Original Article

Scanning Electron Microscopy coupled with Energy Dispersive Spectroscopy applied to the analysis of fibers and particles in tissues from colon adenocarcinomas

Microscopia Elettronica a Scansione con annessa Spettroscopia in Dispersione di Energia applicata all'analisi di fibre e particelle in tessuti derivanti da adenocarcinoma del colon

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Key words: Asbestos, SEM/EDS, colon adenocarcinoma, fibers, particles.

ABSTRACT

Background: The mineral phases regulated as "asbestos" have a well-known role in disease development in the respiratory tract (*e.g.* mesothelioma, pulmonary carcinoma, asbestosis), but it is not clear their role in cancer development in other body sites, as in colon-rectum tract.

Materials and Methods: In this work, seven colon tissues (healthy and neoplastic portions and an area "bridge" between them) from patients affected by colon adenocarcinoma – and living in a highly asbestos-polluted area - have been digested and the inorganic residual components collected on polycarbonate filters analyzed by means of Scanning Electron Microscopy (SEM) with annexed an Energy Dispersive Spectroscopy (EDS) for elemental chemical analysis.

Results: The obtained results allow us to characterize serpentine phases in two of the seven analyzed patients. Moreover, calcium phosphate phases and other metal-rich particles have been observed inside the samples.

Conclusions: SEM/EDS allowed us to morphologically observe and chemically analyze not only asbestos phases, but also other inorganic particles inside tissues deriving from colon adenocarcinomas.

Background: Le fasi minerali normate come "amianti" hanno un ruolo ben definito in relazione allo sviluppo di malattie a livello del tratto respiratorio (ad esempio, mesotelioma, carcinoma polmonare, asbestosi), non non è ancora chiaro il loro ruolo nello sviluppo di tumori in altri siti corporei, quale il tratto del colon-retto.
Materiali e Metodi: In questo lavoro, sette tessuti derivanti da colon (tessuti sani e neoplastici ed una porzione "a ponte" tra questi) di pazienti affetti da adenocarcinoma del colon - e residenti in un'area altamente inquinata da fasi di amianto - sono stati digeriti ed le componenti residue inorganiche sono state raccolte su filtri in policarbonato ed analizzate mediante la Microscopia Elettronica a Scansione (SEM) con annessa Spettroscopia in Dispersione di Energia (EDS) per l'analisi chimica.
Risultati: I risultati ottenuti permettono di caratterizzare fasi di serpentino nei tessuti di due dei sette pazienti analizzati. Inoltre, fasi di fosfato di calcio e altre particelle metalliche sono state osservate all'interno dei campioni.
Conclusioni: La tecnica SEM/EDS permette di osservare dal punto di vista morfologico e di analizzare chimicamente non soltanto le fasi riconducibili all'amianto, ma anche altre tipologie di particelle inorganiche all'interno dei tessuti derivanti da adenocarcinoma del colon.

BACKGROUND

Until the 1990s, the use of asbestos had wide applications all over the world, from the construction industry to objects of common use.[1] As it concerns Italy, law no. 257 of 1992 started the disposal of use, extraction, and production of these dangerous mineral fibers,[2,3] in order to prevent exposure to asbestos, which can be environmental, domestic, or occupational.[4-7] In order to understand the type of asbestos exposure, forensic scientific analysis is used in the legal field when an exposed subject files a claim for asbestos-related illness. Forensic biology consists of several disciplines used to support scientific investigations with the aim of identifying a subject or an object in a crime ascertainment. In this context, forensic geosciences are Earth Sciences disciplines with useful applications in environmental interpretation.[8]

The term "asbestos" groups six fibrous minerals naturally present in metamorphic deposits around the world and it includes five amphiboles (double-chain inosilicates): tremolite, actinolite anthophyllite, amosite, and crocidolite asbestos - and one serpentine, chrysotile asbestos (Table 1).[9-13]

These six minerals have been used for a long time thanks to their availability and for their mechanical and thermal resistance, in particular chrysotile, crocidolite, and amosite.[13]

According to the definition by the World Health Organization,[14] a fiber is considered breathable if its basic (morphological) characteristics fall within the following parameters: i) length equal to or greater than 5 µm, ii) diameter less than 3 µm; iii) length/diameter ratio (aspect ratio) greater than or equal to 3.

