

Review

Asbestos exposure diagnosis in pulmonary tissues

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Summary

The diagnosis of asbestosis requires different criteria depending on whether it is in a clinical or medical/legal setting. In the latter context, only when a "diffuse interstitial fibrosis associated to asbestos bodies (ABs)" is present, it can be said to be asbestosis. Considering the medical/legal setting, the diagnosis must be certain and proven. Unfortunately, it is often difficult to identify ABs by light microscopy (LM), but this does not mean that the diagnosis should be clinically excluded. Other parameters are important, such as working history and/or diagnostic imaging. In addition to LM, normally used for diagnosis, there are other techniques, e.g.: scanning electron microscopy with attached microanalysis microprobe (SEM/EDS), but they require tissue digestion and higher cost. A new approach with micro-Raman spectroscopy and SEM/EDS techniques is able to analyse histological sections without other manipulations that could interfere with analysis of asbestos fibres. In this work, we propose an algorithm for asbestosis diagnosis, especially in the forensic medical field, demonstrating the importance of close collaboration between multiple professionals.

Key words: asbestos, diagnosis, pathologist, mineralogist, lung

Introduction

Asbestosis is a pneumoconiosis related to an excess of asbestos fibres inside pulmonary parenchyma; it is the prototype of diseases caused by mineral fibre inhalation. The diagnosis of asbestosis does not pose difficulties when it is possible to identify asbestos bodies (ABs) in the lung fibrosis ^{1,2}. The diagnosis generally requires: clinical, radiological images, and histopathology with mineralogical analysis (Tab. I). Identification of bilateral pleural plaques and/or fibrotic thickening of the parietal and/or visceral pleura increases the suspicion of asbestosis, but does not permit a diagnosis ¹. The research on pulmonary functionality is not specific for asbestosis. The demonstration of ABs inside sputum or BAL liquid correlates with a strong exposure and the asbestosis degree, but the presence of these exposure markers alone cytological samples is not sufficient to render a diagnosis of asbestosis.

Histological diagnosis is therefore essential by identifying the lung fibrosis with typical asbestos fibres or bodies inside the lung. The close collaboration with an expert in mineralogical researches inside the tissues in both optical and electron microscopies and in micro-Raman spectroscopy becomes fundamental. In the Piedmont Region there are certified centres where it is possible to perform this search, but there are no com-

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Table I. Comparison of the specific characteristics of asbestosis and usual interstitial pneumonia (UIP).

Specific characteristics	
Asbestosis	Usual interstitial pneumonia (UIP)
Bland clinical manifestations.	Severe clinical manifestations.
Moderate respiratory function.	Severe respiratory function.
Slower fibrosis progression.	Faster fibrosis progression.
X-Rays: lower pulmonary lobes. ILO category 1/1 for reticular nodular opacities with restrictive lung disease.	X-Rays: Mild and upper pulmonary lobes. Only UIP is in lower lobes.
HRCT: ground-glass opacities, along with diffuse interstitial fibrosis. It begins centrally and dissipates peripherally following a centrifugal pattern. Bilateral pleural plaques or pleural thickening.	HRCT: evidence of patches of opacities. It begins peripherally following a centrifugal pattern.
Patchy interstitial fibrosis starting from the respiratory bronchioles.	Diffuse interstitial fibrosis.
Peribronchiolar distribution with subpleural accentuation.	Subpleural accentuation, lower lung zone.
Peribronchiolar fibrosis with rare or absent fibroblastic foci. Temporal homogeneity.	Profuse proliferation of fibroblasts. Temporal heterogeneity.
Secondary interest of interlobular septa and visceral pleura may also markedly be thickened by fibrous tissue.	Uncommon diffuse thickening of visceral pleura and/or pleural plaques.
Minimal inflammation.	Minimal inflammation, typically localised to honeycomb foci.
Free ABs observed inside the alveola, phagocyted by the histiocytes, inside the fibrotic interstice or inside giant cells (100%). ¹ In a small percentage of cases, ABs are not demonstrable. In these cases, fibre analysis is indicated	ABs absence.
Uncommon honeycomb changes except in advanced cases.	Common honeycomb changes.

mon protocols. For two decades, the research group in Environmental Mineralogy is active at the Department of Science and Technological Innovation (DiS-IT) of the University of Eastern Piedmont. Since the birth of this group, collaboration with the Santi Antonio and Biagio and Cesare Arrigo Hospital of Alessandria, the Santo Spirito Hospital of Casale Monferrato, and the Interdepartmental Centre for Studies on Asbestos and Other Toxic Particulate “Giovanni Scansetti” led to the use of new technologies. This sharing led to the creation of the “Integrated laboratory for the asbestos research” (LIRicA) in the end of 2022, with the aim of promoting the sharing of the knowledge between academic and hospital personnel about asbestos related diseases. Principal research theme of this team was always the application of innovative techniques, such as Scanning Electron Microscopy annexed to Energy Dispersive Spectrometry (SEM/EDS) and micro-Raman spectroscopy, aimed at the identification (and quantification) of asbestos phases and their localisation inside the tissues from patients affected by asbestos-related diseases ³⁻⁸. Through this close collaboration we want to present a possible protocol to arrive at a diagnosis of asbestosis (Fig. 1), not only for clinical purposes, but also to be routinely used in the medico-legal setting.

