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DIAGNOSIS OF ASBESTOS-RELATED DISEASES: THE MINERALOGIST AND PATHOLOGIST'S ROLE IN MEDICO-LEGAL FIELD

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ABSTRACT

Because asbestos diseases represent a complex pattern of legal, social and political issue, the involvement of the mineralogist and pathologist for a multidisciplinary assess of its diagnosis helps to investigate the relationship between mesothelioma or lung cancer and occupational or environmental asbestos exposure.

In the present study we consider the concentrations of asbestos bodies (ABs) detected by Optical Microscopy (OM) and Scanning Electron Microscopy (SEM), and the burden of different kinds of mineral fibres (among which asbestos) identified by SEM combined with an Energy Dispersive Spectrometer (EDS), in 10 lung tissue samples of subjects with occupational and non-occupational exposure to asbestos.

In all subjects with occupational exposure to asbestos more than 1,000 ABs per gdw (gram of dry weight) were detected both with OM and SEM: this concentration is internationally accepted as suggesting high probability of past occupational exposure to asbestos.

In nine lung samples of the ten investigated by EDS-SEM, different inorganic fibres were found. Asbestos fibres have been identified too, and more than 100,000 ff per gdw were detected in subjects with occupational exposure: this concentration is internationally accepted as suggesting high probability of past occupational exposure to asbestos.

Instead, when the ABs burden is low or moderate (such as in subjects with absent or probable asbestos exposure), the correlation between ABs concentration determined by MO and those determined by SEM is lost. Therefore, when the ABs value in OM is borderline the SEM investigation became essential. Furthermore the mineralogical analysis by SEM-EDS (identification and quantification of inorganic fibres in general, and asbestos in particular) of the fibres detected in the lung tissues is very useful, if not necessary, to complete the pathological diagnosis of asbestos-related malignancies in medico-legal field.

Key words: asbestos fibres, SEM-EDS, lung cancer, mesothelioma.

Introduction

Asbestos has been known and used since prehistoric times. The Ancient Greeks named it "unquenchable, inextinguishable". The Roman naturalist Pliny the Elder wrote about its harmful biological effects: they observed the "sickness of the lungs" in the slaves who worked with asbestos. Asbestos use did not become popular until the Industrial Revolution during the late 1800s. It then began to be used as insulation for steam pipes, turbines, boilers, and other high-temperature products. World War II provided a tremendous boost in the demand for asbestos and multiplied its uses. Starting from 1970 the 70% of world has output goes into asbestos-cement. Ancient observations of the health risks of asbestos were either forgotten or ignored. Only from 1920, asbestos-related disease was associated with some occupations, and it was during this time that reports of asbestosis began to appear in the literature. In 1924, was made the first diagnosis of asbestosis in the U.K. when Cooke [1] reported the case of a woman asbestos worker and in his paper mentioned the presence of mineral particles of various shapes in the lungs. Subsequently the cause of death was called "asbestosis" (Cooke, 1927) [2]. Nevertheless, not until 1929 did he describe the "curious bodies" later called "asbestos bodies" (ABs) (Cooke, 1929) [3]. The pathologist Gloyne (1933) [4] was the first to describe the pleural malignancy in a subject occupationally exposed to asbestos. Reports by Wedler (1943) [5] and Mallory (1947) [6] describing further cases of pleural malignancy associated with asbestos exposure followed subsequently. By the 1950s, malignant mesothelioma became accepted as a distinct clinicopathologic entity. Any remaining doubt concerning the association between pleural mesothelioma and asbestos occupational exposure was removed with the study described by Wagner (1960) [7] on cases of mesothelioma occurring in South Africa in subjects with a documented exposure to crocidolite.

Although asbestos is banned in Italy (L.n°257/1992) [8], as in many other countries, its health consequences are still expected for at least two decades.

Mineral fibers and ABs in lung tissue can be also present in the general population and not only in subjects with specific occupational exposure; actually the dose-response relationship has no established threshold.