Inhalation of asbestos fibers (or ingestion, although this issue is still controversial) can cause several diseases characterized by a prolonged period (called "latency time") between the exposure onset and the disease appearance. The risk of developing an asbestos-related disease is therefore linked to the cumulative dose of inhaled fibers (fibers/liter per year).[15,16]

The main diseases caused by asbestos exposure are asbestosis,[17] mesothelioma,[18-20] lung cancer,[20] and other cancer diseases.[21,22] In this study, we focused on colon adenocarcinoma, starting from the encouraging results obtained from a first case study[23] and considering that only a few studies found in the literature have been carried out to observe the presence of asbestos phases in this body district.[24-26] Moreover, the relationship between asbestos exposure and colon cancer development is still controversial, so observation and identification of asbestos fibers in these types of tissues might allow to better understand the role of these minerals in cancer development.[27]

The colon is an anatomical part of the large intestine which can be divided into six portions: caecum, ascending colon, transverse colon, descending colon, sigmoid colon, and rectum.[28]

The purpose of this work concerns the application of a methodology aimed at the identification of mineral phases and the possible presence of asbestos fibers in tissue samples deriving from colon adenocarcinoma patients by the use of Scanning Electron Microscopy (SEM) with annexed Energy Dispersive X-ray Spectroscopy (EDS) in order to identify and characterize fibers and inorganic phases observed in histological samples and to search a correlation between these compounds and the carcinoma.

MATERIALS AND METHODS

This exploratory investigation aims at searching asbestiform mineral phases in histological colon samples in a group of seven patients with environmental or occupational asbestos exposure. All of them live in an area defined as the "Italian National Priority Contaminated Sites" (NPCS) for land reclamation, recognized by the Italian Republic as sites seriously polluted by asbestos, represented by Casale Monferrato with other 48 municipalities; moreover, many of these people have worked with asbestos or asbestos-containing materials.[29]

The histological colon samples were collected during surgical procedures conducted by the General Surgery Unit of the Santo Spirito Hospital of Casale Monferrato as part of a study authorized by the Ethical Committee of SS. Antonio e Biagio e Cesare Arrigo Hospital of Alessandria after obtaining the patients' written informed consent.

The samples included: neoplastic tissue, healthy tissue, and a "bridge" area between them.

Researchers performed the identification of the phases attributable to asbestos through "qualitative" chemical elemental analysis carried out by SEM/EDS on elongated mineral phases present on the filter after digestion of the biological matrix.

To figure out the amount of incorporated asbestos fibers, the biological matrix has undergone chemical digestion to characterize and quantify the inorganic fibrous phases.[23,30] This traditional procedure involves tissue samples digestion by sodium hypochlorite (NaClO, 12.5%; Carlo Erba, Cornaredo, MI, Italy): for this analysis, 0.5 g of histological colorectal tissue was weighed through a technical balance (BC1000, OrmaEurotek, Milan, Italy), working with an instrumental error of $d = 1 \times 10^{-2}$ g, and the organic component was removed by digestion with 30 mL of NaClO, heated to about 60°C.

The obtained suspension was then filtered on polycarbonate filters (porosity of 0.2 µm and diameter of 25 mm) to collect the inorganic material. To reduce the precipitation of foreign material, *e.g.* NaCl, the solution was diluted with MilliQ water, heated to 60°C, during the filtration process. The obtained filters were then analyzed by SEM/EDS to obtain a chemical microanalysis of each individual observed particle and corpuscle. SEM is an analytical technique for sample observation at magnifications and resolutions thousands of times higher than ordinary optical microscopy. It provides morphological information, *e.g.*, shape, size, and arrangement of the particles on the surface layer, whereas EDS microprobe gives qualitatively and semi-quantitatively compositional information, determining the elements of which the sample is composed.[31-33] Nevertheless, the EDS does not always allow an unequivocal sample mineralogical determination due to their remarkably similar chemical composition (*e.g.* in the case of serpentine phases).

