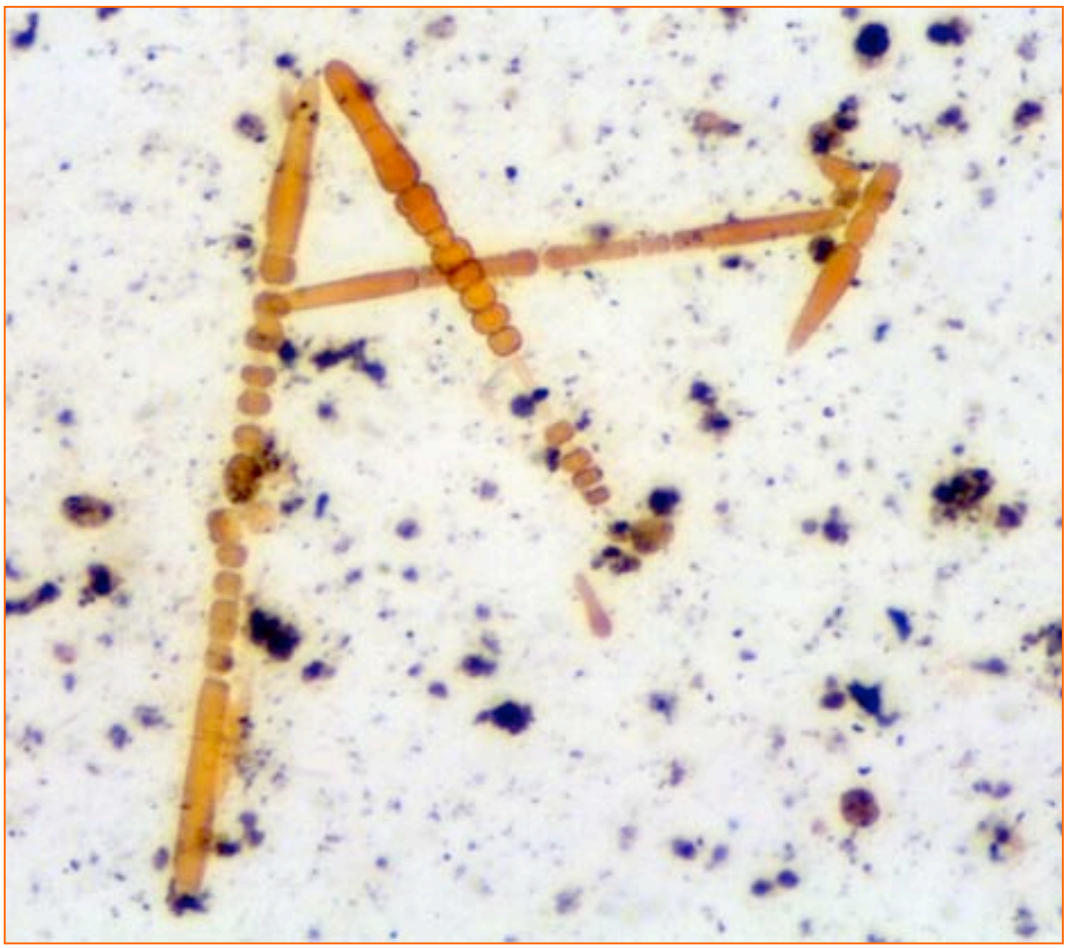
Study of lung asbestos content in a Spanish population



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DOCTORAL THESIS

Study of lung asbestos content in a Spanish population

presented by **María Isabel Velasco García**

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A Andrés

por estar siempre a mi lado

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List of abbreviations

AB: asbestos bodies

AF: asbestos fibres

ARD: asbestos-related diseases

CT: computed tomography

EDS or EDX: energy-dispersive X-ray spectroscopy

EM: electron microscopy

EPA: Environmental Protection Agency

ERS: European Respiratory Society

EU: European Union

k: kappa coefficient

LM: light microscopy (optical microscopy)

MPM: malignant pleural mesothelioma

MR: magnetic resonance

µm: micrometres

PET: positron emission tomography

r: range

RNS: Reactive Nitrogen Species

ROS: Reactive Oxygen Species

rpm: revolutions per minute

SD: standard deviation

WHO: World Health Organization

Introduction

Definition of asbestos

The word “asbestos” (a term of Greek origin, meaning “fireproof”) refers to a group of fibrous meta-silicates of iron and magnesium, among other metals, which are found naturally in the earth's crust. The original Greek word was the verb *sbénnymi* (meaning “to extinguish”), and the prefix “a” in *asbestos* makes the word negative, i. e., “something that cannot be extinguished”.

Asbestos has a number of important properties: it is non-combustible; it provides good thermal and acoustic insulation; and it offers strong resistance to high temperatures, the flow of electricity, abrasion, and microorganisms. These features have made it a very useful element in many different branches of industry.

Asbestos is formed by long, resilient, flexible fibres. Thousands of elementary fibres join together in bundles or aggregates, and can be separated easily into increasingly thinner fibres until they become fibrils of microscopic size (Figure 1).

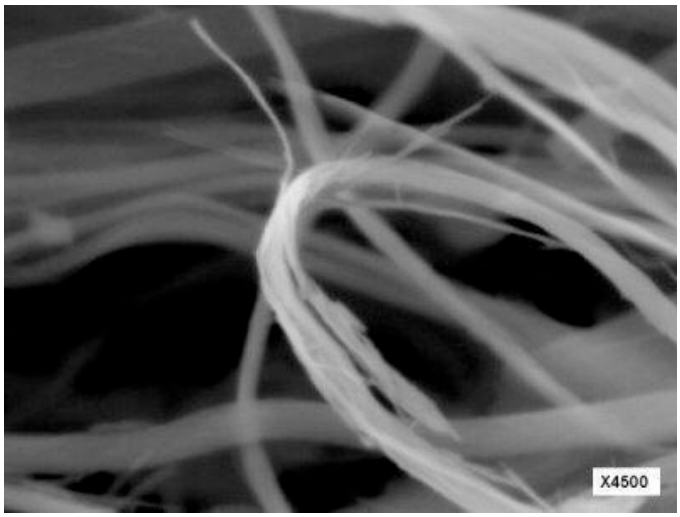

Figure 1. Asbestos fibre, magnified 4500 times.

Once extracted from its natural rocks, asbestos has the property of breaking down into tiny fibres with a length of the order of microns. For example, an inch of the mineral (about 25 mm) can give rise to 1,400,000 invisible fibres.

