

# Asbestos and onset of mesothelioma: case report

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## Summary

Asbestos is a mineral belonging to the group of fibrous silicates. Due to its remarkable resistance to high temperatures, its conformation in fibers and its very low cost, it was used until 1991 to produce materials able of withstanding high temperatures. The silicates of asbestos in the solid matrix were widely used in construction, in engineering, in plumbing, in panels and sheets for ceilings, in plaster and as sound-absorbing material. As-

bestos was also widely used in the manufacturing of household equipment, such as thermal insulation for high and low temperatures, flame retardant ducts, for the production of appliances with use of high temperatures (stoves, hair dryer, etc.), in the clutches brakes, in the baffle plate display screens in the seals, in the production of clothing protecting from heat (gloves, fireproof suits) and in furnishing resistant to elevated temperatures. The exposure to atmosphere containing asbestos can be the cause of several diseases such as pulmonary asbestosis, mesothelioma and lung cancer. The amphiboles are the most dangerous forms of asbestos, and the crocidolite among these is the one with the most neoplastic potential. However, the diagnosis of asbestos related occupational diseases is neither simple nor automatic, as a number of criteria are to be met, such as the study of immunohistochemical markers, and of biological and environmental monitoring, to assess an effective causal relationship. The knowledge about asbestos-related diseases and the diagnostic criteria have evolved over time, but it is still difficult to examine patients who have experienced clinically disease only in recent times, after 10-20 years from the last assumed exposure, due to the latency of the disease, particularly cancer. We present here two case reports of malignant mesothelioma, of assumed asbestos-related occupational origin: their aetiology is debated in the light of literary overview. We analyzed all scientific knowledge about the relationship between human health and exposure to asbestos, since the beginning of its industrial use to the present day. The leading scientific databases (Pubmed, Cochran, Embase, Tripdatabase) were surveyed to reconstruct how and when knowledge on the health hazards of asbestos exposure were published: a total of 13,551 publications were scrutinized. The key topics surveyed were: "*Asbestos and health damage*", "*Asbestos and Malignant Mesothelioma*". The findings learned through our research were applied to the two cases under examination. We found that diagnosis of the malignant mesothelioma attributed to the two patients is not certain because the diagnostic criteria suggested by international literature have not been strictly applied, both from the clinical point of view and from the histopathological and immuno-chemical level.

**KEY WORDS:** *asbestos, mesothelioma, diagnostic criteria.*

## Background

Asbestos is a mineral belonging to the group of fibrous silicates. Asbestos fibers can be found free or weakly bound to each other (friable asbestos) or strongly bound to each other in the case of asbestos in compact matrix. Due to its remarkable resistance to high temperatures, its conformation in fibers and its very low cost, it was used until in Italy 1991 to produce materials able of withstanding high temperatures. The silicates of asbestos in the solid matrix were widely used in construction, in resistant to heat plant engineering, in plumbing for aqueducts and sewers, in panels and sheets for ceilings, in plaster and as sound-absorbing material. It was also widely used in the manufacturing industry for its sound-absorbing characteristics and for the production of manufactured articles and household equipment, such as thermal insulation for high and low temperatures, flame retardant ducts, for the production of appliances with use of high temperatures (stoves, hair dryer, etc.), in the clutches brakes, in the baffle plate display screens in the seals, in the production of clothing protecting from heat (gloves, fireproof suits) and in furnishing resistant to elevated temperatures. The exposure to atmospheres containing asbestos can be the cause of several diseases such as pulmonary asbestosis, mesothelioma and lung cancer. The amphiboles are the most dangerous forms of asbestos and the crocidolite is the one with the most neoplastic potential. The inhalation of even a single asbestos fiber may induce the onset of malignant mesothelioma or other forms of lung cancer. Differently from other substances and dangerous preparations, there is no risk threshold below which the concentration of asbestos fibers in inspired air may be considered safe. Obviously prolonged exposure to asbestos fibers, or inhalation of large amounts, increases exponentially the chances of developing asbestos-related malignancies. Before the eighties, the risk of exposure to asbestos was only considered work-related, but today asbestos is considered as an environmental pollutant. In 1992 legislative decree 257 banned in Italy the use and the processing of asbestos and it gave the standards for decontamination of the areas affected by asbestos pollution.

## Case report

We report here two cases of pleural mesothelioma. Case 1: Male, born in 1956, worked for 18 years on several ships as non-commissioned officer. In 2004 he underwent surgery for right pneumonectomy with partial pericardial and right hemidiaphragm resection and subsequent reconstruction through Goretex implants, followed by successive cycles of radiotherapy. In 2005 the patient presented with "anorexia, worsening dyspnea, and was operated again for mesothelioma, with laterocervical lymphadenopathy". The histopathological examination of biopsy material from laterocervical lymph node was so reported: "localization of malignancy with morphological features consistent with mesothelioma". Subsequently a right pleural effusion was found. After

thoracentesis and cytologic evaluation of pleural fluid a pleural mesothelioma was once again confirmed. The definitive histological examination gives evidence of biphasic malignant mesothelioma infiltrating the lung, with lymph node micro metastasis pT3N1MX .

The patient's condition worsened, with the appearance of severe dyspnea and congestive cardio-circulatory followed by death on September 2005. From the autopsy: "The right parietal pleura is in part thickened and in part replaced by implants. Absence of the right lung. On the left lung the presence of tenacious adhesions is found. The left pleura appears thickened because of the presence of a neoformation of lipidic consistency, that presents in section large necrotic areas; the above lesion expatiates infiltrating diffusely chest wall, left lung, pericardium, diaphragm, up to the falciform ligament and retroperitoneum and incorporating the mediastinal organs (esophagus and trachea). Multiple samples of the lipidic neoformation have been withdrawn in the pleura, the pericardium and the left lung".

From the medical report of the histopathological examination of pleuro-pulmonary biopsy material taken during autopsy: "Fairly cellular neoformation interesting massively visceral and parietal pleura with invasion by contiguity of the soft tissues of the chest wall and the lung parenchyma and interesting diffusely pericardium with infiltration by contiguity of the subepicardial myocardium and aspects of vascular invasion. The neoformation is mainly composed of atypical elements variously twisted and arranged in corrugated, swirling structures, and included in a fibrous stroma. We proceeded to set up preparations of neoplastic tissue treated with immunochemical techniques, in order to highlight the possible mesothelial origin of the tumor".