Materials and methods

A literature search was carried out regarding both the diagnosis of asbestosis and the detection of asbestos in human tissues (database: PubMed; keywords: “practice guidelines and asbestosis” and “asbestos in lung tissues” in the last 10 years).

Taking into account the literature, and our experience, a possible path to achieve a safe diagnosis of asbestosis has been constructed.

The protocol envisaged identifying:

- the most suitable material for asbestos identification;
- the finding of any additional staining on routine histological sections for greater diagnostic accuracy;
- when to contact a research centre where there is a laboratory for asbestos research;
- the most suitable techniques for the best identification of asbestos bodies in lung tissue;
- sensitivity and specificity of techniques used with cost/benefit ratio.

Results

SUMMARY OF LITERATURE RESULTS FOR “ASBESTOSIS”

In the literature, the Asbestosis Committee of the College of American Pathologists and the Pulmonary Pathology Society described minimal criteria for asbestosis diagnosis in 2010 ⁹. Their work represents the collaboration among international committee of North

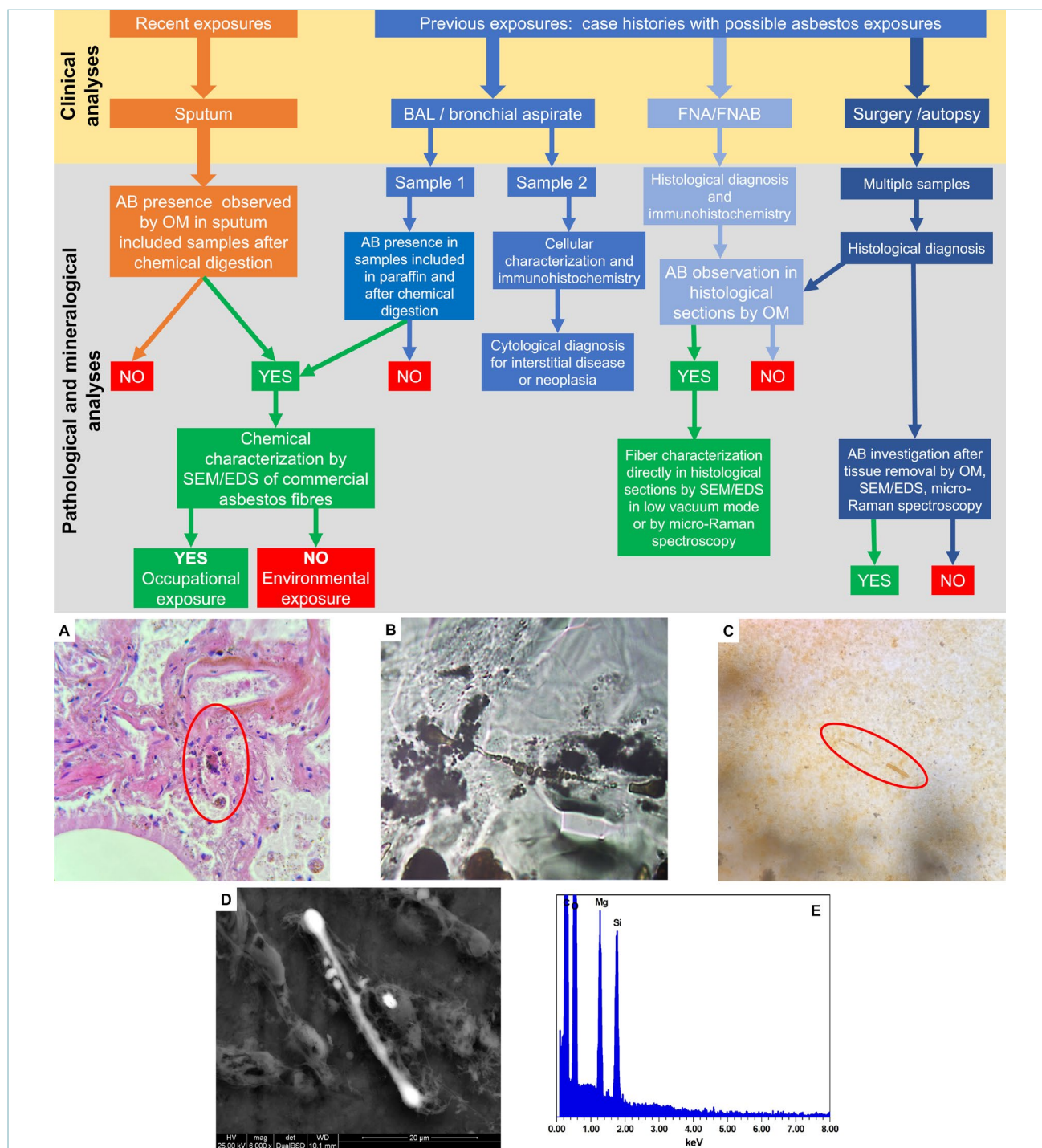


Figure 1. Working scheme for asbestos evaluation from the clinicians to the pathologists and mineralogist evaluations for asbestos in tissues. In this scheme, “NO” does not exclude working exposure, but only that it is not possible to obtain a confirmation of the presence of asbestos. (A) Asbestos body in a H&E stained section from lung tissue (highlighted by the circle, magnification 40X). (B) Asbestos body observed using the LM microscope annexed to the Raman spectroscopy (magnification 100X) in a non-stained histological section from lung. (C) Asbestos body deposited on filters after tissue removal observed by LM at magnification 40X. (D) SEM image of an asbestos body observed using backscattered (DualBSD) detector. Backscattered electrons highlight the morphology and the atomic number of the elements composing the different areas of the samples (a lighter grey corresponds to a higher atomic number of the element). (E) An example of EDS spectrum obtained on a free fibre of chrysotile.

