTOLOGIC EXAMINATION

Doina Havârneanu, Irina Alexandrescu, Doina Popa

Department of Occupational Medicine, Institute of Public Health Iaşi, România

Abstract: Introduction. The permanent existence of large asbestos quantities in many workplaces and the use of asbestos as raw material in different industries expand the number of workers who are occupationally exposed to its noxious effects. Diseases associated with occupational exposure to asbestos are: mesothelioma, lung cancer, asbestosis, and pleural fibrosis/plaques. **Aim.** The present paper illustrates the possibility of revealing occupational exposures to asbestos dusts through sputum cytological examination. This emphasizes the presence of asbestos fibers and bodies in the sputum samples, and also enhances the effectiveness of sputum cytological examination in early diagnosis of possible pretumoral changes in specific groups of employees. **Material and method.** The cytological examination was performed on fresh morning sputum samples (3 smears for each patient) from a group of 39 workers occupationally exposed to asbestos dusts (mean age 38.31±7.12 years, mean work length in asbestos industry 13.10±7.03 years) and a control group of 72 subjects, matched by mean age, mean work length and smoking habit, respectively. **Results.** Asbestos bodies were found in the sputum samples of 29 subjects from the exposed group and, in some of these cases, a high density of free asbestos fibers was also found. According to the Papanicolau classification, the cytological types diagnosed in the samples from the exposed subjects were as follows: 19 cases type I, 16 cases type II, and 4 cases type III. There was a raising tendency (obvious, but statistically not-significant) for the type II inflammatory alterations in those patients from the exposed group with more than 10 years of work length. Related to the smoking habit, an increasing rate (statistically not-significant) of the type II and type III cytology cases appears in the group of the heavy smokers. **Conclusions.** The presence of asbestos bodies in sputum represents an important indicator for occupational exposure to respirable particles. Sputum cytology screening is a practical, noninvasive and inexpensive approach for the diagnosis and assessment of the occupational exposure to asbestos.
Key words: asbestos, occupational exposure, sputum, risk assessment, screening

Rezumat: Introducere. Prezența permanentă în mediul ocupațional a unor mari cantități de asbest precum şi utilizarea acestuia ca materie primă în diferite sectoare industriale extind numărul angajaților expuşi profesional la acțiunea sa nocivă. Bolile asociate expunerii profesionale la asbest sunt constituite în principal de: mezoteliom, cancer pulmonar, azbestoză şi placă/fibroză pleurală. **Scop.** depistarea expunerilor la pulberi de asbest prin examenul citologic al sputei. Acesta evidențiază prezența fibrelor şi corpilor azbestozici în probele de spută şi susține eficiența examenului citologic al sputei în diagnosticarea precoce a unor posibile modificări de tip pretumoral la anumite categorii profesionale. **Material şi metodă.** S-a efectuat examenul citologic al sputei matinale (3 frotiuri pentru fiecare pacient) la un lot

de 39 angajaţi expuşi ocupaţional la pulberi de azbest (vârsta medie 38,31±7,12 ani, vechimea medie în industria de prelucrare a azbestului 13,10±7,03 ani) şi la un lot martor neexpus de 72 subiecţi, echivalent ca vârstă, vechime în muncă şi obicei al fumatului. **Rezultate.** În probele de spută provenite de la 29 subiecţi din lotul expus s-au evidenţiat corpi azbestozici şi, în unele cazuri, şi o mare densitate de fibre de asbest. Din punct de vedere al clasificării Papanicolau, tipurile citologice în care s-au încadrat subiecţii expuşi au fost: 19 cazuri în tip I, 16 cazuri în tip II, 4 cazuri în tip III. S-a remarcat o tendinţă de creştere (evidentă dar nesemnificativă statistic) a frecvenţei modificărilor de tip inflamator specifice tipului citologic II în grupul subiecţilor expuşi cu vechime peste 10 ani. În ceea ce priveşte obiceiul fumatului, caracteristică dar nesemnificativă statistic este creşterea frecvenţei tipurilor citologice II şi III în rândul marilor fumători. **Concluzii.** Prezenţa corpilor azbestozici în spută reprezintă un indicator important pentru expunerea ocupaţional la pulberi respirabile. Examenul citologic al sputei reprezintă o metodă de screening practică, neinvazivă şi ieftină pentru diagnosticarea şi evaluarea expunerii ocupaţionale la azbest.

Cuvinte cheie: asbest, expunere ocupaţională, spută, evaluarea riscului, screening

INTRODUCTION

The permanent existence of large asbestos quantities and the use of asbestos in different industries expand the number of workers who are occupationally exposed to its noxious effects.