The following results have been achieved (Tab. 1):

The data reported are not correctly interpretable as the interpretation key of the symbols -, +, +/- was not provided and, therefore, it is not possible to understand the level of cut-off applied. In fact, as we are going to describe in detail the reactivity scales for the interpretation of the results is usually indicated.

Case 2: Mr. C, a former employee of the Navy, was originally commissioned officer and, as such, embarked from 1959 to 1995. At the age of 59 years, he was hospitalized in the emergency department for the appearance of dyspnea and pain in the left hemithorax. He was discharged with a diagnosis of left pleurobroncopneumonia. In the discharge letter we can read:

**Table 1 - Results of Case 1 neoplastic markers.**

Marker	Result
CK7	+
CK20	-
CK-AE1	+
CK-AE3	+/-
Calretinin	+
CEA	-

"(...) Today we discharge (...) hospitalized in our Division from 17.03.2000 and found affected by left pleuropneumonia. At the entrance the chest X-ray showed extensive pleuro-parenchymal thickening of the lower half of the left lung field. By thoracentesis 1600 cc of purulent exudate liquid were extracted. On this material cytological and bacteriological research for cancer cells was negative. Bronchoscopy was negative as regards direct or indirect signs of neoplastic disease. (...) a detailed diagnostic investigation by thoraco-abdominal CT scan should also be carried out, since other confounding issues can not be completely excluded". Also, from the microbiological exam report: "Material: pleural fluid; Microscopic examination: nothing significant. Bacterial culture (...) no growth (...)". Subsequently, the patient was submitted to multiple biopsies by guided video thoracoscopy in the histological report we read: "the histological and ICH finding directs to mesothelioma, however, it has to be confirmed by appropriate clinical investigations". The patient was treated with chemotherapy, in the same department, he underwent a surgery left pleuropneumectomy with resection of the diaphragm and placement of pericardial and diaphragmatic Gore-Tex patch.

The histological report shows: "(...) Monomorphic epithelial pleural mesothelioma, multinodular, infiltrating adipose tissue subpleural with endolymphatic invasion extended to the pericardium and the peri arteriosus tissue. Sections of hilar bronchi without neoplastic infiltration. Reactive lymphadenitis with anthracosis without metastasis in the lymph node taken and sent separately. Skin flap covered with orthokeratotic skin without cancer sites (...)".

Due to the onset of severe dyspnea with fever, fatigue and worsening chest pain at the site of previous surgery, the patient was readmitted, and, after a short hospitalization for cardio-circulatory failure and supraventricular tachyarrhythmia, he died. In this case it is not possible to carry out a detailed analysis of the type and even of the markers used, which are, however, mentioned in a generic manner, as "guiding" for mesothelioma and, as such, do not comply with the international standards required under a proper medico-legal assessment.

### **The diagnostic quality, i.e. the observation of criteria useful for diagnosis**

The diagnosis of mesothelioma is the result of a process today relatively standardized. The radiological picture in the pleural localization is characterized by the lobulated profile (referable to the tumor mass where this has reached appreciable size) which assumes the profile of the chest wall for the presence of so-called "mamelons". The CT is a useful aid, able to provide a better iconographic definition compared to the standard radiogram. Far from being diriment under the point of view of the diagnosis, especially the differential diagnosis with pleural metastasis of other cancers, it is now considered a valid means to follow the evolution of the disease and the cancer staging. After the thoracentesis,

in cases of pleural effusion it is essential to proceed as early as possible with the radiographic examination to bring out those images, previously veiled by the effusion itself. The cytology on the fluid effusion may provide elements for the diagnostic suspicion (and not the certainty), due to the attitude of the metastatic cells of tumors in other locations to take a mesoteliomatosus look. The examination of the liquid has, however, assumed a greater weight for the development of the cytochemistry and the immunocytochemistry. Basing on the presence of specific enzymes or antigens they can allow to direct the diagnostic suspicion towards mesothelioma or metastatic forms (1). The diagnosis of pleural mesothelioma is based, by common consent, on the histological examination of a targeted biopsy obtainable by pleuroscopy or surgical specimen as a result of possible thoracotomy. The definition of the tissutal origin of the tumor in question, in particular by specific immunohistochemical markers, is fundamental. Nowadays, several tumor markers have been identified, which allow the differential diagnosis with other tumors, in particular bronchial adenocarcinoma (for contiguity) and renal tumors (for distant metastases). It's yet important to bear in mind that not all authors agree on the validity and the preferential choice of the various markers. The complexity of the diagnosis of malignant pleural mesothelioma (MPM) comes from the fact that, almost all the tumors with the only exception of brain tumors, can give pleural metastases (2). It should be noted however that recent data show that in the United States the ratio pleural metastasis / mesothelioma is 50:1. Based on proven scientific evidence, recent French guidelines on the MPM (3) claim that:

- it is not recommended to base the diagnosis of MPM on clinical criteria or on chest X-ray or on analysis of pleural fluid;
- it is recommended for the diagnosis of MPM the execution of a thoracoscopy with pleural biopsies;
- it is always recommended to base the diagnosis of MPM on immunohistochemical investigation;
- it is recommended to use at least two immunohistochemical markers with diagnostic value positive for MPM and two markers with diagnostic value negative for MPM.

As to immunohistochemical markers, substantially the same conclusions have been reached by the most recent literature reviews (4, 5): in any case it is still worth remembering that, despite the pathologists agree that immunohistochemistry is essential for the diagnosis of MPM, there are disputes on the specific value of certain markers and on the choice of markers to be included in the diagnostic battery. The main difficulties persist in the differential diagnosis between MPM and lung cancer (both adenocarcinoma and squamous cell carcinoma). Over the past decade, we have had an increasing availability of antibodies directed against antigens present in mesothelioma more than in other pleural diseases (marker "positive" or "mesothelial").