Types of asbestos

There are several varieties of asbestos. From the mineralogical point of view, there are two main types: the serpentine group and the amphibole group. The serpentines are magnesium silicates structured in the form of flexible fibres, curved, long and thin, with a diameter of 0.02-0.03 microns. Serpentines are

frangible and soluble in organic liquids, and there is a single variety: chrysotile, or white asbestos. The amphibole group includes crocidolite (riebeckite or blue asbestos), amosite (brown asbestos or grunerite) – both silicates of iron and magnesium, although crocidolite also contains sodium – anthophyllite, tremolite and actinolite. All amphiboles are straight, rigid, pointed fibres, insoluble in organic liquids, with diameters ranging from 0. 06 up to 1. 5 μm[1-3] (Figure 2).

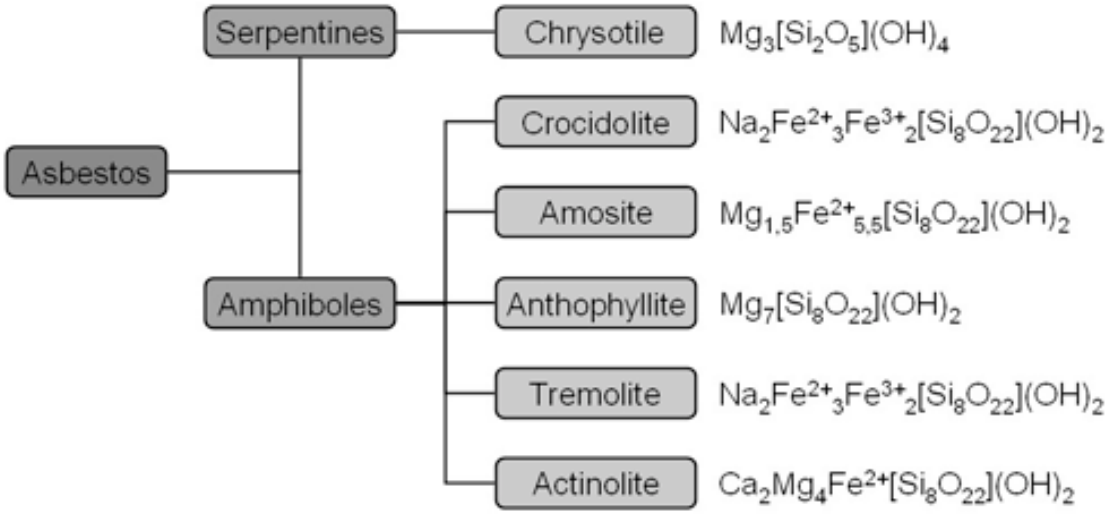

Figure 2. Classification chart of the different kinds of asbestos.

Sources of exposure to asbestos. Use in industry.

The physicochemical properties of asbestos (i. e., its mechanical resistance, and its resistance to heat and electricity) have been known since ancient times. In the twentieth century, the mineral was used in industry in more than 3000 different applications and products.

The asbestos industry in Spain was dominated by a single fibre cement company, called Uralita SA. The firm was so prominent in the field of asbestos that the mineral is often referred to in Spain as *uralita*. Figure 3 displays statistics on the importation of the mineral into Spain in the twentieth century (domestic production was very low).

In Spain asbestos began to be used in the 1940s[4], initially without any regulation. It reached its highest level of use in the 1970s, after the industrial boom, and continued to be used in specific activities until 2001.

Between 1960 and 1984, the use of asbestos in Spain was at its peak. In 1992, the country was Europe's second largest importer, with an annual total of 25,428 tons. The production of asbestos and its industrial use reached their highest levels around the time that many Western countries were beginning to introduce bans on the mineral because of its harmful effects. In Spain, the manufacturing and marketing of the last variety of asbestos, chrysotile, was prohibited on 14 June, 2002[5-6].

Due to its special characteristics, asbestos has been used in a variety of manufactured goods, especially in building materials (roofing and tiling, paper and cement products), friction materials (clutches in automobiles, brakes, transmission components), heat-resistant textiles, containers and packaging, personal protection equipment, paints, vermiculite and talc, and so on. The forms most widely used in construction were chrysotile (white asbestos), amosite (brown asbestos) and crocidolite (blue asbestos).

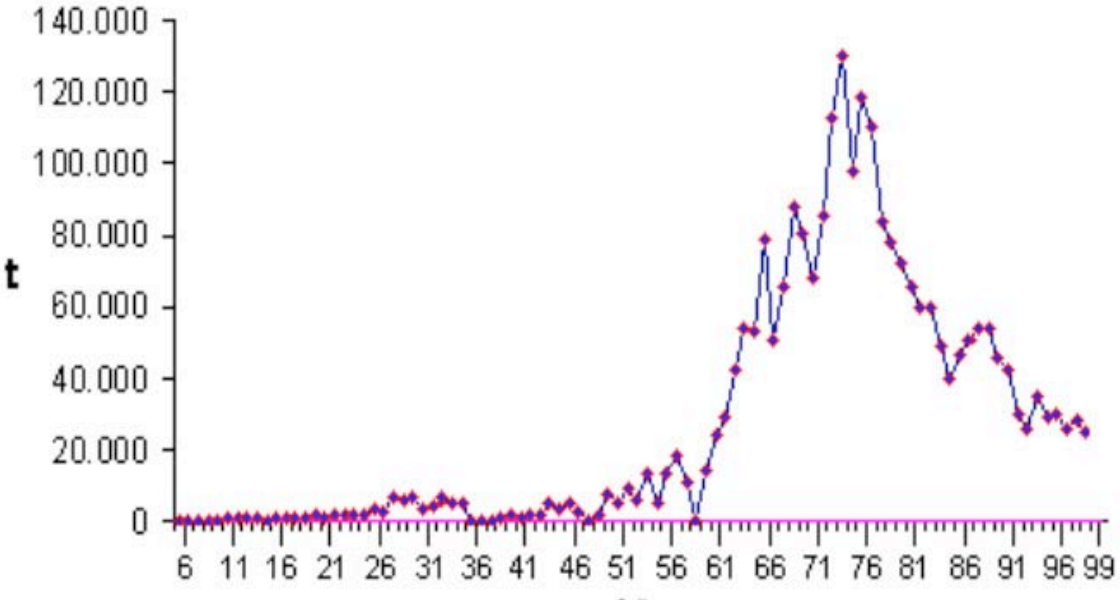

Figure 3. Asbestos imports to Spain in the twentieth Century (tons per year).