"Asbestos" is the general name for a group of mineral hydrous silicates. There are six types of asbestos among which chrysotile (mostly of volcanic origin) represents 95% of the mined and worldwide used asbestos type:

- Chrysotile (white asbestos);
- Crocidolite (blue asbestos);
- Asbestos gruenerite, (amosite, brown asbestos);
- Asbestos actinolite;
- Asbestos anthophyllite;
- Asbestos tremolite.

The first three have been the main commercially used varieties of asbestos. Although they are known by their colour, they cannot be reliably identified solely by colour; analysis in a laboratory is necessary.

At the beginning of the 20th century, Ludwig Hatschek invented a process for combining asbestos fibres with cement to produce asbestos-cement, a material which had excellent technical properties and could be used for a wide range of applications. As asbestos would "last forever," Hatschek named the process Eternit, for eternal, and proceeded to sell the patent to companies all over the world, many of which took the name Eternit (1).

Asbestos may also be incorporated into a big range of products . If the fibres can be released from these products, then danger arises from inhalation of airborne fibres. The microscopic fibres can deposit in the lungs and remain there for many years, and may cause disease many years, usually several decades, later. Several member states have procedures that assign priority to removing the asbestos-containing materials that are considered more dangerous.

All varieties of asbestos are Class 1 carcinogens, that is they are known to cause cancers in humans. The European Asbestos Worker Protection Directive 83/477/EEC as last modified

by Directive 2003/18/EC requires that worker's exposure be kept below 0.1 fibres/ml *for all types of asbestos*. Exposure to all types of asbestos must be reduced to a minimum and in any case below the limit value (2).

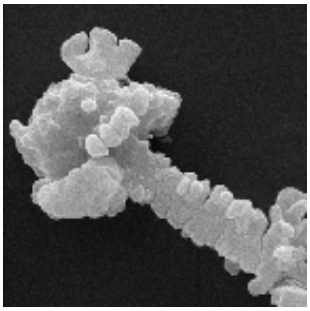
Current state of knowledge

Sputum has widely been used, though it has proved to be a much less reliable data source than others, largely because of the inconsistent quality of individual specimens. The high variability in specimen quality can be circumvented with invasive procedures, such as transtracheal aspirates or suctioning material through a bronchoscope or endotracheal tube, but these techniques are not likely to be chosen as screening techniques for the broader population.

Previous studies have evaluated sputum for tracking exposure to asbestos, heavy metals, and other inhalational agents (3 -7).

Inhaled particles and fibers deposited in the lungs are usually phagocytized by alveolar macrophages. Ferruginous bodies result from the deposition of an iron-rich protein layer at the cell-particle interface of biopersistent fibers or particles that are too large to be completely phagocytized.

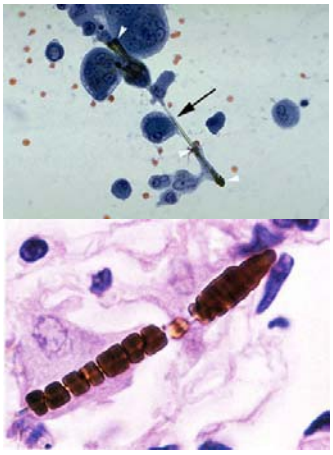
Ferruginous bodies mostly form on particles larger or fibers longer than 10 μm (8, 9). They may occur on a wide variety of materials, including asbestos fibers, sheet silicates, diatomaceous earth, coal particles, metal compounds, and silicon carbide (10). The mechanisms leading to ferruginous bodies formation are not fully understood. Experimental evidence suggests that they could be formed by an exocytotic activity of macrophages or giant cells (11) - see figure 1.



Minerals that interact with lung fluids eventually develop a ferruginous coating that is believed to derive from ferritin. The "asbestos body" shown in the figure is commonly found in the lungs or sputum of individuals exposed to asbestos (including those exposed only to environmental sources). As can be seen from the figure, these coatings are typically thicker at the ends of the fibers (16)

Fig. 1. Asbestos body in sputum, high-resolution transmission electron microscopy

Coated asbestos fibers are referred to as asbestos bodies (ABs). In light microscopy the central core of a "typical" AB is a thin, straight, transparent, and colorless fiber – see figure 2.