In the case of certain positive and negative marker, it is not only the frequency of expression that varies according to the nature of the pleural pathology, but also the mode, or "pattern", with which the positive reac-

tions are distributed within the cell (for example cytoplasmic, nuclear or membrane pattern). In the case, then, of a third category of markers classified by Ordoñez (6) as “miscellaneous”, the pattern of positivization constitutes the essential element of discrimination between the different pleural diseases. It should also be remembered that the same immunohistochemical findings can be read in different ways depending on the cut-off for positivity that has been chosen. Thus, there are authors who make a judgment of positiveness when reactive cells account for 1% or 10%, or 30% of the total. “Scale” of reactivity for the interpretation of the results have also been drawn up. Ordoñez, for example, distinguishes between the following degrees of positivity: ±, when there are cells expressing the marker in percentage less than 1% of the total; +, when the percentage is comprised between 1% and 25%; ++, between 26% and 50%; + + +, between 51% and 75%; + + + +, above 75%.

In any case, despite the high number of markers tested in the course of time, so far none of them has been identified to have any sensitivity and absolute specificity, or nearly absolute, for mesothelioma. The immunohistochemical recognition of this disease, therefore, is carried out with a standard of probability, evaluating the overall results obtained with batteries, or “panel”, of many markers. The composition of the panel is determined by the differential-diagnostic needs, choosing the marker considered most discriminating between the two compared conditions. Over the last decade combinations of positive and negative markers have been proposed, a solution that allows the detection of any suspected mesothelioma on the basis of evidence both affirmative or negative.

The usefulness of a panel is obviously in relation with the level of sensitivity and specificity of the markers that are part of it. In some studies, mostly dating back to the nineties, it was claimed that the panels consisting of two markers carefully chosen are sufficient to distinguish clearly the epithelial mesothelioma from the lung adenocarcinoma, and that their discriminatory power is not significantly increased by the addition of other elements. The most recent literature, however, generally suggests to include in the panel no less than four markers, divided in positive and negative ones, assuming that there is a direct relationship between the diagnostic yield of a panel and the number of its markers. In summary, according to Ordoñez (7):

**a. Differential diagnosis between epithelial MPM and lung adenocarcinoma**

- Top markers positive for mesothelioma = calretinin, Keratin 5/6, podoplanin, WT1
- Top markers positive for adenocarcinoma = MOC-31, Ber-EP4, B72.3, CEA, BG-8, TTF-1

**b. Differential diagnosis between epithelial MPM and lung squamous cell carcinoma**

- Top markers positive for mesothelioma = calretinin, Keratin 5/6, podoplanin, WT1
- Top markers positive for squamous cell carcinoma = MOC-31, Ber-EP4, CEA, BG-8, p63

**c. Differential diagnosis between epithelial MPM and renal carcinoma**

- Top markers positive for mesothelioma = calretinin, Keratin 5/6, podoplanin, WT1
- Top markers positive for renal carcinoma = leu-M1 (CD15), RCC Ma

According to Ordoñez (7), for the differential diagnosis between epithelial MPM and squamous cell lung carcinoma, the best combination is the one with two markers positive for MPM (WT1 and calretinin or citokeratin 5/6) with two negative markers (p63 and MOC-31). Still, according to Ordoñez (7), for the differential diagnosis between epithelial MPM and lung adenocarcinoma, the best combination is the one with two markers positive for MPM (WT1 and calretinin or citokeratin 5/6) with two negative markers (CEA e MOC-31 o B72.3, Ber-EP4, BG-8). In the present cases it can be argued that further laboratory analysis are absolutely necessary to document with the necessary diagnostic certainty the mesothelial matrix of the tumors found, since, in the case of Mr. C, the use of the markers is not defined in detail, while in the case 1, on the basis of the summary table, it appears that the positivity of some specific markers is less than 1%.

**Latency, or the period between the first exposure and the onset of the disease**

The available data on the trends of the incidence show that the risk of onset of mesothelioma increases since the moment of the beginning of exposure, regardless of whether or not the exposure has stopped because unlike chemical carcinogens, asbestos fibers, especially the amphiboles persist indefinitely in the tissues (8). In adequately documented studies the average latency is between 35 and 40 years. Latency periods lower than 20 years are exceptional (9). All tumors caused or jointly caused by known factors appear after a long latency period of years: mesothelioma, compared with these other tumors, is characterized by the longest latency. The latency corresponds to the duration of the pathogenic mechanism of the tumor: the process by which the inhalation of asbestos fibers triggers the mechanism of development of mesothelioma starts immediately after the beginning of the exposure. The model of incidence of mesothelioma developed in epidemiology recognizes the overwhelming importance of the latency after exposure that would dramatically affect the incidence while the dose of the exposure would have only a linear function. This is the model of Peto ( $p = b \cdot t^k$ ), where: p is the incidence of any year; b is a constant, k an exponent ranging between 3 and 4), reanalyzed by Boffetta (10)  $I_m(t) = K_m \cdot E \cdot (t - t_0)^b$ , where:

$I_m(t)$  is the incidence of mesothelioma;

t years after the beginning of exposure;

E is the average exposure expressed in ff / cc;

$K_m$  is a constant that expresses the carcinogenic power on the pleura, which is specific to the type of industry and type of asbestos fiber;

$t_0$  is the minimum required latency to observe an increase of mesothelioma;

$\beta$  is an exponential weighting factor related to the latency, which can also be specific for the specific cohort; the  $\beta$  value is estimated about 3, the  $t_0$  value about 10 years (10) which, substantially, refer to the dose as a factor linearly correlated to the incidence of the mesothelioma, while the latency is exponentially related.