Along with the properties already mentioned, the low cost of asbestos enhanced its use in the manufacture of many types of construction material (for example, in ceiling boards, fibre cement, fire protection, thermal and acoustic insulation and, flooring, etc.).

Types of exposure

There are three main sources of asbestos exposure: the environment, the home, and the workplace. Environmental exposure affects people living near asbestos mines, fibre cement factories, and in general industries in which there is intensive manipulation of the silicate. Domestic exposure is caused by household appliances such as electric blankets and insulating boards, and activities such as washing the clothes of asbestos workers. And finally, the most important source has always been occupational exposure, originating in the workplace.

The exposure generated by the fibre cement factory in Cerdanyola, near Barcelona, was threefold: occupational (for the factory workers), environmental (for the area's residents)[7, 8] and domestic, since the factory workers took their overalls home to be washed.

It should be borne in mind that the forms of exposure to asbestos in Spain may vary, since there are differences between rural and industrial areas. Exposed patients in Spain include workers in the industry and others employed in the repair of structures that contain the mineral. In the medium and long term, the exposed population since 2002 (the year when asbestos was banned) will comprise workers in the asbestos removal and repair sector.

Asbestos in Spain

Since the Ministerial Order of 31 October 1984, which has since been extended by other legislation, work related to the manufacture of products containing asbestos has been regulated along with other activities such as the maintenance or demolition of affected buildings. In Spain, several prevention orders have been issued for the analysis of asbestos in the air and the control of the concentrations to which workers are exposed[9-14]. The Spanish legislation is consistent with international health guidelines and recommends zero doses of asbestos concentration; that is, there is no minimum dose exposure in the workplace.

In recent years, there has been growing concern about the effect of asbestos exposure in the population. In fact, asbestos accounts for 15% of all occupational illnesses recorded in Barcelona between 1987 and 1991. There were 237 fatalities due to mesothelioma in Barcelona province between 1983 and 1990, corresponding to mortality rates of 0. 83 and 0. 47 per 100,000 in men and women respectively[15, 16]. Today in Spain, between 250 and 300 cases of malignant pleural mesothelioma are recorded each year; this is a type of cancer with extremely high mortality rates, linked to occupational exposure to asbestos in 85% of cases and against which at present there is no effective treatment[17]. It is estimated that the cases of lung cancer related to asbestos exposure in Spain will rise to around five hundred a year in 2015.

Due to the long time that mesothelioma symptoms take to appear, the deaths it causes are observed in people who worked with this material for decades in fibre cement industries, foundries, and shipyards in industrial areas like Ferrol (Galicia)[18], Oviedo (Asturias)[19] and parts of the Basque Country. In the Basque Country the incidence of cancer has skyrocketed in recent years, reaching figures well above the national average[20].

In the legal domain, the right of victims and their families to receive compensation is now beginning to be acknowledged, and the condition is now recognized as an occupational disease.

Asbestos in other countries

Amphiboles were recognized as carcinogens in 1973, and in 1977 the International Agency for Research on Cancer (IARC) classified all types of asbestos as group 1 carcinogens (“The agent is carcinogenic to humans”). Since then, scientific data have repeatedly confirmed the dangers of this substance. In its 2006 publication on asbestos[21], the World Health Organization confirmed its carcinogenic potential, and also included chrysotile alongside the amphiboles.

As there is no evidence that the carcinogenic effect of asbestos has a threshold, and in fact the risk of cancer has been seen in populations with very low levels of exposure, the WHO confirms that the best way to eliminate

asbestos-related diseases is to terminate the use of all its varieties. According to the WHO, 125 million people worldwide are exposed to asbestos in their work. In the US, after decades of struggle against the mineral, asbestos regained prominence after the bombings of the World Trade Center in New York on 11 September, 2001, when the collapse of the towers caused the release of large quantities of this pulverized material into the atmosphere.

In the US, it is usually the socially disadvantaged who handle this hazardous mineral. The US Environmental Protection Agency (EPA) stipulates that the removal of any asbestos waste must be done using extremely careful methods. However, these procedures are not always applied, since firms often put potential profits before the safety of their workers.

In Europe, the French and German governments each allocate more than a billion euros per year to compensate people affected by asbestos-related diseases[22]. Indeed, the prospects for the future are alarming: the European Commission speaks of an epidemic of 500,000 deaths in the coming years, an amount 10 times higher than other work-related accidents[23]. In the UK, it is estimated that cancer from exposure to asbestos will cause more than 200,000 deaths over the next decade [24].

Prohibition of asbestos in different countries

The US Department of Health and Human Services, the EPA and the WHO have established that asbestos is carcinogenic to humans.

However, the prohibition of the substance has been a slow, gradual process. In 1991, the World Bank (WB) decided not to fund the manufacture or use of products containing asbestos. On 4 May, 1999, the Technical Committee of the European Union (EU) approved the prohibition of the use of any type of asbestos. In Spain, the industrial use of crocidolite was banned in 1984 and all types of asbestos were prohibited in 2002.

Currently, asbestos is banned by the Rotterdam Convention, which was signed by over one hundred countries and which came into force on 24 February, 2004. As of 1 January, 2005 the use of any type of asbestos was

prohibited in all countries where it had not yet been banned. Under an EU directive, all member states had to prohibit the marketing and use of any type of asbestos.

The pathogenesis of lung disease caused by asbestos

Asbestos is a natural mineral fibre that is a result of a crystallization process. Some of its effects in the body are associated with physicochemical characteristics of the fibres[25, 26]. The danger posed by construction materials containing asbestos depends on their friability (i. e., the ease with which they release asbestos fibres into the air).

How asbestos enters the body

Asbestos fibres that are inhaled progress through the airways and are deposited and drained by the local defence mechanisms. Their ability to penetrate and their size are the two factors that determine their entry into the human body. The ability of a fibre to penetrate the body depends on the ratio between its length and its diameter. Generally, a fibre may be inhaled if its length / diameter ratio is greater than 5 to 10[27, 28]. Fibres of 5 microns or less in length show a higher penetration than longer fibres. But we should also take retention into account, since the shorter fibres can more easily escape barriers of the respiratory system than longer ones.

It is believed that different types of respirable fibres vary in their ability to cause pulmonary disease depending on their length and on their biological persistence in the lungs[25]. Chrysotile forms, which can be broken into multiple microfibers of small length and diameter and are sensitive to acids, are eliminated to a greater extent than the amphiboles, which have greater biodurability and biopersistence[26]. Experimental animal studies suggest that the potential of asbestos to cause fibrosis and cancer is related to the number of fibres longer than 5 to 20 microns in the inhaled air[29, 30]. Some studies consider chrysotile to be the least fibrogenic of the different types of asbestos, and

crocidolite as the most fibrogenic and carcinogenic variant due to its tendency to deposit in the lungs.