American, European, and Australasian pathologists, organized under the auspices of the College of American Pathologists and the Pulmonary Pathology Society. These documents propose the minimal histological criteria for asbestosis diagnosis updating the previous guideline to differentiate asbestosis from Usual Interstitial Fibrosis (UIP). They intended to be used as a basis for communication among pathologists, pulmonologists, oncologists, radiologists, occupational hygienists, and epidemiologists. In the literature, it is difficult to find criteria that also take into account mineralogical analysis. Our objective was to summarise all the literature data, from 2010 to the present, about the minimum criteria for asbestosis (Tab. I), also taking into account the data on mineralogical analysis. To date, “asbestos bodies” (ABs, coated asbestos fibres) inside lung tissues remains the distinctive diagnostic criteria for asbestosis.

ABs are not always observed on histological sections with light microscopy (LM), although staining for iron highlighting their coating is added. Asbestos fibres, principally biopersistent amphiboles, entrapped inside the pulmonary interstitium, have a prolonged permanence time and asbestosis may develop and continue to advance for years after exposure termination. Chrysotile fibres, which are more friable and more easily phagocytosed and eliminated from the pulmonary interstitium, but not less fibrogenic, are difficult to identify in histological sections because they rarely create asbestos bodies. Therefore, more appropriate techniques are needed to identify not only fibres with length $\geq 5 \mu\text{m}$, diameter $\leq 3 \mu\text{m}$, and length/diameter ratio $> 3 \mu\text{m}$ (the so-called breathable fibres), but also fibres with a length $< 5 \mu\text{m}$, which less induce ABs formation but remain present in the lung, if at lower concentrations, in the cytoplasm of macrophages.

CLINICAL DIAGNOSIS, CHEST RADIOGRAPHY, COMPUTED TOMOGRAPHY

In Table II, the literature data about clinical and radiological diagnosis of asbestosis, are summarised⁹⁻²³. Especially that from 2010 (date of the manuscript of Roggli et al., 2010)⁹ to 2024 are summarized²⁴⁻²⁸. Today, unlike the clinical pictures of asbestosis of the past, it can be silent, and asbestosis can only be identified with radiological and/or pathological investigation, especially in cases studied for lung cancer. Spirometry and capillary alveolus diffusion for carbon monoxide can be used for pre-clinical diagnosis of asbestosis. The standard chest X-ray performed with the ILO (International Labour Organization) 2000 criteria can still be considered an excellent examination, given its easy accessibility and because of the lower exposure to ionising radiation, even if it has a low sen-

sitivity (51%)¹³ and a low specificity with a low positive predictive value ($< 50\%$) for asbestosis¹⁹. On the other hand, the sensitivity (70%) and the specificity (91%)²⁰ of chest CT are higher, so HRCT is considered the gold standard technique. CT is more sensitive at detecting the parenchymal changes of asbestosis at an early stage. One useful feature that may aid in the distinction of asbestosis from other fibrosing pneumonitis is the frequent association of pleural abnormalities with the former. The considerable inter-observer variability, especially for the mild frameworks that occur today²¹, has required the definition of criteria similar to the ILO BIT (BIT, Bureau International du Travail) score, but they have not been authorised yet²².

Most cases of asbestosis can be diagnosed as a probabilistic exercise within multidisciplinary groups of diagnosis and treatment and mostly only for clinical and radiological reasons without resorting to histological examination. However, this is not considered sufficient for the Italian national workers' compensation authority (INAIL) and/or for civil and penal legal actions. Therefore, it is essential to always succeed with techniques on lung tissues to demonstrate the presence of asbestos fibres, especially in the case of amphibolic and commercial fibres.

HISTOLOGICAL DIAGNOSIS OF ASBESTOSIS

First of all, it is important to define the type of material used for asbestosis diagnosis. Table III summarises the different types of fluids and tissues and the confidentiality of the material for the cyto/histological diagnosis of asbestosis^{1,2,9,17,18,29-44}.

Acceptable histopathological definitions of asbestosis were provided by the College of American Pathologists-National Institute for Occupational Safety & Health (CAP-NIOSH)³². Criteria have been also defined by a group of specialists in the so-called Helsinki Criteria⁴⁵ and by the American Thoracic Society (ATS)³² for the recognition of occupational pathologies in the forensic medical field. The definitions have been recently updated by the Asbestosis Committee of the College of American Pathologists (CAP) and the Pulmonary Pathology Society (PPS)⁹. Iron staining should be routinely used, especially when the typical ABs are not observed in routinely stained histological sections. ABs distribution in the context of the pulmonary tissue is casual and therefore it is necessary to do more than one section and in different areas of the lung sample. It is clear that the autoptic material is most suitable for a complete diagnosis, allowing the preparation of histological sections of all the lobes and bilaterally the pulmonary segments. Asbestosis diagnosis is confirmed when there is the characteristic fibrosis and the presence of typical ABs on the routine 3-5 μm thick sections^{9,32}.