The concept of dose should however be interpreted not so much as the effect of cumulative dose, but as a result of a critical dose, also highly concentrated in time, able to determine the passage of a certain number of fibers to the parietal pleura. This assumption is supported by the data of an epidemiological survey and by the subsequent follow-up related to a cohort of textile workers, recently published (11, 12), which shows that the relative risk for mesothelioma does not change in the groups exposed for less than a year compared with those who have been exposed for twenty years or more, while the risk increases considerably with the latency after the beginning of exposure. The cumulative dose can be defined, ideally, through the computation of the total lung load of fiber / asbestos corpuscles per unit of lung tissue mass, or by means of an evaluation of the level of environmental exposure, which in turn can be measured or estimated. In the ideal condition the objective assessment of pulmonary load produced summarizes all the aspects of qualitative and quantitative past. In the second case the evaluation can still have its objectivity if it is the result of the summation of situations of exposures measured over time. However, when these data are missing (in the vast majority of cases published in the literature, especially for remote exposures), an "estimate" based on job / exposure matrices is only applied. Sometimes it is possible a "quantitative" estimate, which tends to apply to the reality in question the objective data available, for other working similar contexts. Other times it is only possible to carry out semi-quantitative estimates, such as: high, medium, low exposure, which are not based on measurements. Obviously, the progressively less reliable gradient in the definition of cumulative exposure depending on the approach used is obvious. The above estimates may also be affected by the methodological choices of the authors. For example, in the study by Hansen (13) on a cohort of residents in areas near the crocidolite mine of Wittenoom in Australia, the possible domestic exposure for cohabitation with workers in the mine was not considered. The authors justify their choice with a number of reasonings, but considering that 24 of the 27 cases of mesothelioma examined lived in the same house for workers, it is difficult to admit that the domestic exposure did not lead a more important exposure than the environmental one, which is instead the only one taken into account by the authors.

In Iwatsubo et al.'s research (14), a case-control, the different classes of exposure, from which the authors derive the conclusion of a significant excess of mesothelioma for increasing levels of "cumulative exposure", are in fact "constructed" retrospectively on the basis of matrices job / exposure without the availability of any environmental

measure. This does not prevent authors from "proposing" "quantitative" levels of cumulative exposure, levels that furthermore, as a precautionary measure, the authors themselves indicate in quotation marks, indicating that these values are defined on the basis of subjective evaluations of a pool of experts, in the absence of any objective measure levels of environmental concentrations of asbestos. The cumulative dose, however assessed, can of course derive both from a high exposure concentrated in a narrow span of time, or from a less important exposure prolonged in time. There is no way to distinguish in the lung tissue what proportion is attributable to a remote exposure and what to a recent exposure, especially in the case of amphiboles. Therefore the assessment of pulmonary loads received from the lung over time can only be the result of environmental measures carried out during the lifetime of a professionally exposed worker. As exemplified this condition is difficult to be found in the literary data and, in epidemiological studies, it's applied only in cohort studies, where data on exposure conditions during the different periods of employment of the exposed persons are available. If such data are available, in epidemiological studies, it's usual to proceed to the "construction" of different "exposure categories" obtained by integrating the environmental concentrations of fibers in different periods with the duration of exposure equal to those specific levels. For example, a cumulative exposure of 30 fibers / years could be the result of an exposure for 1 year of 30 ff / cc, or of an exposure for 3 years at a level of 10 ks / cc, of 10 years at a level of 3 ff / cc, or still of 30 years at a level of 1f/cc. We still want to point out what Richard Doll and Peto Julian claimed (15) in their monography "Asbestos. Effects on health of exposure to asbestos". About their model on estimate of the incidence of mesothelioma in relation to the latency they write: "The estimate of risk increases roughly in proportion to the duration of exposure up to about 10 years but much weakly subsequently and there is only a small difference among the expected effects if the exposure stops or continues after 20 years".

One of the major contributions to the development of knowledge of the time of latency parameter is provided by the work of Lanphear and Buncher (16) who examined 21 case studies documenting a total of 1690 cases of mesothelioma. 99% of these had a latency period longer than 15 years, and 96% had a latency period of at least 20 years. This observation indicates that the minimum latency to be considered is 20 years and that cases with latency between 15 and 20 years are unusual. Even the Italian Mesothelioma Registry data indicate periods of very long latency: in fact, in the recent publication of Marinaccio et al. (17) the average latency 2544 cases registered in Italy from 1993 to 2001 was 44.6 years. The work that follows has the essential aim of connecting the large mass of scientific information that has been produced worldwide about asbestos and its effects on human health. Crucial knowledge in the medical-legal has been realized internationally, because of the many causes of recognition of damage to person. Our work wants to provide an update on many aspects of this delicate issue.

## Materials and Methods

To assess, and analyze, all scientific knowledge about the relationship between human health and asbestos, produced since the beginning of its industrial use to the present day, we consulted the literature network by search engines with the use of keywords. A search in the world leading scientific databases (Pubmed, Cochrane, Embase, Tripdatabase) identified a total of 13.551 publications. We reconstructed how and when the knowledge of the diseases related to asbestos exposure has developed.

## Results

The number of surveyed studies is less than ten until 1964, about one hundred from 1972 to 74, about 150 until 1978. Since 1982 there has been a growing interest with an average production per year of about 350 scientific papers. There are two peaks, in 1982 and 2001 respectively, with 430 and 562 publications. Through these works the human health effects following exposure to asbestos were identified and defined:

- diffuse interstitial fibrosis or parenchymal asbestosis;
- non-malignant disorders of the pleura or pleural asbestosis (thickening, plaque deposits);
- skin lesions (warts) on hands and forearms.

## Asbestosis

Parenchymal Asbestosis is a chronic progressive, irreversible pneumoconiosis, characterized by a linear diffuse interstitial pulmonary fibrosis, resulting in prolonged inhalation of asbestos fibers. It is usually related to the duration and the significant levels of exposure. Asbestosis impairs organ function, is irreversible and has a tendency to evolve even after and of the exposure. This ability of progression is likely due to the persistence of biological activity of the fibers held in the lung and of immune phenomena. It is a diffused lung disease that results in the formation of fibrous tissue in the areas delegated to the gas exchange and that may reduce the oxygen from breathed air into the blood. The diagnosis is at least 10-15 years after the first exposure, although symptoms can occur even before in case of particularly intense exposure. The earlier and the most common symptom is the dyspnoea on exertion that increases with the worsening and broadening of parenchymal fibrosis.

## Pleural plaques

They represent a late sign of exposure to asbestos, as they appear at least 20 years after the first exposure. The plates are small areas of fibrous thickening, often located in the lower part of the rib cage, in the posterolateral region and in the diaphragmatic dome. They are generally asymptomatic, they do not lead, unless they are very large, to changes in the lung function and they may calcify.