Because asbestos diseases due to occupational or environmental asbestos exposure represent a complex pattern of legal, social and political issue, a multidisciplinary assess of its diagnosis is a valid approach in legal discussions.

When an asbestos disease is identified, it is important both to research the type of asbestos exposure (occupational or paraoccupational, anthropogenic or natural environmental) and to

found all informations about job history and other possible asbestos exposure. The presence of some morphological asbestos markers of exposure (i.e. mesothelioma, pleural plaques, asbestosis, and asbestos bodies) helps in the study of a causal relationship between their presence and health impairment or death. The clinical and radiological data are useful in demonstrating that an asbestos disease is present, but there is nothing more persuasive than visual evidence that asbestos is in the lung tissue in elevated concentrations. So, one of the best way to demonstrate that a subject has developed an asbestos-related disease is to obtain a lung tissue specimen to analyze, and highlight the presence of ABs or asbestos fibers.

ABs are the histological hallmark of exposure to asbestos (DeVuyst et al., 1998) [9]. They develop when asbestos fibers are inhaled and deposited in the lung parenchyma and have a characteristic microscopic appearance that is readily recognized by the pathologist. But also a large number of other different types of fibrous dusts (e.g. sheet silicates, carbon fibers, fibrous aluminium silicate, cosmetic talc, fibrous glass, etc.) can be covered (Churg and Warnock 1981) [10], therefore the term “ferruginous bodies” (FBs) is generally used when the nature of the fibrous core was not known (fibrous dusts other than asbestos can become coated with iron such as sheet silicates, carbon fibers, metal oxides, man-made mineral fibers, diatomaceous, zeolite) (Churg, 1983) [11].

ABs identification in histological sections are i) very low expensive methods; ii) are possible to do in any laboratory with a gold standard for the identification of asbestos exposure; iii) are important component of the pathologic diagnosis of asbestosis and their presence serves to alert the pathologist that the patient has been presumably exposed to airborne asbestos fibers (Roggli et al 2010) [12].

Three main methods to assess the presence of ABs and asbestos fibers in lung tissue for their subsequent quantification are used. These employ chemical digestion of the tissue with the use of either potassium hydroxide or sodium hypochlorite, and plasma ashing techniques. The potassium hydroxide digestion is the better method to fixed tissue, while sodium hypochlorite produces the best results with fresh lung specimens (Davis, 1984) [13]. Instead the ashing method destroying the organic component of the lung tissue but it has been suggested that can breakup long fibers so their amount tend to be higher than those where chemical digestion has been used.

The digested samples observed under optical microscopy (OM) is a simple and inexpensive technique, and is more readily available than electron microscopy (EM). ABs amount is usually at last an order of magnitude lower than the corresponding counts made by EM

(Pooley and Clark, 1980) [14]. Furthermore, OM technique cannot distinguish throw ABs from pseudo ABs and asbestos fibers from other inorganic fibers (because does not permit to identify the nature of the fiber in the core), and cannot resolve fibers less than 0.20 μm in diameter; therefore, several fibers are too fine to be detected by this technique. Scanning and Transmission Electron Microscopy (SEM and TEM) have a greater resolution, magnification and can be combined with an Energy Dispersive Spectrometer (EDS) therefore allow measurement of fibers size, and the chemical composition of the fibers burden can be determined too.

The distinction between asbestos fibers and many other fibers present in human lung tissue is essential in establishing a diagnosis in order to correlate the tissue asbestos burden with various asbestos-related diseases. Their identification and quantification is pivotal to complete the pathological diagnoses.

TEM-EDS is the more suitable procedure to identify fibers type and value their size but this method is very time-consuming and requires skilled operators. In addition the amount of material studied for each field of observation is very small and the particle quantification is almost impossible. Instead SEM-EDS is the best method for routine analyses in term of cost/benefit ratio. Because analytical methods to identify and quantify asbestos fibers are regulated by laws only for airborne and bulk samples (PCM, XRPD, FTIR, SEM-EDS), to detect inorganic fibers in samples of biological tissue by SEM-EDS we used the protocol proposed by Belluso et al. (2006) [15].