The relationship between asbestos and smoking

The combination of asbestos exposure with tobacco increases the risk of lung cancer by 50 times compared to non-smokers and unexposed people. Although the carcinogenic potential of asbestos is recognized, the attribution of cancer to this cause is hindered in many cases by the coexistence of smoking. This uncertainty regarding the involvement of asbestos exposure to the development of cancer makes it mandatory to objectively identify the presence of asbestos fibres deposited in the lung[31].

The body's response to asbestos fibres

The fibres of larger diameter and length are typically deposited in the bronchial and bronchiolar branches. Fewer fibres reach the alveolar compartment. The fibres deposited on the mucociliary epithelium may be partially eliminated with the mucus, while the ones that reach the alveolar or interstitial space may be eliminated or modified by macrophages.

The mucociliary system of the bronchial epithelium removes the fibres from the respiratory tract and facilitates swallowing. The remaining fibres are engulfed by macrophages, which are transferred via the lymphatic system or pass into the pulmonary interstitium. The macrophages attempt to phagocytize the fibres, although in many cases the dimensions of the fibres exceed the capacity of the macrophage, leading to incomplete phagocytosis, cell disruption, and subsequent enzymatic damage to the tissue. Asbestos fibres accumulate in the lungs when the exposure load exceeds the drainage capacity[32].

The mechanisms by which asbestos fibres reach the pleura are still pending investigation[33], although a direct passage through the lung or retrograde redistribution through the lymphatic system in the parietal pleura have been proposed[34, 35]. Some studies suggest that the fibres may pass from the alveolar space to the visceral pleura either directly or via the lymphatic system, due to the existence of connections between pulmonary and pleural

lymphatic systems. In any case, the distribution of asbestos fibres in the pleura is not homogeneous, but is concentrated in specific points in the parietal pleura near the lymphatic vessels rich in macrophages and lymphocytes, similar to the "black spots" in anthracosis[36].

Although chrysotile asbestos is the most commonly used, the fibre that is found most often in the lungs of patients with mesothelioma is crocidolite. This is because, as noted above, chrysotile is soluble in organic liquids, which facilitates its fragmentation and elimination over time. In animal studies, levels of accumulation of crocidolite in the lung have been reported to be three or four times higher than those of chrysotile[37].

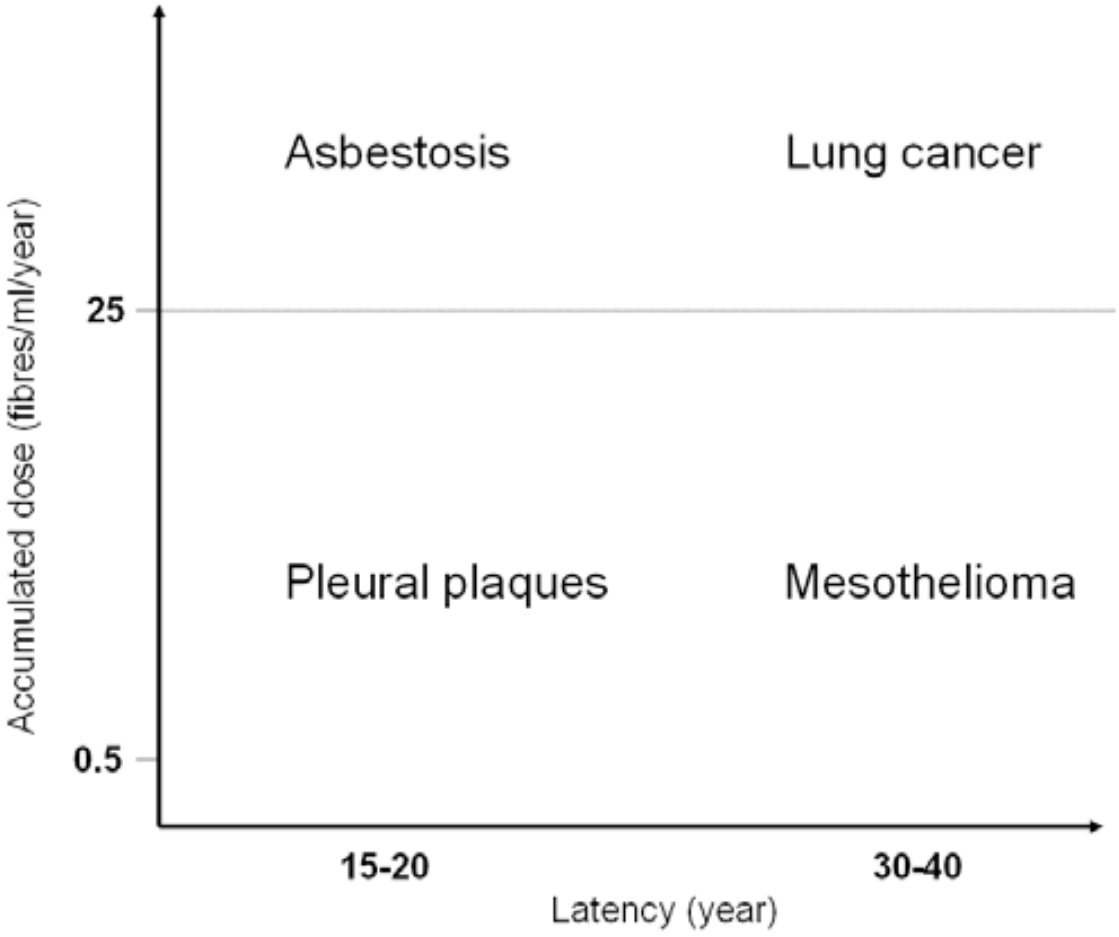

Figure 4. Chart showing the most frequent asbestos-related pathologies in relation to the number of asbestos fibres respired annually.

The pulmonary carcinogenicity of asbestos is widely accepted today, but not all mechanisms and markers involved in its development have been accurately described[38-40]. One aspect that accounts for the higher biological activity of amphiboles is their composition: the presence of iron on the surface

of amosite, and especially of crocidolite, determines to a large extent the production of free radicals (ROS and RNS) with power to cause oxidative damage; some authors propose that the ROS affect DNA through a persistent chronic inflammatory process, resulting in carcinogenesis associated with fibrosis[41-42]. The genotoxicity of asbestos may be due to the mechanical action of the fibres; however, this would mean that asbestos-like structures (carbon nanotubes, for instance) should produce lung diseases similar to those caused by asbestos, but this phenomenon has not been observed to date.