## Benign asbestos pleuritis

Pleural effusion is a benign pathological event relatively uncommon in clinical practice, although it is considered to be the earliest pleural manifestation due to asbestos, as it can also occur after a few years after exposure.

## Rounded atelectasis

The rounded atelectasis is a benign lesion, localized in sub-pleural site, with usual nodular radiological appearance. It may be associated with exposure to asbestos as a result of recurrent benign pleural effusions, followed by an invagination of the visceral pleura with the collapse of the surrounding tissue, or it may result from an area of visceral pleural fibrosis exercising traction on the near lung parenchyma. Through these works all aspects of carcinogenesis by asbestos are identified and defined. Here a brief history of the developing of knowledge. The number of printed research surveyed in worldwide data banks is around or below ten from 1960 to 1972 with two exceptions: for 1966 and 1969 respectively with 18 and 27 articles. Between 1974 and 1979 there are about 40 or so per year, while the growth of interest began in 1980 with a total of 86 studies. From 1990 to 2004 scientific interest and production will definitely raise to touch a record in the years 2004 to 2007. The first report of international mesothelioma is by Wagner in 1960 (18), on a highly specialized Anglo-Saxon journal. He describes 33 cases of mesothelioma in patients who had probable exposure to blue asbestos in the Griqualand West in Asbestos Mountains. Wagner himself (19), reported in 1993 that after the publication of his first 33 cases a dispute arose in the first place about the diagnostic appearance and secondly about the hypothesis of an association of mesothelioma with a genetic factor rather than with the exposure to crocidolite (actually a genetic susceptibility was subsequently detected) (20). Only after the first report of Wagner, of the diversity of biological effects raised at the international level as well as the issue of the different power in the determination of mesothelioma by the different types of asbestos fibers, i.e. the amphiboles that is crocidolite belongs to and the coil that is chrysotile. Wagner, at the time of the first report had found cases of mesothelioma in workers from the district placed west of Kimberley, where crocidolite was mined, but not east of Kimberley where amosite and chrysotile were extracted (21). Hence, the authors did not have elements to believe chrysotile and amosite etiologic agents of mesothelioma. Only later Sluis-Cremer et al. (22) observed, 7.8 cases in subjects exposed to amosite, with an incidence of 44.6 cases of mesothelioma per 100,000 persons / year exposed to crocidolite, and realized concluding that crocidolite had a toxicity for mesothelioma much higher than amosite. They also observed the relatively low risk of mesothelioma in workers with amosite. There can be no doubt that crocidolite is far more dangerous than amosite, at least for mesothe-

lioma. So at the time of the first observations – and until 1980 – crocidolite, namely the blue asbestos from South Africa, was the kind of asbestos related to the onset of mesothelioma (23). After the publication, in December 1965, of the acts of conference held in 1964 on “Biological effects of Asbestos”, the International Union Against Cancer (UICC) (24), a non-governmental organization leader in the global control of cancer, recommended to continue the investigations on the association between asbestos and cancer (25). The UICC program itself shows that knowledge of that time required further investigation and, in any case, we were far from having elements that could indicate rules of practical behaviour in the presence of low doses of asbestos fibers other than crocidolite.

Not all the specialists who had dealt with these problems, using different techniques (tests on animals, inhalation, intratracheal, subcutaneous, intraperitoneal or pleural direct administration, epidemiological investigations substantiated or not substantiated by quantitative data on the entity of exposure) agreed on some of the data, in particular the kinds of asbestos and the intrapulmonary deposition of the different kinds of fibers as to the size, length, diameter and shape of the fibers themselves, as well as the assessment of the intensity of environmental exposure. With regard to the quantitative assessment of the effects of the exposure to asbestos, more recently, in 1998, Boffetta (10), on the basis of the known data of duration of exposure, of the type of asbestos and of the environmental concentration assessed, as already said, the carcinogenic power of the different types of asbestos used in the different fields of activity. Considering carcinogenic power equal to 1 for exposure to mixtures of chrysotile asbestos at 98% and of crocidolite at 2% in the textile, he calculated a power equal to 1.5 for exposure to mixtures of chrysotile 60%, amosite 40% in the field of insulation, a power equal to 3.2 in the production of manufactured at amosite 100% and a power equal to 12 in the cement industry of asbestos with a mixtures of chrysotile 89%, crocidolite 10%, amosite 1%. In these jobs environmental concentrations of airborne fibers ranged from 9 to 20 fibers / ml. Let's say something about the information on mesothelioma Italian occupational doctors have had. At least until all the 80s, the possibility to have information came from journals in English, because published in United Kingdom, Canada, Australia and South Africa, from the attendance of the annual Congress of the Italian Society of Occupational Medicine and Industrial Hygiene and from the reading / study of specialized treatises. The magazine “Medicina del Lavoro” in the years 1960 to 1967 only published some synthetic reviews of scientific articles issued abroad. Only in 1968 the first Italian scientific contribution (but in English in a time when the knowledge of the language was not as widespread as today) was published: 6 cases of mesothelioma in the cohort of 288 patients compensated by INAIL for asbestosis in the period 1943/1967 and died in Piedmont, Liguria and Lombardy are reported (26). As regards, however, the appearance of contributions on mesothelioma-asbestos, it's necessary to wait, as the first and only con-