Filters were examined by a Quanta 200 ESEM (FEI Company, Hillsboro, OR, USA) instrument coupled with the EDAX (Mahwah, NJ, USA) EDS, in Variable Pressure (VP) conditions and acquiring imaging information by the Backscattered Electron (BSE) detector.

The experimental conditions were set as follows: pressure of 90 Pa, working distance of 10 mm and acceleration voltage of 20 kV. 600 microscopic fields were analyzed with a magnification of 5500X (corresponding to a total analyzed area of approximately 12 mm^2). The microscopic fields were displayed along 6 parallel horizontal stripes and the distance between the horizontal stripes was previously defined as indicated in the work of Belluso *et al.* (2006),[30] in order to fix the random distribution of the fibers and par-

Table 1. Chemical composition of different asbestos types.

Asbestos types	Chemical formula[13]
Actinolite	$Ca_2(Mg,Fe^{2+})_5[Si_8O_{22}](OH)_2$
Amosite	$(Fe^{2+},Mg)_7[Si_8O_{22}](OH)_2$
Anthophyllite	$(Mg,Fe^{2+})[Si_8O_{22}](OH)_2$
Crocidolite	$Na_2Fe_2^{3+}(Fe^{2+},Mg)_3[Si_8O_{22}](OH)_2$
Tremolite	$Ca_2Mg_5[Si_8O_{22}](OH)_2$
Chrysotile	$Mg_3[Si_2O_5](OH)_4$

ticles deriving from the digestion procedure. The backscattered images, characterized by a gray scale contrast produced by the samples, allowed an easy detection of the inorganic phases: in fact, the inorganic phases deposited on the polycarbonate filter are easily detectable because they appear brighter in the images due to their higher atomic number (Z).

On all inorganic fibers/particles, EDS spectra were collected and processed using GENESIS software, version 3.6.

For each analyzed particle the chemical composition was evaluated using the EDS probe which allowed to conduct various elemental microanalyses on the inorganic phases, both inside the observed foreign body (IN-analyses) and in the areas outside and close to the particles (OUT-analyses).

OUT-analyses allowed us to outline the composition of the fiber surrounding areas, which is mainly made up of carbon and oxygen. Typically, in the areas outside the fiber there are no chemical elements that can be included in the mineral phases under analysis (such as Si, Mg, Al, Fe, Na, Ca), but there are fundamental elements of the composition of the polycarbonate support on which the residual inorganic material adheres.

The spectra obtained from the OUT-analysis were compared to the IN-analysis values to obtain the effective elemental composition of the analyzed phase: in fact, it is possible that during washing NaCl precipitated near the particles and fibers and the collection of OUT analyses allowed to ascribe the presence of these elements to the preparation procedure and not to the inorganic phase itself.

Considering the operative conditions related to the not conductivity, not polishing of the samples, and VP operating condition, the obtained results must be considered only as qualitative information. The identification of the mineral phase was confirmed by comparison with the EDS spectra database acquired on pure samples of asbestos phases, analyzed in SEM/EDS under the experimental conditions mentioned above.[23,31,34-38]

RESULTS

In the present study, the results obtained from a cohort of seven patients affected by colon adenocarcinoma and living in an asbestos-polluted area are presented. In Figure 1, an example of elongated particle (about 4 µm in length and <1 µm in thickness, so not definable as "breathable fiber") is reported. This particle was observed in a healthy portion of colon tissue from patient #2.

The EDS analyses obtained from this particle, indicated the presence of Mg and Si (Figure 1b; in this spectrum Na and Cl peaks are due to the crystallization of NaCl from sodium hypochlorite solution). The ratio between the intensities of the peaks ascribed to magnesium and silicon is consistent with the chemical composition of a serpentine phase. Nevertheless, the morphology of the particle does not allow to distinguish between the two mineral elongated phases of this mineralogical group, chrysotile (that is regulated as "asbestos") and antigorite (not asbestos).[2]

In the neoplastic tissue of the same adenocarcinoma case, other elongated morphologies were observed (Figure 2a): these crystals appeared composed by microfibrils with diameter <1 µm and a length >10 µm.