An asbestos body is composed of a faintly visible central translucent core (black arrow) that is the long, thin asbestos fiber itself and an outer surface coating of iron and protein that gives the structure a "beaded" character or clubbed appearance (white arrow heads). Because of the characteristic iron/protein coat, they are sometimes referred to with the more general term, ferruginous bodies. (Other inhaled foreign materials in the lung such as talc and mica may become ferruginated and appear in lung specimens as ferruginous bodies, but these bodies have a different microscopic appearance because they lack the characteristic long slender central lucent core of the asbestos body (17)

Fig. 2. Asbestos bodies in sputum, light microscopy
(author image collection)

The fiber is covered by a regularly segmented or continuous golden yellow to red brown coating. Some branched or curved forms can be observed (8, 10, 12). The validity of this definition is supported by numerous electron microscopy analyses which have demonstrated that 95 to 98% of the core fibers of structures corresponding to this definition are indeed asbestos fibers (8, 13). Most ABs are built on amphibole asbestos fibers and the AB burden correlates with the amphibole content of the lung (14). ABs on chrysotile have been observed in subjects recently exposed to this type of fiber despite its shorter biopersistence (12). Concentrations above 1 AB/ml in bronchoalveolar lavage fluid (BALF) or above 1,000 AB/g dry lung tissue indicate nontrivial asbestos exposure, and the concentrations of ABs in BALF and lung tissue are correlated (15).

RESULTS

We found asbestos bodies in the sputum samples of 29 subjects from the exposed group and, in some cases, a high density of free asbestos fibers (see figures 3–12, author image collection).

Table 1. The presence of asbestos bodies in the sputum samples of the exposed group versus the control group

Group	Asbestos bodies present	Asbestos bodies absent	Total
Exposed	29 (74.35%)	10 (25.64%)	39 (100%)
Control	6 (8.3%)	66 (91.66%)	72 (100%)

$\chi^2_{(1)} = 51.08$, $p < 0.0001$

The presence of asbestos bodies in sputum of the exposed subjects doesn't significantly correlate neither with the work length nor the smoking habit.

Figure 3. MGG staining, x200

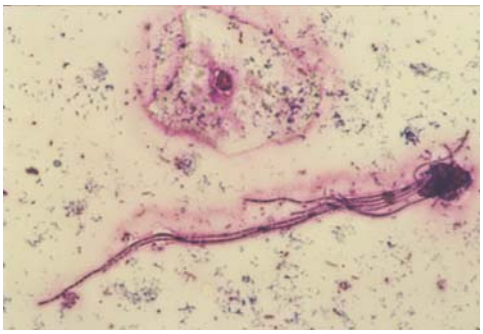


Figure 4. MGG staining, x400

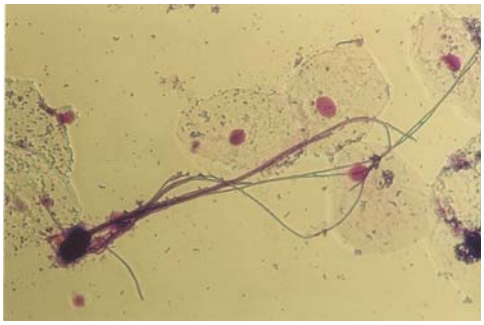


Figure 5. MGG staining, x200

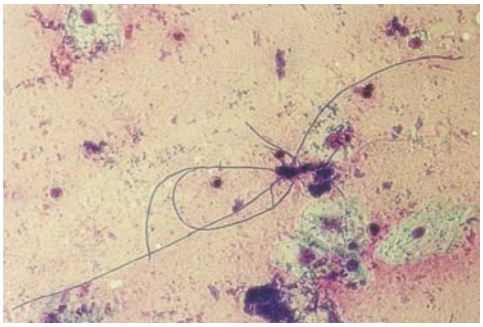


Figure 6. MGG staining, x200

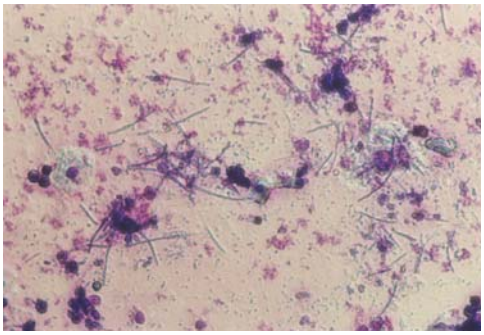


Figure 7. MGG staining, x200

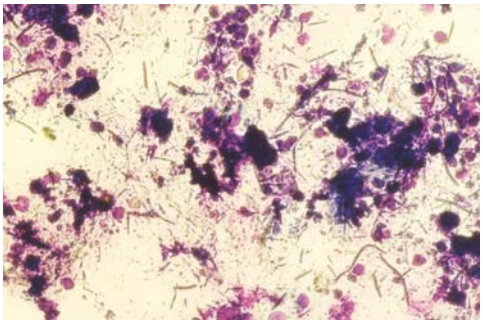


Figure 8. MGG staining, x200

Figures 3 – 8. Asbestos fibers in sputum (from the collection of the Department of Occupational Medicine, Institute of Public Health Iasi, Romania)