tribution, the holding of a conference of specialist area, the Italian Society of Occupational Medicine and Industrial Hygiene, held in Saint Vincent in 1971, where was the relationship of Ruby and co-workers was exposed (27). The duration of the uncertainty regarding diagnosis and causation is clearly evident in Italy by reading only texts of occupational medicine for the use of experts. In fact Vigliani (28) concluded that the possibility that pleural mesothelioma, and also apparently peritoneal one, are connected with accidental or occupational exposure to inhalation of asbestos fibers is a very interesting topic of research for both occupational physicians and oncologists. So it is a topic of research and not an acquired certainty to be used as a guide in daily practice. It should be noted, however, as the quoted text is, rather than a treaty, a “dispensation” for students of medicine and, therefore, with limited diffusion. The edition of 1981 of the Treaty of Sartorelli (29) and the text of Vigliani and Bonsignore of 1981 (30) report historical updates regarding foreign literature that allow us to see how many and what are the aspects of the relationship asbestos-mesothelioma which is object of conflicting views still far from an unequivocal recognition. Murray in 1991 (31), recalls the difficulties encountered in the UK by the British Occupational Hygiene Society (Bohs) in 1968 suggesting the adoption of a standard of occupational exposure limit value of 2 fibers per milliliter and the reasoning according to which it was established in 1969 a limit of 0.2 fibers / milliliter as regards the crocidolite. Two years later, in 1993, Carnevale and Chellini (32) in the chapter on Mesothelioma wrote that the IARC evaluations are probabilistic, qualitative and therefore of limited use in the discrimination of the carcinogenic agents for which has not been held account the quantitative frame, implying that the IARC had not been offered an assessment for the purposes of public health. The many controversies about the danger of exposure to chrysotile asbestos and on the dose-effect relationship, are again resumed in 1995 by Englund (33). As evidence of the lack of knowledge we have the testimony of the General Directorate of Fire Prevention Services at the Ministry of Interior, which issued a series of circulars relating to the safety standards for protection against fire that allowed or required the use of asbestos. We get further confirmation of regulatory delay and then of the delay of knowledge for the entrepreneurs paying attention to the dates of publication of the Community Directives relating to restrictions on the marketing and use of certain dangerous substances and preparations. The 1980/1107/CEE Directive (implemented in Italy eleven years later by Legislative Decree 277/91) lists asbestos between agents for which “when member states adopt, for the protection of workers, measures concerning an agent, they take prevention measures provided for in Article. 4 (these are the general measures) and additional measures”.

The general measures are: 1) limited use of the agent in the workplace; 2) limiting the number of workers who are or may be exposed, and 3) technical prevention measures; 4) establishment of limit values (here we observe that the general measures are more neces-

sary as are exceeded the limit values that had still not been fixed at that time); 5) protection measures involving the application of procedures and appropriate working methods; 6) collective protection measures; 7) individual protection measures; 8) sanitation; 9) information for workers; 10) use of warning and safety signs; 11) monitoring workers' health; 12) keeping and updating records indicating the level of exposure; 13) emergency measures; 14) if necessary, total or partial prohibition of the agent if the use of other means available is not sufficient to ensure adequate protection. The 1983/477/CEE Directive on the protection of workers from risks related to exposure to asbestos finally fixes, in the art. 8, the limit values for occupational exposure: 1 fiber / cc for asbestos fibers other than crocidolite and 0.5 fibers / cc for crocidolite. It's important that in Annex II of this directive is for the first time indicated mesothelioma among diseases that can be caused by asbestos. In Italy, however, the entrance of mesothelioma among tabulated diseases for which there is a presumption of legal risk (and thus a clear knowledge) happened 11 years after the above-mentioned EC directives, so only with the Presidential Decree 336 of 13 /4/1994 to the voice 56 of the new table Annex 4. This disease was not specifically indicated nor in the DPR 482 of 06.20.1975 or, even less, in the Presidential Decree 1124 of 30.06.1965. In other words, until 1994 the legislature ignored the communications of Selikoff and the Acts of the New York Academy 1964. The Directive 1983/478/CEE forbids, for the first time, the placing on market and the use of crocidolite and provides for the labeling of all products containing asbestos fibers. 18 years have passed since the publication of volume 132 of the NY Academy of Sciences. The 1985/610/CEE Directive forbids the use of all asbestos fibers listed in Annex 1 regarding: A) toys, B) materials intended to be applied by spraying; C) materials containing asbestos to be sprayed on the bottom of the vehicle body; D) finished products in the form of powder sold to the general; E) merchandise for smokers such as tobacco pipes and F) catalytic filters; G) paints and varnishes. These same restrictions were taken in Italy by order of the Ministry of Health of 06.26.1986, but only the DPR 05/24/1988 n. 215 will implement the directives 1983/478/CEE and 1985/610/CEE. The 1987/217/CEE Directive on the prevention and reduction of environmental pollution by asbestos public methods of sampling and analysis hitherto not regulated. The 1991/382/CEE Directive lowers the limit value for chrysotile to 0.6 fibers / cc and for the other asbestos fibers to 0.3 fibers / cc. The 1999/77/EC Directive aims to ban the use of chrysotile asbestos fibers and Art. 2 provides that Member States must comply with the Directive by 1 January 2005. The 83/477/EEC Directive of 19 September 1983 "on the protection of workers from the risks related to occupational exposure to asbestos" marks a fundamental advance for knowledge and management of the asbestos problem: "Current scientific knowledge is not such as to enable to establish a level below which there are no risks to health - one fiber per cm<sup>3</sup> not crocidolite and 0.5 fiber/cm<sup>3</sup> crocidolite".

In Italy since March 1993 the law 257/92 forbids:

- extraction;
- import and export;
- marketing;
- the production of asbestos and asbestos-containing products.

### The risks from asbestos in maritime professions

These risks have been analyzed much later than those of other professions; epidemiological surveys have been conducted in various countries.

- In the U.S. Jones et al. (34) analyzed the chest radiographs of 5041 naval engineers. They revealed asbestos-related pleural abnormalities in 12% of cases. The prevalence of pleural lesions rose to 27% in those with longer working periods.
- An extensive research was conducted on 3324 seamen of the merchant marine in the United States (35). In 34.8% of cases were observed radiologically pleural and / or pulmonary alterations due to asbestos. According to Seidman (35), the prevalence was higher in the machinery sector staff (42.5%) than in the maritime of deck (36.6%), stewards (28.4%) or staff who had worked in several sectors (30.9%).
- In Japan, Kagamimori and Hosoda (36) revealed a marked difference in the prevalence of pleural plaques in the maritime sectors. The prevalence of radiographically detectable plaques was 10% in drivers (90 examinees) and 2% in the deck crew (136 examinees).
- In Greece Velonakis et al. (37) studied a series of 141 seamen in retirement. In 41% of cases were radiologically detectable pleural and / or lung asbestos related alterations.
- In Iceland, as regards incidence and mortality from cancer, a research was conducted in 1988 (38). A cohort of 477 drivers and mechanics on board was considered. The study showed a significantly increased mortality rate for cancer of the trachea, bronchi and lungs. An investigation was also conducted in parallel to determine the smoking habits in this occupational category, and from the survey emerged that the seamen in question did not differ from the rest of the population, so the smoking habit could not be held responsible for the excess of cancers found.
- In a study conducted in the United States causes of death in 1922 deaths of seamen of the merchant marine were considered (39). It was observed an excess of cancers of the respiratory system.
- In Italy mortality in a cohort of seafarers in Civitavecchia was examined (40). Also the results of this study deposed for an increase in mortality from cancer of the respiratory system. Among the tumors observed there was also a case of pleural mesothelioma.
- Pleural mesotheliomas in maritime have been observed in different series (41, 42).
- In a series of 70 pleural mesotheliomas examined in 1982, in 5 cases the tumor could be attributed to maritime activities (43).