In this particular morphology, analyzing the different points of the bundle of fibers, it was possible to observe the presence of Ca as main constituent (Figure 2b), especially in the thickest parts of this aggregate (point 1 in Figure 2a), whereas a higher abundance of magnesium and silicon was detected when the EDS spectra were acquired on the thinnest parts of the fibrils (point 2 in Figure 2a, EDS spectrum reported in Figure 2c). It may be due to a covering composed substantially of calcium, but the point must be further deepened. Nevertheless, the contained phase showed an EDS spectrum with Mg>Si peak intensities, which is ascribable to a serpentine phase also in this case.

It must be underlined that these morphologies were also observed in case #6 reported in this work and the chemical elements ascribable to the possible silicate phase were always magnesium and silicon.

In case #2, rounded morphologies with a micrometric diameter were observed in the healthy tissue, Figure 3a.

Addressing the electron beam onto these particles, the obtained EDS spectra showed that they are composed of calcium, phosphorus, and traces of magnesium: this chemical composition is qualitatively comparable with the chemical composition of calcium phosphates. It is interesting to highlight that similar morphologies were observed inside histological section of lung from patients affected by malignant pleural mesothelioma by Rinaudo *et al.*:[39] in that case, the

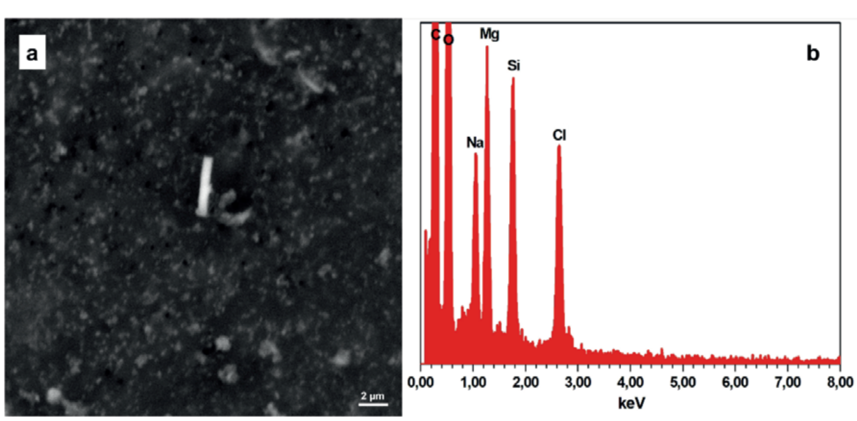

Figure 1. (a) Elongated particle observed in the healthy portion of colon tissue; (b) EDS analysis obtained on the fiber: the ratio of the intensities of the Mg and Si peaks are consistent with a serpentine phase (Na and Cl are ascribed to the crystallization of NaCl from sodium hypochlorite solution).

application of micro-Raman spectroscopy allowed to characterize the phases as apatite and β-tricalcium phosphate and, in future studies, application of this technique might allow to better define the mineral phase associated to these morphologies. In the case of lungs, these formations were observed in pleural thickening related to malignant mesothelioma and the calcium phosphate phase (mineralogical name "apatite") is involved in the asbestos body composition, together to the iron proteins (hemosiderin and ferritin).[40]

The same calcium phosphate rounded morphologies were observed also in cases #3, #4, and #5.