Table II. Clinical diagnosis, chest radiography, computed tomography, cytology and histology, and mineralogical characterization: advantages and disadvantages.

Symptoms	Asymptomatic Symptomatic with: Dyspnoea and dry cough; Basic rale and digital clubbing; Chronic pulmonary heart in the terminal stage.	Advantages: correct working and environmental medical history.
		Disadvantages: Aspecific. General asbestosis is less severe than UIP.
Respiratory function	Restrictive Lung Diseases: Reduction of pulmonary volumes; increased work of breathing; inadequate ventilation and/or oxygenation; decrease in the forced vital capacity. FEV1/FVC ratio is either normal or increased.	Advantages: It adds more diagnostic confidence with low costs and risks.
		Disadvantages: Aspecific.
Radiology (X-ray/CT imaging)¹⁰⁻¹²	X-ray: lower lung zone; diffuse reticulonodular infiltrates. "Shaggy" cardiac silhouettes. Indistinct diaphragmatic contours. 1/1 ILO category (presumptively diagnostic but not unequivocal) with reductions in predicted FVC and diffusion capacity less than the lower limits of normal, it is sufficient for the diagnosis of asbestosis. ILO 0/1 category is suggestive but not presumptively diagnostic. HRCT (increased sensitivity but not specificity): ground-glass opacities, along with diffuse interstitial fibrosis. Bilateral pleural plaques or pleural thickening.	Advantages: X-Ray adds more diagnostic confidence with low cost and risk. CT is more sensitive at an early asbestosis stage. Sensitivity: 70%; specificity 91%. ¹⁹
		Disadvantages: Sensitivity of only 51%. ¹⁹ HRCT , it has costs and X-ray absorptions higher than those of X-rays.
Cytology and Histology²⁹⁻³¹	Cytology: prevalence of histiocytes. Presence of ABs. Histology: 1 or more ABs in routine lung sections or in the routine 3-5 µm thick sections. Lung fibrosis: diffuse; bilateral; - inferior lobes > superior lobes; - peribronchial; subpleural tiers of alveoli; alveoli in proximity to the bronchioles; occasionally honeycomb change A histological diagnosis for asbestosis, to be sure, requires the identification of a peribronchiolar /interstitial fibrosis and the presence of the typical ABs in histological sections (more than 1 per cm²). In the absence of a mineral fibre analysis, the diagnosis of asbestosis by light microscopy is usually debated only in borderline cases.	Advantages: Cytology: rapid initial test. 95% of patients with asbestosis have ABs in BALF. Histology: Surgical biopsy is a Gold Standard when exposure history is not compelling and uncertainty exists on the basis of radiographic and/or clinical grounds
		Disadvantages: Cytology: not diagnostic. Histology: diagnostic but not representative of the entire lung damage.
Mineralogy³⁻⁸	Identification and quantification of coated (ABs) and uncoated asbestos fibres. Distinction between industrial asbestos and asbestos source increases the confidence limit of the diagnosis of asbestosis	Advantages: LM: (H&E and Pearls): diagnosis of asbestosis. ABs count by digestion techniques. Low costs and times. Crystalline structure with polarised a light microscopy. SEM: high resolution; chemical analysis (EDS). Micro-Raman spectroscopy: identification of asbestos phases.
		Disadvantages: LM: low resolution. SEM/EDS: high costs and long analysis times. It often requires tissue digestion. Micro-Raman spectroscopy: low signal of Raman spectra.

Histopathological asbestosis can show nonspecific features like Mallory bodies identified inside alveolar cells (7%), bronchiolar metaplasia or "pulmonary adenomatosis" (10% of cases)⁴⁴, dendriform pulmonary ossification (DPO, osseous metaplasia - 2% of cases),^{34,35} and pulmonary "blue bodies" (1% of cases)³⁶. Moreover, there is association with other infections, especially aspergillus (rarely)³⁸, DIP-like area (6%)¹. These aspects do not help in diagnosis. Even today, both radiologists and pathologists do not

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Table III. Types of fluids and tissues and the confidentiality of the material for the cyto/histological diagnosis of asbestosis.

Material type	Utility	Sensitivity/ Specificity	Cost/benefit ratio
Sputum	ABs research to study possible recent exposure.	Low sensitivity and specificity to asbestosis.	Very good.
BAL/bronchial aspirate	Limited role to asbestosis diagnosis. ABs research to study possible recent exposure and cellular type distribution. ABs demonstration inside BALF is possible in more than the 95% of patients affected by asbestosis. Negative BALF exam for ABs does not exclude the possibility of an asbestos-related pulmonary pathology.	Low sensitivity and specificity to asbestosis.	Good (very low adverse event rate).
Fine needle aspirate	Insufficient to diagnose asbestosis. The presence of ABs is indicative of considerable occupational exposure to asbestos. ⁴³	Low sensitivity. High specificity to asbestosis.	Good.
Needle biopsy	Insufficient to diagnose asbestosis. The presence of asbestos bodies is indicative of considerable occupational exposure to asbestos.	Low sensitivity. High specificity to asbestosis.	Good.
ENDOBONCHIAL BIOPSY TBLB (transbronchial biopsy) TBLC (transbronchial criobiopsy) VAT (video-assisted thoracoscopic lung biopsy) SLB (surgery lung biopsy with thoracoscopy) EBUS-TBNA	Good for asbestosis diagnosis. Recommended in cases with dubious exposure and/or suspects on clinical/radiological data. SLB is a Gold Standard for asbestosis diagnosis but not all patients can cope with it. TBLC is preferable to other techniques since it is the most suitable for obtaining histological data with less risk.	TBLC/SLB: diagnostic performance 80%/90%, but TBLC has less risks. VAT: High sensitivity and specificity to asbestosis.	Endobronchial: high diagnostic value and low risk. Transbronchial: High risk. VAT: high diagnostic value but high cost and expertise requirement. TBLC: less invasive than VAT and SLB.
Surgical specimen	Good for asbestosis diagnosis. To be found in cases of excised lung tissue for lung cancer.	High sensitivity and specificity to asbestosis.	Good in selected cases of lung cancer resection.
Autopsy material	Very good asbestosis diagnosis. Recommended in cases where there was a diagnostic doubt in life and for the recognition of occupational disease.	High sensitivity and specificity to asbestosis.	Good in selected cases of deceased people.