Epidemiological studies on exposed workers in the international navies lie between 1982 and 2001. Before

there wasn't adequate knowledge and awareness of the risks of mesothelioma on board ships. Another element consistent with what has been expressed is the fact that the engineering of marine construction in 1972 was required of the use of asbestos, strictly imposed by the International Convention for the Safety of Life at Sea and the shipping registers of the various countries, as the ship had to possess precise characteristics of isolation, incombustibility, resistance to fire and heat, to prevent the spread of fire and damage to persons in case of fire. The insulating materials also were necessary to cover thermal plants, engines, pipes, to avoid heat loss and to prevent vibrations and noises.

### Duration and natural history of the disease

The disease progress is very slow; according to studies on cell growth, considering the cellular doubling time of malignant mesothelioma (275 days) and the number of doublings (30) to reach in plan a diameter of 10 mm, the median interval estimated that runs from the first exposure to onset of the first clinical manifestation and the clinical diagnosis of cancer is 22.6 years (44). In general, therefore, combining biological annotation with epidemiological observations on large series we deduce that the latency period is between 20 and 40 years after exposure and cases observed between 15 and 20 years are sporadic, exceptional and are probably due to exposure previous to the first identified, misdiagnosed because of the modest character and occurred in the general living standard.

The exposure may not be necessarily of professional kind. The unexposed population of large industrial cities or that living in areas near to the place of production of asbestos is also exposed to the risk of mesothelioma. Wives or family members of asbestos workers, brushing at home dusty overalls are considered at risk. More than 30% of the people who come into contact at home with asbestos workers have radiological features of pulmonary exposure. The risk of mesothelioma in the relatives is estimated by 1% compared with 8-13% of workers directly exposed. In addition to those from asbestos, other exposures are held responsible for the onset of malignant pleural mesothelioma, in particular the erionite. Other factors that may promote the onset of malignant mesothelioma are chronic lung infections, pulmonary tuberculosis and radiation. Finally, it has been recently suspected a possible etiologic role of the simian virus 40 (SV40). Sequences that encode for antigens of such viruses were indeed extracted from samples of human mesothelioma and not from the adjacent normal lung tissue. Furthermore, tumors histologically identical to human mesothelioma arise when SV40 DNA is injected into the pleural cavity of mice (44).

### Discussion

The analysis of world literature clearly demonstrates that the estimates expectations for the next 20 years, given the extent of asbestos in various fields of human

activities, is rising, as are rising the claims for personal damage in the entire world. The international literature in this regard, poses a series of recommendations and guidelines for accurate diagnosis of Malignant Mesothelioma liable to confusion with other diseases as cancer or not, that have nothing to do with Mesothelioma. The literature also indicates the dose-independence of the initiation of the process of carcinogenesis, experience having shown that even a very small dose may represent, according to the genetic predisposition or concomitant factors, such as infection with SV40, necessary and sufficient cause to promote the complex process of carcinogenesis of the mesothelium. In other words, the exposure to so-called "Trigger Dose" is an event that takes place at the beginning of the work course in all those people who work in environments where asbestos contamination is higher than the intrinsic defence mechanisms. The literature data on growth (doubling time of the cellular malignant mesothelioma – 275 days – and the number of doublings – 30 – to reach a diameter of 10 mm in plan) demonstrate that the initial lesion has a very slow growth and that requires to give pathological manifestation from functional point of view, from 20 to 40 years with a median of 22.6 years, without considering all the biological extrinsic and intrinsic variables of the organism, under the definition of "individual susceptibility". As previously explained, in this process there is no certainty of diagnosis of malignant mesothelioma attributed to our two cases as diagnostic criteria suggested at international level have not been strictly applied both from the clinical point of view and from the histopathological immuno-chemical point of view. The modern scientific knowledge on the timing of growth of the tumor, according to the natural history of the disease, would put the beginning of the disease in the two cases we are examining, in times very near to the beginning of the work history and in any case in time where, of course, the knowledge on the pathogenetic MPM were non-existent (case 2), or definitely not consolidated (case 1) so as not to allow the implement or training, information, or prevention measures of any kind. It is clear that during the early years of the working life of the two patients epidemiological knowledge, that matured after the 80s especially after 1988 and after 2000, were very limited. In previous years the knowledge related were not consolidated. In fact, in international law, only Directive 83/477/EEC of 19 September 1983 "on the protection of workers from the risks related to occupational exposure to asbestos" marks an essential advance for knowledge and management of the problem asbestos: "Current scientific knowledge are not such as to enable to establish a level below which there are no risks to health - one fibre per cm<sup>3</sup> not crocidolite and 0.5 fibre/cm<sup>3</sup> crocidolite." In 1987 IARC has classified as carcinogenic to man all types of asbestos and in 1988 the carcinogenicity of asbestos has been reported on the texts of medicine. The abolition of the use of asbestos has been enshrined in law by the European Union in the EU Directive 96/62. In Italy, Law 257/92 prohibits the extraction, import, export, marketing and production of asbestos and the European legislation on face masks with high filtering

power (P3) is of 1991. We remind in the United States Jones et al. (34) In Japan Kagamimori et al. (36); in Greece Velonakis et al. (37); in the United States (24, 39); in Iceland in 1988 (38); in Italy Rapiti et al. (40), White (41, 43). The occasion of the first exhibition of our two cases, as it is recognized that even a very small dose may be cause a necessary and sufficient to promote the complex process of mesothelium carcinogenesis, can probably be traced back, in the performance of their duties, to 'inhalation of microfibers freed occasionally in the ship, and at the time of the conjectured assumption of "trigger dose" neither case 1 or case 2 could be aware of the consequences of exposure to asbestos fibers, nor could implement an effective prophylaxis individual with the use of effective means of protection. The European standard that defines the different types of mask at negative pressure according to their filtering capacity is of 1991 (EN 149). The EN 149 provides for three classes of protection: FFP1, FFP2 and FFP3 based on the efficiency of the filter, according to European standard EN 143, 1990. The classes of protection and the filtering efficiency are summarized in Table 2.