A multidisciplinary approach helps to investigate the relationship between the occurrence of mesothelioma or lung cancer and occupational or environmental asbestos exposure at the individual level, to resolving a critical medico-legal question.

The present study aims to evaluate the efficiency of a pathological and mineralogical approach in the identification of asbestos exposure in some medico-legal cases of mesothelioma and lung cancer.

Material and methods

Through a retrospective studies we selected 10 cases of pleura-pulmonary neoplasia (mesothelioma and lung cancer) of which have been request anatomo-pathological consulting to an expert pathologist in asbestos associated diseases (dott.ssa Bellis) and there were an adequate quantity of material for mineralogical analyses and complete clinical date.

The histological diagnosis of mesothelioma and lung cancer have been confirmed by immunohistochemistry, following international guidelines (Pinto et al., 2013) [16].

An accurate anamnesis to define a possible asbestos exposure (occupational or environmental) has been assessed for each subject according to the questionnaire by the National Mesothelioma Registry (ReNaM, quarto rapporto, 2012) [17]. The study of the patient's history includes all the information about the occupational activity, family history, and life style (i.e. smoke).

The successive step has been to prepare the lung tissue samples for OM and SEM-EDS investigation to quantify the presence of ABs and, by SEM-EDS to quantify and identify the asbestos fiber in according to Belluso et al. (2006) [15]. Two portions of lung tissue from each subject (respectively of 0.5 g and 0.25 g) were digested in NaClO in order to eliminate the organic matrix and to produce a suspension of inorganic material. Both portions were filtered through a mixed cellulose esters membranes with a diameter of 25 mm and respectively pore size of 3 μm for LM examination and pore size of 0.45 μm for SEM-EDS observation. A portion of 2,5 g of lung tissue for each subject was dehydrated, to obtain the dry weight useful to determine the concentration of the Abs and fibers per gram of dry weight (gdw).

ABs counting by OM was carried out observing the whole membrane at 400 magnification. Identification and quantification of inorganic fibers were carried out by SEM (Cambridge Stereoscan S-360) with EDS (Oxford INCA Energy 200, EDS-SDD) at 2000 magnification observing only a portion of filter (corresponding about 2 mm^2). All the inorganic particles corresponding to fiber definition (greater than 5 μm in length and less than 3 μm in diameter, with an aspect ratio length/diameter \geq of 3:1), were considered (Directive 2003/18/EC; WHO, 1986) [18,19]. A chemical analysis was conducted after the observations were completed. The ABs and asbestos fibers amounts was normalized to 1 gdw, according to the international standard (De Vuyst et al., 1998) [20].

Results

The lung samples of the 10 cases selected were investigated: seven subjects with mesothelioma and three subjects with lung cancer, with different kinds of asbestos exposure. The results about ABs and asbestos fibers detected by both OM and SEM-EDS observation are reported respectively in table 1 and table 2.

About ABs

ABs were detected by OM and SEM (figure 1), respectively in 90% and 80% of the selected cases. SEM detected none ABs in the lung samples of two subjects with mesothelioma: one with probable occupational asbestos exposure and the other without asbestos exposure (table 1). OM detected none ABs in the lung sample of the subject with lung cancer without asbestos exposure (table 2).

In the two cases where ABs were observed by OM but not by SEM we can hypothesize: i) the ABs observed by OM are not really ABs but they are FBs; ii) when the ABs burden is lower than 1000 ABs/gdw it is possible that they are not detected by SEM because of the minor percentage of material prepared and observed.

More than 1,000 ABs per gdw, value considered as indicative of high probability of occupational exposure to asbestos [9,21] were detected by OM e SEM respectively in 50% e 80% of the selected case.