always use the same criteria for asbestosis diagnosis, especially in early-stage cases, thus underestimating it.

Mineral identification inside tissues

Coated and uncoated asbestos fibres are the subject of the mineralogical identification. ABs must be distinguished from the pseudo-asbestos bodies referring to the work published by the Italian National Institute of Health ⁴⁶. The test for the detection of ABs in sputum may be of some use as a marker of exposure, although it has very high specificity, but low sensitivity, and positivity correlates with the intensity of exposure. The search for asbestos bodies directly in lung tissue is more sensitive, as well as more specific. ABs may be observed inside hilar or mediastinal lymph nodes,

often associated with lymph nodal parenchyma fibrosis ^{39,47}. This curious observation is largely limited to patients with a heavy load of pulmonary parenchymal fibres, and it is probably related to an overload of the clearance mechanisms ³⁹. Mineralogical research can also be performed after chemical digestion of lung tissue, looking not only for asbestos bodies but also for free fibres.

OPTICAL MICROSCOPY TECHNIQUES

Different sample preparations are possible ⁴⁸.

In Figure 1A, a classical 3 µm thick histological section stained with haematoxylin and eosin (H&E) for LM analysis is shown.

In Figure 1B, an example of ABs observed in an unstained 5 µm section is reported: in this case, the section is not coloured for the successive analysis by

micro-Raman spectroscopy because the staining reagents give vibrational bands in the spectra that do not allow an optimal condition for asbestos signal detection. In Figure 1C, an example of digested tissue filtered on polycarbonate filter is reported: in this case, the sample can be used for asbestos fibres and bodies counts by both LM and SEM/EDS (the sample preparation is the same, there are different preparations prior to the analyses).

ELECTRON MICROSCOPY TECHNIQUES

A few ultrastructural studies have been reported in patients affected by asbestosis, especially for the analysis of alteration of the parenchyma. More numerous are studies that identify the mineral fibres covered and not in the lung parenchyma after chemical digestion. Shelburne et al., in 1983, observed that transmission electron microscopy (TEM) is an inefficient technique to detect ABs, even in patients with heavy load of tissutal asbestos, because of the minimum amount of tissue that can be examined using this technique⁴⁹. The scanning electron microscopy technique has greater sensitivity and specificity for the search for mineral fibres.

SCANNING ELECTRON MICROSCOPY WITH ENERGY DISPERSIVE SPECTROSCOPY (SEM/EDS)

Scanning Electron Microscopy (SEM) with Energy Dispersive Spectroscopy (EDS) microprobe is a technique allowing high resolution images with magnification up to hundreds of thousands of times of the studied object and to collect spectra on chemical composition. The evolution of SEM, especially with the acceleration of the technological development of the last decades, now allows to observe samples both specifically prepared for the analysis (with lapping and deposition of a metallic target to make the samples more conductive to obtain higher magnification and resolutions) and samples without any particular manipulation. This proved to be very important in the application of this technique to the research and identification of asbestos fibres and bodies inside respiratory and extra-respiratory tissues deriving from patients affected by asbestos related diseases (Figs. 1D and 1E).

Discussion and conclusion

In the past, asbestosis was diagnosed in the form of advanced interstitial fibrosis with a worsening evolution. Today it is diagnosed in a pauci-symptomatic form and with extremely mild radiological findings (forms 0/1, 1/0 and 1/1 of the ILO BIT 2000 classifi-

cation) even in subjects with previous and documented asbestos exposures. Any concomitant exposure to cigarette smoke can affect fibrosis progression degree and, if associated with other toxicants for the respiratory system (e. g.: dust, silica, fumes), induce the onset of chronic obstructive pulmonary disease. Pleural plaques occur very slowly after 3 to 4 decades from initial asbestos exposure, and can be demonstrated in more than 2/3 of exposed individuals without asbestosis. Diffuse pleural thickenings are very rare, involving only 5% of patients with a much shorter latency.

A histological diagnosis for asbestosis requires the identification of a bronchiolar/interstitial fibrosis and the presence of typical ABs in histological sections (more than 1 per cm²). This is at least the criterion required for the recognition of asbestosis as an “occupational disease”. Clinical/radiological diagnosis alone is not sufficient to respond to medico-legal questions. It is clear that the more material available (surgical or autopsy specimens), the easier the diagnosis of asbestosis.