In Italian law (DM 06/09/94 - Standards and technical methods of application of Article 6, paragraph 3, and Article-12, paragraph 2 of the Law of 27 March 1992, No. 257, on the cessation of use of asbestos) prompted the adoption of "mask with filter type P3 or full face masks, depending on the level of potential exposure". Recently, it has been raised the question of the role of the microfibers in the genesis of the disease. It was therefore developed a debate (45-47) on the possibility that pleural mesothelioma is associated in a prevalent or even exclusive way, according to some authors, with the inhalation of asbestos fibers with special dimensional characteristics, that is to say with a length less than 5 m and ultrafine diameter, on the order of fractions of microns. In particular, in 2005, Suzuki in a study on samples of lung and mesothelial tissue conducted by electron high resolution microscopy reported a percentage of the total close to 90% of asbestos fiber of length less than 5 m and diameter less than 0, 25 microns. Given the predominance of this fraction of the total fiber found, Suzuki (48) concluded that it is not prudent to maintain a position that assumes that the short fibers and thin to induce only a modest risk of mesothelioma. Also in 2005, Chiappino (49) postulated an explanation of the pathogenicity of the so-called ultra-short and ultra-fine fibers (length of few microns, diameter of the order of 0.2 m), based on two observations:

- 1 Only the portion of ultrafine fibers is able to overcome the barrier-lung pleura and reach the parietal pleura (when originates mesothelioma).

- 2 The distribution of the pleura is not random because these fibers are concentrated in focal points of the parietal pleura (black spots) corresponding to the absorption of the lymphatic stomata and therefore at such points the fiber concentration is far lower.

This hypothesis needs further evaluation, and if confirmed, would imply some reflections on medical-legal issues. In fact, assigning a predominant etiologic role in the genesis of mesothelioma for fibers ultrafine and less than 5 micron in length, would be necessary to answer to questions concerning both the technical possibility to produce reliable estimates of exposure (since, by definition, these fibers dimensions are not taken into account in the calculations) and the technical possibility to carry out effective measures to reduce exposure by the environmental and personal preventive devices, designed for the removal of fibers with different and "classic" size characteristics. This especially applies to occupational exposures incurred up to the 80s. On this subject, there was a fierce debate about the fact that the fibers USW / ultrafine detected level pleuropulmonary are inhaled as those that is to say are fragmented in the lung when inhaled. In support of the first hypothesis (which of course should be related to the medico-legal and hygienistic considerations above), among others, the results of research of Gibbs and Hwang (50), Pooley and Clark (51), Wagner JC (52) and Lee and Van Orden (53) are reported. The first show that in the work areas of the mines of crocidolite, amosite and chrysotile fibers of length less than 5 microns represent 96% for crocidolite, 88% for amosite and to 99% for chrysotile. Similar results were reported by Pooley and Clark that, in mines of crocidolite and amosite, revealed a percentage of fibers of length less than 4 microns equal to 85,4% for crocidolite and to 68.2% for amosite. The data of Lee and Van Orden concern samplings in not strictly occupational environments, that is not proper asbestos industry, such as public buildings, schools, homes, and the data recorded refer to a percentage of fibers of length less than 5 microns equal to 90% for amphiboles and to 99% for chrysotile. Scientific information published in the last three years (2005-2008) are of great value for the understanding of basic concepts of etiology, exposure and forensic assessment.

## Conclusions

- There is no certainty of the diagnosis of malignant mesothelioma attributed to the 2 cases reported above, as diagnostic criteria suggested by the literature both in terms of the clinical and histopathological

**Table 2 - Classes of protection and filtering efficiency.**

Filtering facepieces	Particle filters	Minimal filtering efficiency
FFP1	P1	78%
FFP2	P2	92%
FFP3	P3	98%

FFP1: dust / harmful aerosol; FFP2: dust / fumes / low speed aerosols; FFP3: dust / fumes / toxic aerosol

and immunochemical have not been rigorously applied.

- On the basis of epidemiological data and cell biology of the natural history of mesothelioma, it is clear that in these cases, where the diagnosis was confirmed histo-pathologically, it is unlikely that asbestos exposure occurred in the 20 years preceding death. Thus, the mesotheliomas were likely to have clinically manifested relatively late in the disease process..
- The first exposure to asbestos in working life of the cases presented above likely occurred in the first few years of activity (2/4 years), as it has been amply demonstrated that even a very small exposure dose of asbestos can trigger the mechanism of initiation and progression of Malignant Pleural Mesothelioma.
- Knowledge about health risks of exposure to asbestos by respiratory route was only consolidated in the scientific literature around the 80s. In 1987 IARC classified asbestos of all types as carcinogenic to humans. In 1988 the dissemination of knowledge on the carcinogenicity of asbestos has been reported on the textbooks of medicine. The use of asbestos was abolished in Europe by the the European Union Directive 96/62. The European legislation that identifies the type of face masks according to their filtering capacity is of 1991.
- Epidemiological studies on the risks from asbestos in the Italian and foreign navies began in the period 1982 - 2001.
- The two cases described above could not be aware of the consequences of exposure to asbestos fibers, nor could implement a valid individual prophylaxis with the use of effective protective devices.
- Effective personal protection equipment were not on the market until the early 1990s, when the development, patenting and production of a mask effective to 98% in the interception and capture of asbestos fibers, and the mask with P3 filter became available.

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