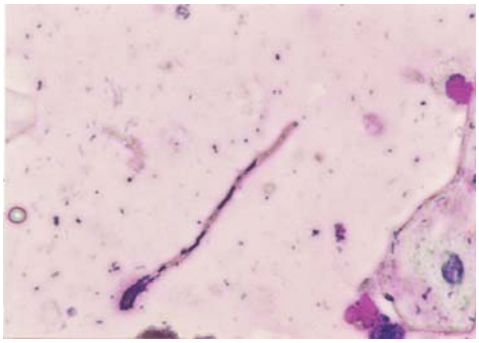


Figure 9. MGG staining, x400

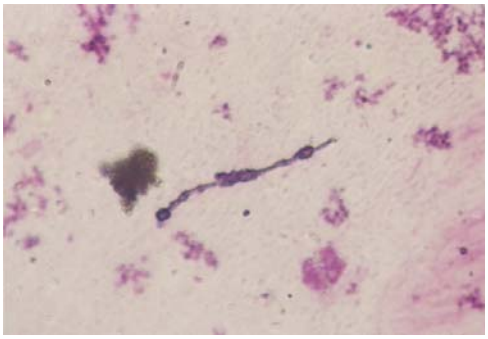


Figure 10. MGG staining, x400

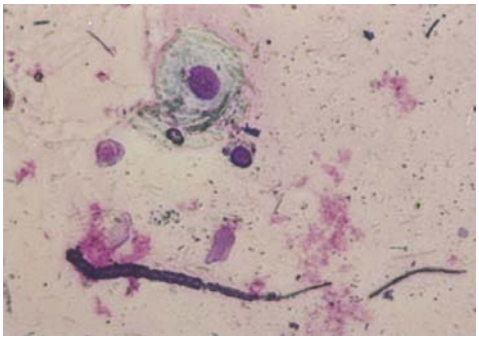


Figure 11. MGG staining, x400

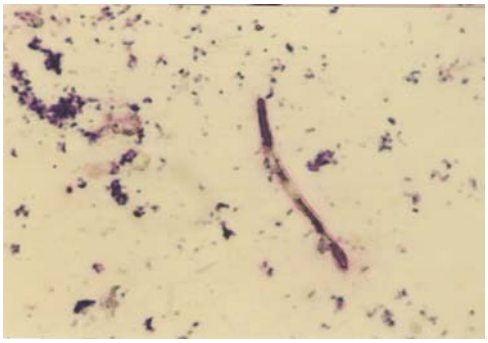


Figure 12. MGG staining, x400

Figures 9 – 12. Asbestos bodies in sputum (from the collection of the Department of Occupational Medicine, Institute of Public Health Iasi, Romania)

According to Papanicolau classification of the cytological aspects for the exposed subjects, we found the following cytological types:

- 19 cases (48.71%) - *Type I cytology* (no sign of cellular atypia)
- 16 cases (41.02%) - **p < 0.02,** if compared with the control group - *Type II cytology* (benign atypia: epithelial cells with inflammatory changes, polymorphonuclear leukocytes present, lymphocytes, histiocytes etc);
- 4 cases (10.27%) - **p < 0.027,** if compared with the control group - *Type III cytology* (suggestive but inconclusive for malignancy – "suspicious atypia").

We found a raising tendency (obvious, but statistically not-significant) for the type II inflammatory alterations in the group with more than 10 years of work length (42.85% of the subjects from this group with type II Papanicolau cytology).

Related to the smoking habit, we found an increasing rate (but statistically not-significant) for the type II cytology cases (46.15%) and

type III cytology cases (23.7%) in the group of the heavy smokers (subjects with the value of the Brinkman index– daily cigarette numbers multiplied by smoking years - more than 200).

CONCLUSIONS

The presence in sputum of asbestos bodies represents an important indicator for occupational exposure to respirable particles

The 4 cases of cytological Papanicolau class III plead for the existence of certain pathological processes which aren't conclusive for malignancy, but must be surveyed in dynamics

Sputum cytology screening represents a practical, noninvasive and inexpensive approach for the diagnosis and assessment of the occupational exposure to asbestos. That is why cytological sputum diagnosis must be used much more frequently for surveying the high-risk groups. Only this way the stages that are prognostically more favorable of preneoplasm and carcinoma in-situ, can be detected and possibly treated curatively.

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