Transformation of an asbestos fibre into an asbestos body

An asbestos fibre is transformed into an asbestos body by the action of macrophages. Larger fibres, that is, those with a length above 10 microns, are often attacked by more than one macrophage, and they become encased by iron-rich material and form the core of an asbestos body (AB). Thus, the term "asbestos body" is used to designate fibres which have been coated with ferroproteins by macrophages in the lung. The coating of the fibres is part of the lung's defence system in order to make them inert and non-immunogenic. The presence of ferruginous bodies in the lung is considered a good indicator of past exposure to asbestos[43].

The asbestos retained in the respiratory organs exerts its damaging effects very slowly. There is a significant latency period between the start of exposure and diagnosis of the disease. The toxicity of asbestos is related to its fibrous structure, since it has been shown that pulverized asbestos does not cause disease[44]. Even low-intensity or short-term exposure to asbestos may be pathological, and there are factors of individual susceptibility which have not been fully described[45]. Pulmonary asbestos content is a reflection of various combined effects; in addition to exposure and retention, the metabolism of the individual and environmental agents also exert an influence[46].

The diseases with earliest onset are benign pleural plaques and pleural effusions, which may occur only 20 years after exposure[47]. As mentioned above, asbestosis, malignant mesothelioma and lung cancer usually appear 30 years after contact.

Another aspect to consider is the strength of the relationship between exposure, lung asbestos burden, and disease. It is recognized that benign pleural alterations may occur even with mild exposures, while asbestosis and lung cancer require longer periods of contact[48].

Finally, the frequency with which different diseases appear may vary widely. Benign pleural diseases are common in exposed individuals, especially pleural plaques, while malignant mesothelioma is a rare disease. The finding of bilateral pleural thickening on the chest radiograph has a predictive value as an indicator of asbestos exposure of 81%, if other known causes of thickening[49] are excluded.

Future projections

A study commissioned by the EU concluded that half a million Europeans will die before 2030 due to asbestos-related diseases, between 40,000 and 56,000 of whom will be Spanish. In a study conducted in Catalonia, a first estimate of the number of buildings affected reported 6,000 buildings and 14,000 underground garages constructed using asbestos for fireproofing purposes, and 19,000 other buildings which contain asbestos, and 100,000 s.q.m. of false ceilings in office buildings[50, 51].

The expectations for the future are a matter of deep concern, especially because of the time lag of more than 20 years between exposure and manifestation of disease. In the next few years, diseases due to exposures occurring 30 years previously will appear. And it should not be forgotten that a significant number of workers still handle asbestos structures and will continue to so in the coming years: despite the ban on the industrial use of crocidolite imposed in 1984, and the ban on chrysotile in Spain in 2002, the negative impact of asbestos in our population remains very high.

Diseases caused by asbestos

Over the years in which asbestos was used, the health risks associated with the inhalation of its fibres become known and its various uses were prohibited.

Asbestos mainly affects the respiratory system; it is in the lungs are where the greatest amounts of fibres are deposited. The main pathologies caused by inhalation of asbestos are asbestosis, benign pleural diseases (such as plaques, diffuse fibrosis, pleural effusion or rounded atelectasis), malignant mesothelioma, and lung and other cancers[52, 53].

Non-malignant pleural diseases are the most common and are classified into three groups: pleural plaques, pleural thickening, and pleural effusions.

Pleural plaques are collagen formations and are distributed either unilaterally or bilaterally. They generally affect the parietal pleura: either costal, mediastinal or diaphragmatic. From the histological point of view, they are made up of acellular hyalinized collagen structures, covered by a layer of mesothelial cells.

In general, the plaques do not cause respiratory difficulties but are evidence of asbestos exposure, even at low levels. The pathogenesis has been postulated to be transpleural migration of inhaled asbestos fibres. Plaques usually appear 20 to 30 years after initial exposure and the incidence increases with the amount of asbestos inhaled. The plaques may increase in size as the patient grows older, and calcification is common.

In workers exposed to asbestos, chest radiography does not detect plaques in the first 10 years. After 19 years, however, plaques are visible on chest X-ray in 10% of workers, and the figure rise sharply to 58% after 40 years[54]. In populations with environmental exposure to asbestos such as northern Greece, 47% of the population has pleural calcifications[55]. Thus, the presence of plaques is considered a reflection of an individual's exposure, and so detection by X-ray is of great diagnostic and epidemiological value.

The best diagnostic imaging technique is computed tomography (CT), which allows good identification of the plaques and of possible concomitant pulmonary involvement. In terms of prognosis, the possible malignant development of pleural plaques has been the subject of many studies. The evidence accumulated so far is insufficient to conclude that malignancy will definitely occur[56]. However, since the presence of plaques is an indicator of exposure to asbestos – whose carcinogenic potential is undeniable – patients

with plaques have a high risk of developing pleural or lung tumors, so they should avoid asbestos exposure and should stop smoking.

Benign pleural effusion is a known consequence of exposure to asbestos. It is the disease with the earliest onset because, although it usually appears after 20 years of contact, it may appear in the first 10 years. It is also the most frequent complication in the first two decades after exposure. A study conducted in 1982 showed a direct relationship between the degree of exposure and the frequency of effusion[54].

To establish the diagnosis of benign asbestos-related pleural effusion other causes must be ruled out, especially malignant pleural mesothelioma and metastatic cancer. Therefore, if after an initial assessment the effusion persists, thoracoscopy is recommended. In any case, effusions are only diagnosed as benign after a follow-up control lasting at least three years.

Unlike plaques, diffuse pleural fibrosis mainly affects the visceral pleura without clearly defined margins. Its frequency and rate of progression increase with the intensity and duration of exposure to asbestos[57]. Histological examination shows collagen deposition with few cells and hyperplasia of mesothelial cells. There is usually subpleural pulmonary fibrosis, at depths not greater than 1 cm. In severe cases, this fibrosis can cause ventilatory restriction and on occasion respiratory failure.

X-ray examination should include an oblique projection. In addition to pleural thickening, which predominates in the middle and lower fields, parenchymal lines are often seen on the periphery of the lung perpendicular to the thickened pleura, producing an image known as "crow's feet". Chest CT allows better visualization of these abnormalities, and of any accompanying lesions such as rounded atelectasis.

Rounded atelectasis is a lesion in which an area of the peripheral lung is trapped by the underlying pleural thickening. Its characteristic image on chest CT shows a peripheral location, pleural thickening and curvature of vessels and bronchi converging toward the pulmonar hilum[58]. This allows differentiation from cancer, and thus avoids the need for aggressive diagnostic tests.