In addition to the asbestos identification, other particles and fibers were characterized on the collected filters. Based on the observed particles and fibers, it was possible to estimate the amounts of particles in the different types of analyzed tissues, these data are reported in Table 2). As it concerns the concentration of the different inorganic phases, in this case, the high number of particles did not allow a precise quantification, due to the optimization of the times and costs of the analyses, so a limited number of particles were analyzed, compensating the subjectivity of the analysis fixing the number and the localization of the point of analysis before image acquisition. As it can be seen in Table 2, not only silicate phases were observed, but also other phases: it is interesting to highlight the presence, in particular, of different types of metal-rich particles, among which the iron rich ones are the most represented. Moreover, Ti-rich phases were observed in all the studied cases. The provenience of these different particles might be further studied considering other patients' information (*e.g.* smoking, food preferences, other sources of exposures, living habits), that were not considered in this study, being focused on asbestos exposures.

CONCLUSIONS

Through the analysis of the samples and from the acquired data about the chemical composition of each phase obtained via SEM/EDS, it was possible both to confirm the presence of various inorganic phases in the analyzed tissues and to obtain a characterization of the particles and fibers from a morphological and chemical/mineralogical point of view.

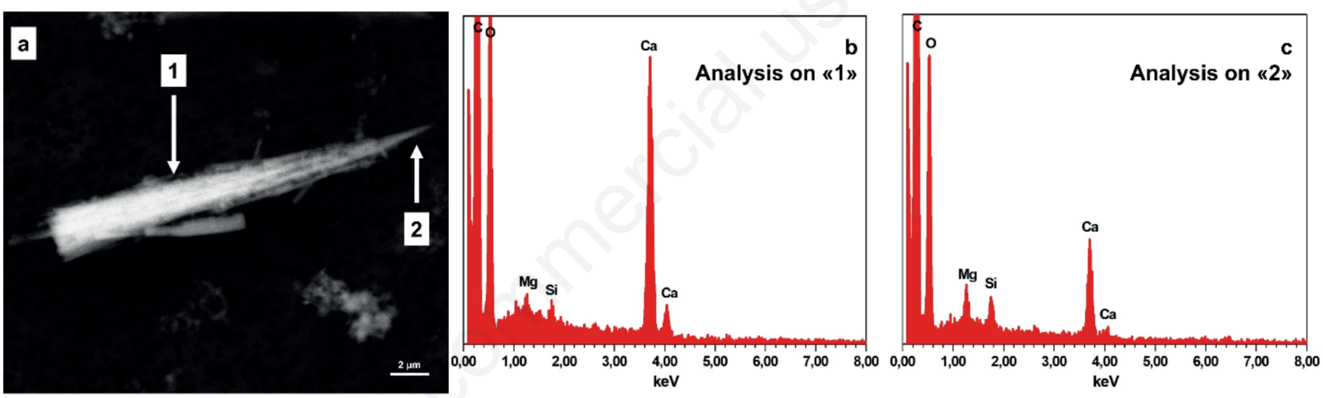

Figure 2. (a) Another characteristic morphology observed in the neoplastic tissue; (b) EDS spectrum obtained on the point indicated by "1" in Figure 2a; (c) EDS spectrum obtained on the point indicated by "2" in Figure 2a.

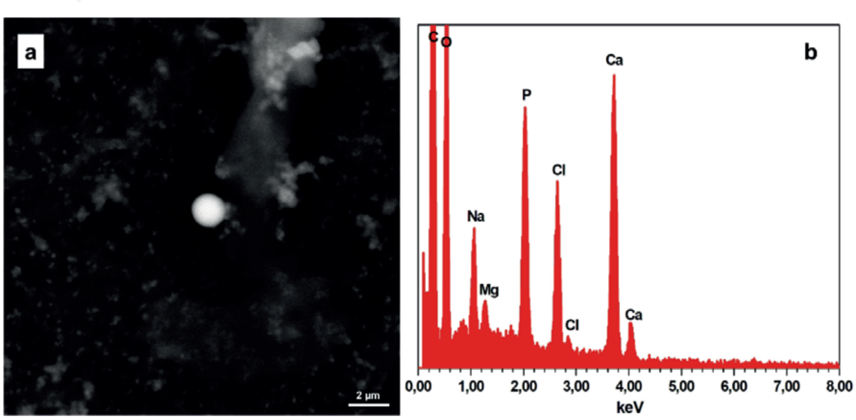

Figure 3. (a) Rounded morphology observed in the healthy tissue of case #2. (b) EDS spectrum obtained on the particle reported in Figure 3a. The chemical elements ascribed to the spherical particle are Ca, P, and traces of Mg; Na and Cl are due to the crystallization of NaCl from the solution used for tissue digestion.