The diagnosis of that is accepted in legal questions depends by ABs or asbestos fibres in the lung. For this reason, it is necessary to obtain sufficient tissue sample for analysis. It must be representative of the lesion to identify both specific asbestos fibrosis and ABs within the lung tissue. For this reason, some methodologies and techniques (LM, SEM/EDS, and micro-Raman spectroscopy) can be applied and the techniques that maintain the structure of the lung parenchyma should be preferred. The removal of the biological material with chemical digestion is useful to value fibre load. It is possible to characterise asbestos directly inside the typical histological sections utilised in Pathological Anatomy using both micro-Raman spectroscopy and SEM/EDS.

To do this, interaction among mineralogists, biologists, clinicians, radiologists, pathologists is necessary. SEM/EDS is one of the techniques provided by the Italian Law for mineral phase analysis ruled as asbestos by the Legislation in air samples¹⁶. In fact, considering environmental analysis, it is possible not only to identify the observed asbestos fibres, but also to quantify them obtaining a numeric value corresponding to the number of fibres per litre (or cm³) of sampled air on specific filters. For this reason, fibre analysis also inside the tissues from patients affected by asbestos-related diseases using SEM/EDS was widely developed both in scientific and in medico-legal scopes. Thus, there is more than one advantage respect to LM: firstly, it is possible to couple morphological analysis to the chemical analysis, obtaining compositional spectra allowing mineralogical identification of the different crystals; moreover, the higher resolution of

the technique permits to detect phases with a much lower diameter (SEM lateral resolution can reach also the nanometre, in optimal operative conditions). Lastly, counting methods developed over the years allow definition how many fibres are present inside the analysed organ and to hypothesise the possible origin of patient's exposure.

Moreover, the development of the low vacuum mode allows the analysis of samples without any particular manipulation: this peculiarity allows SEM/EDS application even in the search for asbestos fibres directly inside the tissues, in histological sections routinely used by the pathologists for asbestosis diagnosis, giving the possibility of asbestos fibre identification even if they are covered by the iron-rich covering typical of the ABs ⁷. The second fundamental advantage of this application consists in the possibility of coupling analyses obtained by other techniques to achieve further information. For example, coupled application of LM allows to identify the type of tissue where the inorganic phases ² are embedded or the combination of an investigation by micro-Raman spectroscopy ⁸ permits to obtain a confident attribution of the mineral phase also in case of very similar chemical compositions ⁵⁰, as in the case of serpentine minerals, among which only chrysotile is defined as asbestos by the Law.

Therefore, in consideration of the literature and our experience, we have built a scheme (Fig. 1) to hypothesise a possible path to be followed in cases of pulmonary fibrosis where it is necessary to identify a possible asbestosis, both for therapy (different from UIP) and for possible legal compensation.

CONFLICTS OF INTEREST STATEMENT

The authors declare no conflict of interest.

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AUTHORS' CONTRIBUTIONS

Conceptualization DB; methodology DB, AC; validation DB; formal analysis AC, AG; investigation DB, AC, AG; data curation DB, AC, AG; writing-original draft preparation DB, AC, AG; writing-review and editing, AC, DB, AG; visualization AC, AG; supervision MB; project administration DB, MB, AM; funding acquisition AM.