In the lung samples of all subjects that have had occupational exposure to asbestos (two cases of mesothelioma and two cases of lung cancer), more than 1,000 ABs per gdw were detected by both OM and SEM observation, as expected. ABs were also detected by OM observation in the lung samples of all subjects that have had probable occupational exposure to asbestos (three cases of mesothelioma); in two of these cases ABs were detected by SEM investigation too. However only in one case more than 1,000 ABs per gdw were counted both by OM (2,500 ABs/gdw) and SEM (6,120 ABs/gdw). In the other two cases this value result respectively slightly below (832 ABs/gdw) and very low (143 ABs/gdw) when the observation was carried out by OM; ABs concentration is over the limit (3,825 ABs/gdw) and not present (0 ABs/gdw) when the investigation was carried out by SEM.

In the only case of probable environmental exposure to asbestos (case of mesothelioma) ABs amount is lower than the limit (415 ABs/gdw) when the investigation was carried out by OM, and over (33,444 ABs/gdw) when the investigation was carried out by SEM.

The lung samples investigation of the two subjects without asbestos exposure (one case of mesothelioma and one case of lung cancer) has shown that the ABs amount is respectively lower of the limit (800 ABs/gdw) and absent (0 ABs/gdw) when the observation was carried out by OM, as expected; while, when the investigation was carried out by SEM-EDS, ABs are also absent (0 ABs/gdw) in the subject with mesothelioma but over the limit (4,936 ABs/gdw) in the subject with lung cancer.

Some studies point out the correlation between ABs count by OM and by SEM (in subjects with occupational exposure) [21,22]. We have to consider that when the ABs burden is low or

moderate (such as in subjects with absent or probable asbestos exposure) the correlation between ABs concentration determined by MO and those determined by SEM is lost. Therefore, when the ABs value in OM is borderline the SEM investigation became essential.

About uncoated fibers

In nine lung samples of the ten investigated, different inorganic fibrous uncoated species has been determined by EDS-SEM (figures 2,3). Among these, six were identified as asbestos. Five of them are amphiboles: asbestos tremolite, asbestos actinolite, asbestos grunerite, asbestos anthophyllite, and crocidolite. As it concerns tremolite and actinolite asbestos, they are grouped together because their chemical characterization cannot be determined by qualitative EDS-SEM analyses.

Due to the difficulty in distinguishing chrysotile (asbestos) and antigorite (non asbestos) using this technique we considered them as a sole group, named chrysotile-antigorite group. Between the subjects with occupational exposure, in two cases (one of mesothelioma and one of lung cancer) fibers could not be chemically identified because their diameter was too thin. In the other case of lung cancer 45,900 ff/gdw asbestos grunerite (57%), 27,540 crocidolite (34%) and 6,120 ff/gdw chrysotile-antigorite (7.7%) were detected. Crocidolite (79.940 ff/gdw) was the only fiber chemically identified in the last case while 70% of the fibers was too thin. The concentration of asbestos fibers in these two cases are about 80.000 ff/gdw, a value slightly below the limit of 100,000 ff/gdw (quantities internationally established as indicative of significant amphibole asbestos exposure).

Discussion

Unfortunately, legal aspect is not considered in the first step of diagnosis of malignant mesothelioma or lung cancer. Only after the death of the patient can turned out evident the necessary of retrospective studies. Nevertheless, sometime the material (i.e. lung tissue) for a thorough investigation is not available because an autopsy was not been request.

The new technologies in the field of pathology (e.g. molecular therapies, endoscopy, and molecular radiology) could not forget the conventional investigation by OM and by SEM-EDS in the diagnosis of occupational asbestos diseases (i.e. mesothelioma).

Mesothelioma and lung cancer (by asbestos exposure) diagnosis can be made with cytology, histology of biopsy, histological examination of the surgical specimen, or histological examination of autopsy material (pathologists' involvement). In 1981, Mark [23] was thw first

to suggest the “second diagnosis” as another important aspect of the pathologist’s role: the identification of other abnormalities related to asbestos fibers inhalation.

The aim of our study is to underline that the demonstration of the presence of asbestos (mineralogical identification by SEM-EDS investigation) and its burden in lung tissue of subjects with possible asbestos neoplastic diseases (mineralogists’ involvement) represent an important medico-legal value because confirm a past exposure.