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Table 2. Inorganic phases observed in the colon tissues analyzed. The number of "x" indicates the greater number of particles observed based on the number of analyzed particles (x<2; 2<xx<5; xxx>5).

	Case 1		Case 2			Case 3		Case 4			Case 5		Case 6			Case 7	
	Bridge	Neoplasia	Healthy	Bridge	Neoplasia	Healthy	Neoplasia	Healthy	Bridge	Neoplasia	Healthy	Neoplasia	Healthy	Bridge	Neoplasia	Healthy	Neoplasia
Possible asbestos phases					Sepentine inside Ca aggregate								xx	x		xx	
Other silicates						x			x	xx	xx	xx	xxx	x	xxx	xxxxx	xx
Iron phase				xx				xx	xx	xxxx	xxxx	xxxx	xx	xx	xxxx	xx	x
Titanium phases	xx		xx	xx		x			xx	xxxx		x	xxx		xxxx	xxx	xx
Fe particles	xx	xxx				xxx			xx	xxx	xx	xxx	xx	xx	xxx	xxx	xx
Ca particles		xx	x	x	xx		xx		x		xx	xx	xx			xx	
Al particles																	
Si particles				xx		x	x	x		x	x			x		xx	
Cr particles	xx																
Mg particles				x				xx					x				
Bi particles											x						
Ni particles													x		x		
PCa particles			x	x	x	x		x		x	x						x
PMg particles			x														
SBa particles													xx				
Zr particles													x				
CaMg particles													x		x		
CaMgTi particles															x		
CaMgAl particles																x	

In these samples, particles of composition not attributable to asbestos and elongated fibrous phases presenting the typical composition of "asbestos" have been observed. In the seven cases considered in this study, no "asbestos bodies" and no well-developed asbestos fibers, as those observed in the case reported in Rinaudo *et al*. (2021),[23] were observed. Being the presented cases the first part of a greater project, that considers a total number of 20 patients, it will be interesting to observe the differences among them and to relate the relative exposure information collected with the interviews. The data will be presented in a future publication.

Considering the inorganic content observed in the presented cases, Table 2 shows all the most meaningful results that have been obtained comprising those not reported in this paper. Also, these data will be evaluated with interviews related to the professional and life histories of the individual subjects in order to better define the possible origin of the other compounds.

This study is only an exploratory investigation since the limited number of samples analyzed is not sufficient to provide a scientific report able to definitively explain the mechanism that allows fibers or particles once inhaled (or ingested) to settle in the colorectal tract. The asbestos fibers identification is a first significant result, useful to understand in the future the mechanisms that they can establish with tissues.

Further studies are necessary to develop solid and statistically relevant investigations based on SEM/EDS. This technique could be a method in the forensic field for the identification of mineral phases and the obtained data can be used to prove a correlation between asbestiform fibers and the onset of asbestos-related diseases in those anatomical districts for which these relationships are still discussed.

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Authors' contributions: MB, supervision of the project, drafting and revision of the work; AC, design of the work, acquisition, analysis, and interpretation of data, drafting and revising the work critically for important intellectual content, image composition; SC, drafting and revising the work critically for important intellectual content; MA, surgical intervention and sample collection, medical support and drafting and revising of the work; EN, surgical intervention and sample collection, medical support and drafting and revising of the work; FG, supervision of the project; LC, acquisition, analysis, interpretation of the data, graphical support and revising of the work; CR, acquisition, analysis and interpretation of data, critically revising the work for important intellectual content; GG, support in data interpretation and presentation, revising of the work and scientifical support; AM, substantial contributions to the conception, design of the work, acquisition, analysis and interpretation of data, drafting and revising the work critically for important intellectual content. All the authors have read and approved the final version of the manuscript, and agreed to be held accountable for all aspects of the work.