Asbestosis was the first pulmonary disease associated with asbestos, in 1929[59]. It is defined as diffuse interstitial pulmonary fibrosis caused by asbestos exposure and is therefore common in workers exposed to this mineral. People

with asbestosis report cough, dyspnoea, and a restrictive ventilatory disorder which leads to death in around 50% of cases.

The two main types of cancer caused by asbestos exposure are lung cancer and mesothelioma. The first reports linking asbestos and lung cancer date back to 1932[60]. Lung cancer is the leading cause of death in exposed patients: in Spain, it is estimated that about 4% of cases of lung cancer are asbestos-related[61]. In most cases, the exposure occurs in the workplace[62], and the latency of onset after the first exposure ranges between 20 and 40 years.

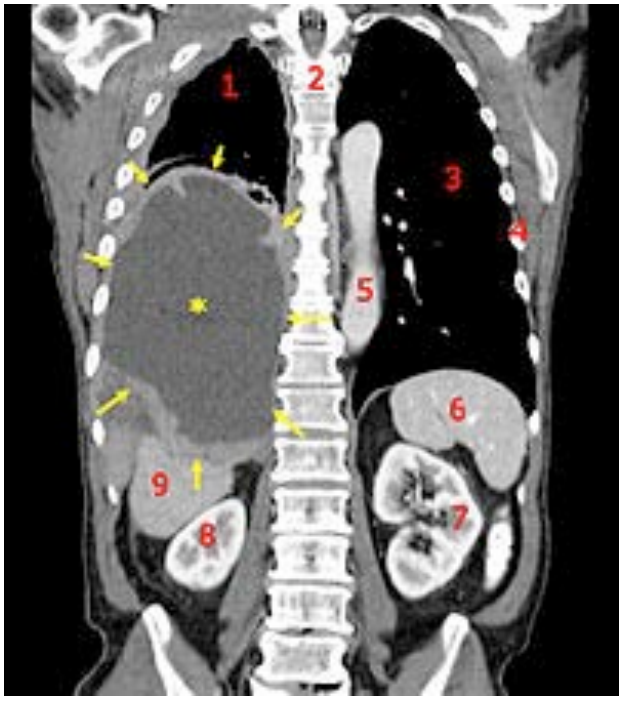

Figure 5. Malignant mesothelioma (yellow arrows) with pleural effusion (yellow asterisk).
1. Right lung 2. Spinal column 3. Left lung 4. Ribs 5. Aorta 6. Spleen 7. Left kidney 8. Right kidney 9. Liver

Although the main risk factor for developing lung cancer is smoking, the inhalation of asbestos also independently increases the risk of this condition[63]. The risk is increased even in the absence of pulmonary asbestosis[64]. Another important aspect is the synergy between tobacco smoking and exposure to asbestos, a combination which multiplies the risk of lung cancer[65-68]. In general, one year of high exposure (e. g., involvement in the manufacture of asbestos products, in insulation using asbestos products, demolition of buildings) or ten years of moderate exposure (e. g., in construction, shipyards, and so on) may be sufficient to double the lung cancer risk[69].

Lung cancer caused by asbestos does not differ from other types in terms of clinical symptoms, radiological imaging, diagnosis, prognosis or treatment. A history of exposure together with the presence of suggestive pulmonary or pleural radiographic lesions facilitates diagnosis. One aspect of particular interest is the attribution of disease to asbestos, especially due to the legal repercussions and the possible compensation that this may involve. The

main difficulty is that the majority of patients with lung cancer who have been exposed to asbestos have also been smokers, and so asbestos cannot be considered as the sole cause. To prevent lung cancer, both smoking and exposure to asbestos should be avoided.

As regards other malignancies, asbestos exposure was at one point associated with laryngeal, oesophageal, gastric and colorectal cancer. However, recently it was reported that there is no evidence of an increased risk in people exposed to asbestos[70, 71].

Pleural mesothelioma is a primary malignant tumour of the mesothelium that usually affects the pleura and more rarely, the peritoneum. Pleural mesothelioma may have a latency period of 20 to 40 years. This is a rare disease whose incidence increases in direct proportion to the use of asbestos. Its prevalence is estimated at between 10 and 30 cases per million per year in men, and two per million per year in women[56]. In Spain, no reliable figures are reliable. A causal relationship with asbestos was clearly demonstrated in the report by Wagner et al in 1960[72]. Malignant mesotheliomas have been reported after radiotherapy and chronic pleural inflammation; although in these cases it is difficult to conclusively establish a causal relationship.

In most instances, patients with malignant mesothelioma have had intensive exposure to asbestos in the workplace, although cases have also been reported after mild or occasional environmental or domestic exposure[73, 74]. The types of fibre most closely related to mesothelioma are amphiboles, particularly crocidolite, although the condition may also be caused by chrysotile. The latency period is usually between 30 and 40 years; in 99% of cases it is more than 15 years, and in 96% more than 20 years[75]. If the intensity of exposure is low, the latency period before the appearance of mesothelioma is longer.

There are three histological types of mesothelioma: epithelial, mesenchymal and mixed. The defining signs of malignant mesothelioma on CT are nodular pleural thickening, affecting mostly the diaphragmatic pleura. According to the degree of invasion of adjacent structures, a staging is performed which is useful for deciding on treatment and prognosis of the

disease. Obviously the diagnosis of mesothelioma is histological, and so a pleural biopsy is required.

The prognosis of mesothelioma is poor. The median survival of patients is less than 1 year, although some patients survive for several years. Unfortunately, at this time malignant mesothelioma has no cure.

Diagnosis of diseases caused by asbestos

The diagnosis of asbestos-related diseases is based on the confirmation of exposure by clinical interview, physical examination, imaging tests such as chest radiography or computed tomography (CT), lung function tests, and biopsy studies when required. Chest radiography is the best tool for detecting lung and pleural abnormalities resulting from exposure to asbestos. The plausibility of attributing a respiratory illness to asbestos according to the intensity of exposure and the latency is shown in Figure 4.

People who have been exposed to asbestos should undergo regular checks. Below is a list of techniques that can be used to diagnose asbestos-related conditions and to monitor exposed patients, depending on the medical assessment in each case:

• Clinical history and physical examination: the risk factors and symptoms presented by the patient are recorded in the clinical history. The physical examination provides information on the signs of asbestos-related diseases, lung cancer, and other health problems.

• Radiological studies: the most commonly used test is chest radiograph with anteroposterior, lateral and oblique views.