In fact, it is not possible to attest that the mesothelioma or lung cancer are due to asbestos exposure only known the work activity or other possible exposure (e.g. environmental) of the subject. Therefore, it is important to be able to attest the relation between asbestos fibers or other kind of fibers exposure and the pathology of the patient for a prevention question. For this reason, when the patient dies, a correct diagnosis must be done on autopsy material, especially in the case of suspected occupational disease.

The chemical analysis by EDS-SEM of the fibers detected in lung samples of subjects with pulmonary ills can be correlated with the life background (occupational and/or environmental).

The histopathological diagnosis of mesothelioma and lung cancer, and the mineralogical investigation of fibers inhaled, require a multidisciplinary group of study. The results obtained represent an important service to the society (e.g. reducing number and costs of litigation process).

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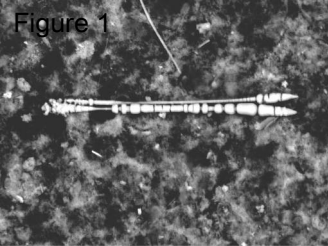
Figure legend

Fig 1. Backscattered electron SEM image of twin ABs (2000X)

Fig 2. Backscattered electron SEM image (2000X) of crocidolite

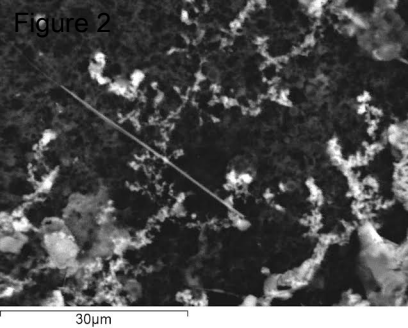
Fig 3. EDS/SEM spectrum of crocidolite

Figure 1



60µm

Figure 2



30µm

Figure 3

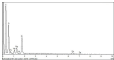


Table 1

MESOTHELIOMA				
cases	asbestos exposure	OM ABs/gdw	SEM AB/gdw	SEM-EDS asbestos ff/gdw
1	occupational	>1,000	363,375	fibers too thin to investigate by SEM-EDS
2	occupational	42,840	228,400	70% fibers too thin to investigate by SEM-EDS 79,940: crocidolite
3	occupational probable	2,500	6,120	18,000 tremolite-actinolite asbestos
4	occupational probable	832	3,825	111,807 anthophyllite asbestos 695 tremolite-actinolite asbestos 695 crocidolite
5	occupational probable	143	0	4,370 anthophyllite asbestos 8,743 tremolite asbestos 17,486 crocidolite
6	absent	800	0	6,120 tremolite-actinolite asbestos
7	environmental probable	415	33,444	16,722 tremolite-actinolite asbestos 33,444 chrysotile-antigorite 16,722 crocidolite

Tab 1. ABs detected by both OM and SEM observation, and asbestos fibres investigated by EDS-SEM in lung samples of subjects with mesothelioma.

ABs, Asbestos Bodies

gdw, gram of dry weight

OM, Optical Microscopy

SEM, Scanning Electron Microscopy

EDS-SEM, Energy Dispersive Spectrometer

Table 2

LUNG CANCER				
cases	asbestos exposure	OM ABs/gdw	SEM AB/gdw	SEM-EDS asbestos ff/gdw
1	occupational	>1,000	342,720	45,900 grunerite asbestos 6,120 crysotile-antigorite 27,540 crocidolite
2	occupational	48,000	11,946,240	fibers too thin to investigate by SEM-EDS
3	absent	0	4,935	0

Tab 2. ABs detected by both OM and SEM observation and, asbestos fibres investigated by EDS-SEM in lung samples of subjects with lung cancer

ABs, Asbestos Bodies

gdw, gram of dry weight

OM, Optical Microscopy

SEM, Scanning Electron Microscopy

EDS-SEM, Energy Dispersive Spectrometer