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Ethics approval and consent to participate: The histological colon samples were collected during surgical procedures conducted by the General Surgery Unit of the Santo Spirito Hospital of Casale Monferrato as part of a study authorized by the Ethical Committee of SS. Antonio e Biagio e Cesare Arrigo Hospital of Alessandria after obtaining the patients' written informed consent.

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REFERENCES

1. Park EK, Takahashi K, Jiang Y, et al. Elimination of asbestos use and asbestos-related diseases: an unfinished story. Cancer Sci. 2012;103:1751-5.
2. Legge 257 del marzo 1992. Norme relative alla cessazione dell'impiego dell'amianto. S. Ord. alla G.U.N. 087 Serie Generale Parte Prima del 13/04/1992 Supplemento 064 del 13/04/1992 - Legge 27 marzo 1992, n. 257.
3. Decreto Ministeriale del 06/09/1994, Normative e metodologie tecniche di applicazione dell'art.6, comma 3, e dell'art.12, comma 2, della legge 27 marzo 1992, n. 257, relativa alla cessazione dell'impiego dell'amianto, GUI – Supplemento Ordinario n° 220.
4. Decreto legislativo n° 277 del 15 agosto 1991, 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'articolo 7 della legge 30 luglio 1990, n° 212.
5. Decreto Legislativo 152/2006, Norme in materia ambientale, GUI n° 88.
6. Decreto Legislativo n° 81 del 9 aprile 2008, Attuazione dell'articolo 1 della legge 3 agosto 2007, n. 123, in materia di tutela della salute e della sicurezza nei luoghi di lavoro, G.U. n° 101 del 30 aprile 2008.
7. Directive 2003/18/EC (2003) of the European Parliament and of the Council of 27 March 2003 amending Council Directive 83/477/EEC on the protection of workers from the risks related to exposure to asbestos at work, 2003, OJ L 97, 48–52.
8. Di Maggio R, Barone P, Pettinelli E, et al. Geologia forense. Introduzione alle geoscienze applicate alle indagini giudiziarie. Palermo, Italy: Dario Flaccovio Editore; 2013. 465 pp.
9. Hawthorne FC, Oberti R, Della Ventura G, Mottana A. Amphiboles: crystal chemistry, occurrence, and health issues. In: Reviews in Mineralogy and Geochemistry (Rosso JJ ed.) vol. 67; Chantilly, VA, USA: Mineralogical Society of America; 2007. 545 pp.
10. Palmieri EL, Parotto M. Il globo terrestre e la sua evoluzione. 2nd ed. Bologna, Italy: Zanichelli; 2017.
11. Pauling L. The nature of the chemical bond and the structure of molecules and crystals. An introduction to modern structural chemistry. Ithaca, NY, USA: Cornell Univ Press: 1960. 664 pp.
12. Putnis A. Introduction to mineral sciences. Cambridge, UK: Cambridge University Press; 1992. 480 pp.
13. Deer WA, Howie RA, Zussman J. Introduzione ai minerali che costituiscono le rocce. Bologna, Italy: Zanichelli; 1994. 680 pp.
14. World Health Organization (WHO). Environmental Health Criteria 53 – Asbestos and other natural mineral fibres, International programme for chemical safety. Geneva, Switzerland: WHO; 1986. Available from: https://apps.who.int/iris/bitstream/handle/10665/37190/9241541938-eng.pdf
15. Solbes E, Harper RW. Biological responses to asbestos inhalation and pathogenesis of asbestos-related benign and malignant disease. J. Investig. Med. 2018;66:721-7.
16. International Agency for Research on Cancer (IARC). Asbestos (chrysotile, amosite, crocidolite, tremolite, actinolite, and anthophyllite). In IARC monographs on the evaluation of carcinogenic risks to humans, Volume 100C; Lyon, France: IARC; 2012; pp. 219–309.
17. Fazzo L, Binazzi A, Ferrante D, et al. Burden of mortality from asbestos-related diseases in Italy. Int. J. Environ. Res. Public Health. 2021;18:10012.
18. Wadowski B, De Rienzo A, Bueno R. The molecular basis of malignant pleural mesothelioma. Thorac. Surg. Clin. 2020;30:383-93.