• CT Scan: gives very precise information about the existence of pulmonary and mediastinal disorders, and has high sensitivity for detecting early disease.

• Magnetic resonance imaging (MRI): provides high quality images for detecting abnormalities in the brain, chest wall and spinal cord.

• Positron emission tomography (PET): allows evaluation of the radiolucent emission of positrons by the lesions detected, especially tumors, and is therefore a useful diagnostic test.

• Sputum cytology: a simple, non-invasive study for detecting bronchial malignancy, but less sensitive than other tests.

• Needle biopsy: used to obtain a cellular or histological diagnosis of pulmonary and pleural lesions. It is usually CT-guided.

• Bronchoscopy: comprises endoscopic examination of the bronchial tree with the possibility of cytological examination of bronchial aspirate, bronchoalveolar lavage and biopsy of possible endobronchial or pulmonary lesions (transbronchial biopsy).

• Other tests available are thoracoscopy, mediastinoscopy, bone marrow biopsy, and in general the examination of any other organ affected.

In our setting, the level of diagnosis of asbestos-related disease is low. The reason for this is the difficulty of establishing a history of exposure to asbestos through a clinical examination. In a high percentage of cases, diagnosis can be established through a medical history, radiographic examination and, if required, a conventional histological examination. However, sometimes there are doubts about the history of exposure, or the clinical history is unclear; what is more, the medical-legal considerations mean that it must be conclusively demonstrated that the patient's condition is caused by asbestos. Non-systematized clinical interviews have a lower sensitivity than structured questionnaires, as data from our group have shown[43]. One of the causes of this low sensitivity is the latency period between exposure and disease, as this may hinder the patient's accurate recall of the activities performed years before[76]. In countries like Finland and Belgium, the attribution of lung cancer to asbestos includes systematic analysis of asbestos in lung tissue.

Attribution of disease to asbestos

As far as patient management is concerned, it must be decided which cases require analysis of asbestos in the lung, and which techniques should be used. With regard to the first point, if the patient reports exposure or if the disease is specifically caused by asbestos, as is the case of malignant mesothelioma, further studies are not necessary.

However, the manifestations of the disease are often non-specific and the information obtained by the physician is incomplete, inaccurate, or inconsistent with the clinical and radiological picture presented by the patient. In these cases, the study of asbestos retained in the lung is particularly useful. Another important point is the fact that asbestosis, malignant mesothelioma, lung cancer and recently pleural and pericardial fibrosis in patients with demonstrated work-related exposure asbestos are classified as occupational diseases, with all the economic benefits that this represents for the patient[77].

The identification of asbestos fibres in the lungs of individuals with lung cancer or pulmonary interstitial fibrosis can attribute the disease to occupational exposure, even if the patient presents other risk factors such as smoking, or if the degree of exposure does not reach the accepted criterion for cumulative exposure estimated at more than 25 fibre-ml-year[78]. By the same token, their absence argues against a causal attribution.

A recent study compared the percentage of exposure to asbestos using a comprehensive questionnaire, which included occupational, domestic and environmental exposure, and determined the presence of ferruginous bodies (FB) in the lung in the general population and in patients with lung cancer. The sensitivity of the questionnaire for detecting a deposit of ferruginous bodies of 1000/g or more was 86%, and its specificity was 66%[43]. The ability to remove chrysotile after inhalation may explain its absence in the lung many years later; as a result, the finding of elevated levels of asbestos in the lung demonstrates exposure, but negative values do not completely rule out previous exposure to chrysotile.

Determination of asbestos bodies and asbestos fibres in lung tissue

A direct method for verifying previous exposure, and at the same time for establishing the magnitude of the risk, is the analysis of asbestos content in the lung. Asbestos can be detected by light microscopy in the form of ferruginous bodies encased by ferrous material inside the macrophages. Values of 1000 FB per g of dry lung tissue in lung biopsy or of one FB per millilitre in bronchoalveolar lavage are indicative of demonstrable occupational exposure[31]. To detect asbestos fibres, electron microscopy is required. If the aim is to

identify the chemical composition of the fibre and thus its type, a range of methods may be used, including energy dispersive X-ray spectroscopy (EDS). These methods must be carried out in a laboratory by trained staff who prepare the samples and count the fibres or FB. In addition, each laboratory must establish its reference values according to the population in its environment. Therefore, it seems clear that the asbestos content in the lung should be determined in specialized centres.

Another issue of practical importance is to decide the method of obtaining samples for analysis. In patients with diffuse lung disease, pleural injury, or lung cancer who are not candidates for surgery, bronchoscopy with bronchoalveolar lavage is recommended, as there is a good correlation between the number of FB in the bronchoalveolar lavage and in the lung[31]. In patients with resectable lung cancer, a piece of tissue can be extracted to analyze the asbestos content.

Ultimately, the most difficult aspect of the diagnosis of asbestos-related disease remains the determination of exposure. To improve diagnosis it is essential to train physicians, both primary care physicians and specialists, in the skill of taking occupational histories[79]. It is also vital to have access to specialist centres where exposure can be assessed through the analysis of lung asbestos content. At the time of writing, there was no centre of this kind in Spain.

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Hypotheses and objectives

Hypotheses

Chapter 1: People living in the city of Barcelona present asbestos deposition in the lung.

Chapter 2: The methods for quantifying asbestos in the lung using light microscopy are accurate and reproducible.

Chapter 3: Patients with lung cancer and patients with asbestosis have a higher AB content in the lung than healthy, unexposed individuals.

Chapter 4: Most asbestos fibres in the lung in the Spanish population are amphiboles.

Objectives

Chapter 1: To analyse the possible existence of AB deposits in the urban population of Barcelona and to determine the distribution of this mineral in three areas of the lung.

Chapter 2: To assess the reliability of the method currently used for AB counting based on sample examination by a single observer.

Chapter 3: To analyse the pulmonary levels of asbestos in shipyard workers from El Ferrol with pulmonary pathology and to study the possible relation with the years of exposure and smoking.

Chapter 4: To study pulmonary asbestos content via a characterization of its fibres.

Chapter 1

Prevalence and distribution of asbestos lung residue in a Spanish urban population

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Arch Bronconeumol. 2010 Apr;46(4):176-81

Abstract

Background

The purpose of the present study is to analyze the prevalence and distribution of asbestos lung residue in the Barcelona urban population.