References

- Sporn TA, Roggli VL. Asbestosis. In: Oury TD, Sporn TA, Roggli VL (Ed.) *Pathology of Asbestos-Associated Diseases*. Third Edition. Springer-Verlag Berlin Heidelberg, 2014, pp. 53-80. <https://doi.org/10.1289/ehp.80341>
- Cooke WE. Fibrosis of the lungs due to the inhalation of asbestos dust. *Br Med J* 1924;2(3317):147-148. <https://doi.org/10.1136/bmj.2.3317.147>
- Grosso F, Croce A, Trinchieri NF, et al. Asbestos fibres detected by scanning electron microscopy in the gallbladder of patients with malignant pleural mesothelioma (MPM). *J Microsc* 2017;266:48-54. <https://doi.org/10.1111/jmi.12517>
- Croce A, Capella S, Belluso E, et al. Asbestos fibre burden in gallbladder: a case study. *Micron* 2018;105:98-104. <https://doi.org/10.1016/j.micron.2017.12.001>
- Grosso F, Croce A, Libener R, et al. Asbestos fiber identification in liver from cholangiocarcinoma patients living in an asbestos polluted area: a preliminary study. *Tumori J* 2019;105:404-410. <https://doi.org/10.1177/0300891619839305>
- Rinaudo C, Croce A, Erra S, et al. Asbestos fibers and ferruginous bodies detected by VP-SEM/EDS in colon tissues of a patient affected by colon-rectum cancer: a case study. *Minerals* 2021;11:658. <https://doi.org/10.3390/min11060658>
- Croce A, Musa M, Allegrina M, et al. Environmental Scanning Electron Microscopy technique to identify asbestos phases inside ferruginous bodies. *Microsc Microanal* 2013;19:420-424. <https://doi.org/10.1017/S1431927612014390>
- Rinaudo C, Allegrina M, Fornero E, et al. Micro-Raman spectroscopy and VP-SEM/EDS applied to the identification of mineral particles and fibres in histological sections. *J Raman Spectrosc* 2010;41:27-32. <https://doi.org/10.1002/jrs.2403>
- Roggli VL, Gibbs AR, Attanoos R, et al. Pathology of asbestosis - An Update of the diagnostic criteria. Report of the Asbestosis Committee of the College of American Pathologists and Pulmonary Pathology Society. *Arch Pathol Lab Med* 2010;134:462-480. <https://doi.org/10.5858/134.3.462>
- Finkelstein MM, Virgilis JJ. Radiographic abnormalities among asbestos-cement workers: an exposure-response study. *Am Rev Respir Dis* 1984;129:17-22. <https://doi.org/10.1164/arrd.1984.129.1.17>
- Ducatman AM, Withers BF, Yang WN. Smoking and roentgenographic opacities in US Navy asbestos workers. *Chest* 1990;97:810-813. <https://doi.org/10.1378/chest.97.4.810>
- Barnhart S, Thornquist M, Omenn GS, et al. The degree of roentgenographic parenchymal opacities attributable to smoking among asbestos-exposed subjects. *Am Rev Respir Dis* 1990;141:1102-1106. https://doi.org/10.1164/ajrccm/141.5_Pt_1.1102
- ILO. Guidelines for the use of the ILO International Classification of Radiographs of Pneumoconioses, 2000 edition. Geneva, International Labour Office, 2002, Occupational Safety and Health Series, No. 22 (rev. 2000).
- McMillan GHG, Pethybridge RJ, Sheers G. Effect of smoking on attack rates of pulmonary and pleural lesions related to exposure to asbestos dust. *Br J Ind Med* 1980;37:268-272. <https://doi.org/10.1136/oem.37.3.268>
- Kilburn KH, Lillis R, Anderson HA, et al. Interaction of asbestos, age and cigarette smoking in producing radiographic evidence of diffuse pulmonary fibrosis. *Am J Med* 1986;80:377-381. [https://doi.org/10.1016/0002-9343\(86\)90709-6](https://doi.org/10.1016/0002-9343(86)90709-6)
- Decreto Legislativo 15 agosto 1991, n. 277. Attuazione delle direttive n. 80/1107/CEE, n. 82/605/CEE, n. 83/477/CEE, n. 86/188/CEE e n. 88/642/CEE, in materia di protezione dei lavoratori contro i rischi derivanti da esposizione ad agenti chimici, fisici e biologici durante il lavoro, a norma dell'art. 7 della legge 30 luglio 1990, n. 212. *Gazzetta Ufficiale Supplemento Ordinario* n° 200, 27 Agosto 1991.
- Roggli VL, Sharma A. Analysis of tissue mineral fiber content. In: Oury TD, Sporn TA, Roggli VL, eds. *Pathology of Asbestos-Asso-*