19. Wilk E, Krówczyńska M. Malignant mesothelioma and asbestos exposure in Europe: evidence of spatial clustering. Geospat. Health. 2021;16:1.
20. Kwak K, Kang D, Paek D. Environmental exposure to asbestos and the risk of lung cancer: a systematic review and meta-analysis. Occup. Environ. Med. 2022;79:207-14.
21. Nowak D, Schmalfeldt B, Tannapfel A, Mahner S. Asbestos exposure and ovarian cancer - a gynaecological occupational disease. Background, mandatory notification, practical Approach. Geburtshilfe Frauenheilkd. 2021;81:555-61.
22. van Zandwijk N, Reid G, Frank AL. Asbestos-related cancers: the 'Hidden Killer' remains a global threat. Expert Rev. Anticancer Ther. 2020;20:271-8.
23. 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.
24. Ehrlich A, Rohl AN, Holstein EC. Asbestos bodies in carcinoma of colon in an insulation worker with asbestosis. JAMA 1985;254:2932–3.
25. Ehrlich A, Gordon RE, Dikman SH. Carcinoma of the colon in asbestos-exposed workers: Analysis of asbestos content in colon tissue. Am. J. Ind. Med. 1991;19:629–36.
26. Kobayashi H, Ming ZW, Watanabe H, et al. A quantitative study on the distribution of asbestos bodies in extrapulmonary organs. Acta Pathol. Jpn. 1987;37:375–83.
27. Gamble JF. Asbestos and colon cancer: A weight-of-the-evidence review. Environ. Health Perspect. 1994;102:1038–50.
28. Barbatelli G, Bertoni L, Boccafoschi F, et al. Anatomia umana. Fondamenti con istituzioni di istologia. Milano, Italy: Edi. Ermes; 2018. 976 pp.
29. Binazzi A, Marinaccio A, Corfiati M, et al. Mesothelioma incidence and asbestos exposure in Italian national priority contaminated sites. Scand. J. Work Environ. Health. 2017;43:550-9.
30. 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.
31. Croce A, Rinaudo C. Appunti per biologi su cristalli e minerali. Metodi non distruttivi per la loro identificazione. Roma, Italy: Tab Edizioni; 2020. 168 pp.
32. Goldstein JI, Newbury DE, Echlin P, et al. Scanning electron microscopy and X-ray microanalysis. 3rd ed. New York, NY, USA: Springer; 2017. 708 pp.
33. Welton JE. SEM petrology atlas. Tulsa, OK., USA: The American Association of Petroleum Geologists; 1984.
34. 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. 2019;105:404-10.
35. Croce A, Capella S, Belluso E, et al. Asbestos fibre burden in gallbladder: A case study. Micron. 2018;105:98-104.
36. Grosso F, Croce A, Trincheri 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.
37. Grosso F, Randi L, Croce A, et al. Asbestos fibers in the gallbladder of patients affected by benign biliary tract diseases, Eur. J. Gastroenterol. Hepatol. 2015;27:860-4.
38. Croce A, Musa M, Allegrina M, et al. Environmental scanning electron microscopy technique to identify asbestos phases inside ferruginous bodies. Microsc. Microanal. 2013;19: 420-4.
39. Rinaudo C, Croce A, Musa M, et al. Study of inorganic particles, fibers, and asbestos bodies by variable pressure scanning electron microscopy with annexed energy dispersive spectroscopy and micro-Raman spectroscopy in thin sections of lung and pleural plaque. Appl. Spectrosc. 2010;64:571-7.
40. Jaurand MC, Renier A, Daubriac J. Mesothelioma: do asbestos and carbon nanotubes pose the same health risk? Part. Fibre Toxicol. 2009;6:16.