- ciated Diseases, Third edition. Springer, Berlin, Germany, 2014, pp. 309-354.
- 18 Belluso E, Bellis D, Fornero E, et al. Assessment of inorganic fibre burden in biological samples by scanning electron microscopy - energy dispersive spectroscopy. *Microchim Acta* 2006;155:95-100. <https://doi.org/10.1007/s00604-006-0524-y>
 - 19 Huuskonen O, Kivisaari L, Zitting A, et al. High-resolution computed tomography classification of lung fibrosis for patients with asbestos-related disease. *Scand J Work Environ Health* 2001;27:106-112. <https://doi.org/10.5271/sjweh.596>
 - 20 Ross RM. The clinical diagnosis of asbestosis in this century requires more than a chest radiograph. *Chest* 2003;124(3):1120-8. <https://doi.org/10.1378/chest.124.3.1120>
 - 21 Ferretti GR, Bricault I, Coulomb M. Virtual tools for imaging of the thorax. *Eur Resp J* 2001;18:381-392. <https://doi.org/10.1183/09031936.01.00217701>
 - 22 Arakawa H. A Glossary of HRCT Terms. In: Kusaka Y, Hering KG, Parker JE, eds. *International classification of HRCT for occupational and environmental respiratory diseases*. Springer; Tokio, Japan, 2005, p. 12.
 - 23 Schwartz DA, Davis CS, Merchant JA, et al. Longitudinal changes in lung function among asbestos-exposed workers. *Am J Respir Crit Care Med* 1994;150:1243-1249. <https://doi.org/10.1164/ajrccm.150.5.7952547>
 - 24 Apostoli P, Boffetta P, Bovenzi M, et al. Position Paper on Asbestos of the Italian Society of Occupational Medicine. *Med Lav* 2019;110(6):459-485. <https://doi.org/10.23749%2Fmdl.v110i6.9022>
 - 25 Miller A. Recognizing the pleura in asbestos-related pleuropulmonary disease: known and new manifestations of pleural fibrosis. *Am J Ind Med* 2024;67(1):73-80. <https://doi.org/10.1002/ajim.23553>
 - 26 Spagnolo P, Ryerson CJ, Guler S, et al. Occupational interstitial lung diseases. *J Intern Med* 2023;294(6):798-815. <https://doi.org/10.1111/joim.13707>
 - 27 Matyga AW, Chelala L, Chung JH. Occupational lung diseases: spectrum of common imaging manifestations. *Korean J Radiol* 2023;24(8):795-806. <https://doi.org/10.3348/kjr.2023.0274>
 - 28 Järholm B, Burdorf A. Asbestos and disease - a public health success story? *Scand J Work Environ Health* 2024;50(2):53-60. <https://doi.org/10.5271/sjweh.4146>
 - 29 Teschler H, Friedrichs KH, Hoheisel GB et al. Asbestos fibers in bronchoalveolar lavage and lung tissue of former asbestos workers. *Am J Respir Crit Care Med* 1994;149:614-645. <https://doi.org/10.1164/ajrccm.149.3.8118631>
 - 30 IAC-IARC-WHO. WHO Reporting System for Lung Cytopathology. IAC-IARC-WHO Joint editorial Board, 1^o ed., 2022.
 - 31 Schwartz DA, Galvin JR, Burmeister LF, et al. The clinical utility and reliability of asbestos bodies in bronchoalveolar fluid. *Am Rev Respir Dis* 1991;144:684-688. https://doi.org/10.1164/ajrccm/144.3_Pt_1.684
 - 32 Craighead JE, Abraham JL, Churg A, et al. The pathology of asbestos-associated diseases of the lung and pleural cavities: diagnostic criteria and proposed grading schema. Report of the pneumoconiosis Committee of the College of American Pathologists and the National Institute for Occupational Safety and Health. *Arch Pathol Lab Med* 1982;106:544-596. PMID: 6897166
 - 33 Roggli VL. Pathology of human asbestosis: a critical review. In: Fenoglio-Preiser CM ed. *Advances in pathology*, vol 2. Yearbook Med Pub, Chicago, 1989, pp 31-60.
 - 34 Joines RA, Roggli VL. Dendriiform pulmonary ossification: report of two cases with unique findings. *Am J Clin Pathol* 1989;91:398-402. <https://doi.org/10.1093/ajcp/91.4.398>
 - 35 Ndimbie OK, Williams CR, Lee MW. Dendriiform pulmonary ossification. *Arch Pathol Lab Med* 1987;111:1062-1064. PMID: 3117014
 - 36 Koss MN, Johnson FB, Hochholzer L. Pulmonary blue bodies. *Hum Pathol* 1981;12:258-266. [https://doi.org/10.1016/S0046-8177\(81\)80127-X](https://doi.org/10.1016/S0046-8177(81)80127-X)
 - 37 Brody AR, Hill LH. Interstitial accumulation of inhaled chrysotile asbestos and consequent formation of microcalcifications. *Am J Pathol* 1982;109:107-114. PMID: 7124904
 - 38 Hillerdal G, Hecksher T. Asbestos exposure and aspergillus infection. *Eur J Respir Dis* 1982;63:420-424. PMID: 6754414
 - 39 Roggli VL, Benning TL. Asbestos bodies in pulmonary hilar lymph nodes. *Mod Pathol* 1990;3:513-517. PMID: 2217155
 - 40 Raghu G, Collard HR, Egan JJ, et al. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. *Am J Respir Crit Care Med* 2011;183(6):788-824. <https://doi.org/10.1164%2Fajrccm.2009-040GL>
 - 41 Raghu G, Remy-Jardin M, Myers JL, et al. Diagnosis of Idiopathic Pulmonary Fibrosis. An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline. *Am J Respir Crit Care Med* 2018;198(5):e44-e68. <https://doi.org/10.1164/rccm.201807-1255st>
 - 42 Raghu G, Remy-Jardin M, Richeldi L, et al. Idiopathic Pulmonary Fibrosis (an Update) and Progressive Pulmonary Fibrosis in Adults: An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline. *Am J Respir Crit Care Med* 2022;205(9):e18-e47. <https://doi.org/10.1164/rccm.202202-0399st>
 - 43 Leiman G. Asbestos bodies in fine needle aspirates of lung masses. Markers of underlying pathology. *Acta Cytol* 1991;35:171-174
 - 44 Kuhn C 3rd, Kuo TT. Cytoplasmic hyalin in asbestosis. A reaction of injured alveolar epithelium. *Arch Pathol* 1973;95(3):190-194.
 - 45 Henderson DW, Rantanen J, Barnhart S, et al. Asbestos, asbestosis and cancer: the Helsinki criteria for diagnosis and attribution. A consensus report of an international expert group. *Scand J Work Environ Health* 1997;23:311-316. <https://doi.org/10.5271/sjweh.3462>
 - 46 Gruppo Biofibre. Istituto Superiore di Sanità. Corpuscoli dell'asbesto nel tessuto polmonare umano e liquidi biologici: metodo analitico e atlante fotografico. 2017, Rapporti ISTISAN 17/12.
 - 47 Vincent JH, Jones AD, Johnston AM, et al. Accumulation of inhaled mineral dust in the lung and associated lymph nodes: implications for exposure and dose in occupational lung disease. *Ann Occup Hyg* 1987;31:375-393. <https://doi.org/10.1093/annhyg/31.3.375>
 - 48 Gurina TS, Simms L. Histology, Staining. [Updated 2023 May 1]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK557663/>
 - 49 Shelburne JD, Wissemann CL, Broda KR, Ret al. Lung- non-neoplastic conditions. In: Trump BF, Jones RJ, eds. *Diagnostic electron microscopy*, vol 4. Wiley, New York, 1983, pp. 475-538.
 - 50 Rinaudo C, Croce A. Micro-Raman spectroscopy, a powerful technique allowing sure identification and complete characterization of asbestiform minerals. *Appl Sci* 2019;9:3092. <https://doi.org/10.3390/app9153092>