Methods

Lung autopsy samples were obtained from 35 individuals who had lived in Barcelona. The close family was interviewed in order to rule out asbestos exposure. Samples were obtained from three areas of the right lung during the autopsy: upper lobe apex, lower lobe apex, and lower lobe base. The samples were treated to remove organic material. The inorganic residue was analyzed using a light microscope. The results were expressed as asbestos bodies per gram of dry tissue. Levels greater than 1000 AB/g of dry tissue were considered as potentially causing disease.

Results

AB were detected in 29 (83%) of the subjects, of which 86% had levels less than 300 AB/g. Only one individual (3%) had values greater than 1000 AB/g dry tissue. The asbestos residue was higher in the lower lung lobe in 17 individuals (48%) than in the rest, although no significant differences were seen as regards AB residue in the three lung areas studied.

Conclusion

The results of this study show that the urban population of Barcelona has asbestos levels in the lung that vary between 0 and 300 AB/g dry tissue. No differences in the asbestos residues were detected in the lung areas studied in this population.

Keywords: Asbestos. Light microscope. Lung. Urban population.

Introduction

Asbestos is a natural silicate with a fine fibre structure. Sufficiently intense inhalation of asbestos fibres increases the risk of contracting various respiratory diseases, including benign lesions such as pleural plaques, pleural effusion, pleural fibrosis, and more severe pathologies such as malignant mesothelioma, lung cancer and asbestosis.[1]

Asbestos was a frequently used material due to its wide industrial utility[2] .The boom in use of asbestos in Spain took place between 1970 and 1990; in 1992 Spain was the second largest European importer with 25,428t[2] and the total ban on its use did not come until 2002. Therefore, a large number of workers have been exposed to this mineral and will continue to be so into the future given the incorporation of asbestos into numerous structures and buildings. According to the voluntary registration of occupational respiratory diseases in Catalonia, diseases resulting from exposure to asbestos fibres are the second most common.[3]

The diagnosis of diseases caused by inhalation of asbestos is based on 3 factors: knowledge of exposure, patient manifestation of a compatible clinical profile and the exclusion of another disease that would justify the profile. In certain cases, there is a mismatch between the exposure and the clinical profile, which makes diagnosis difficult and often raises medical-legal issues. In these cases, it is necessary to determine the amount of asbestos in the lung tissue,[4,5] which requires a lung examination and the establishment of reference levels in each population through an analysis of individuals with no known occupational exposure.[6,7]

In Spain, the data relating to the pulmonary asbestos deposit in the population is limited to a study published by Monsó et al[8] in which necropsic samples were studied from 33 patients, 16 of which were residents of rural areas and 17 were from urban areas, along with samples from 8 patients with lung cancer without occupational exposure to asbestos. In this study, 50% of the individuals living in urban areas had asbestos bodies (AB) in the lung, compared to only 2 of the 16 (12.5%) living in rural areas.

To date there has been no study that provides more information on the Spanish population. This lack of evidence contrasts with the need for reference values, from which to differentiate the concentrations with pathological potential from those attributable purely to environmental exposures and that do not confer disease risk.

On the other hand, a key element in the interpretation of pulmonary AB values is the importance of the sample. Thus, to obtain representative samples for pulmonary asbestos deposits, it is crucial to know whether this mineral is deposited uniformly in the lung. The studies carried out so far, mostly on subjects exposed to asbestos, have yielded inconsistent results. The accumulation of asbestos in the various areas of the lung follows different patterns depending on the type of asbestos. Sebastien et al[9] showed a greater accumulation of chrysotile asbestos in peripheral areas. Morgan and Holmes[10] observed a greater concentration of anthophyllite in the lower pulmonary lobes, whereas Churg et al[11] found higher levels of asbestos in the upper regions of the lung. More recent studies continue to provide conflicting results, since Kishimoto et al[12] found chrysotile and amosite forming AB, both in the upper and middle lobes, while Teschler et al[13] concluded that the lower lobes contained the most asbestos. It should be noted that the population examined in these studies[9-13] had known exposure to asbestos, the majority of which was occupational. So far, in our area, there has not been a single study done on the distribution of asbestos in the lung.

The objective of this study was to analyze the possible existence of AB deposits in Barcelona's urban population and to determine the distribution of this mineral in 3 areas of the lung.

Material and Methods

Study Population

The study was conducted on 35 necropsic lung samples from individuals residing in the city of Barcelona, collected prospectively from June 2004 to June 2005 at the Instituto Anatómico-forense of Barcelona (Forensic Lab of Barcelona), (Table 1). Information on possible exposure of the patient was obtained through interviews with next of kin: spouse, children, parents or siblings. The sampling criteria were set as follows: lack of lung disease and residency in Barcelona for at least 10 years.

Table 1
Characteristics of the study population

Patient number	Age	Sex	Smoking habit	Job	Cause of death	Area 1 (ABs/g)	Area 2 (ABs/g)	Area 3 (ABs/g)
1	87	Male	Non smoker	Cable factory	Ischaemic heart disease	62	19	437
2	79	Female	Non smoker	Housewife	Heart failure	0	20	22
3	43	Male	Smoker	Messenger	Ischaemic heart disease	0	0	0
4	35	Male	Smoker	Messenger	Traffic accident	0	0	0
5	67	Male	Former smoker	Printing press	Aneurysm	0	89	20
6	77	Female	Non smoker	Housewife	Heart failure	321	245	667
7	43	Male	Non smoker	Teacher	Ischaemic heart disease	22	0	0
8	44	Male	Former smoker	Metallurgy	Ischaemic heart disease	0	40	146
9	44	Male	Smoker	Panel beater	Suicide	44	0	21
10	76	Female	Former smoker	Administrative work	Ischemic heart disease	62	0	120
11	78	Female	Unknown	Ironer	Head trauma	77	195	266
12	37	Male	Smoker	Waiter	Ischaemic heart disease	20	0	0
13	54	Male	Smoker	Electrician	Cerebral haemorrhage	22	0	0
14	78	Female	Non smoker	Metallurgy	Unknown	0	20	0
15	64	Female	Non smoker	Podiatrist	Heart failure	82	90	21
16	43	Male	Smoker	Journalist	Traffic accident	0	0	0
17	72	Female	Non smoker	Housewife	Suicide	0	38	66
18	44	Female	Smoker	Housewife	Pulmonary thromboembolism	0	22	22
19	49	Male	Smoker	Waiter	Heart failure	0	0	0
20	54	Male	Smoker	Graphic artist	Traffic accident	0	19	40
21	85	Female	Non smoker	Textile worker	Unknown	170	260	283
22	74	Male	Former smoker	Lathe operator	Sepsis	21	0	0
23	74	Male	Former smoker	Administrative work	Ischaemic heart disease	0	19	19
24	85	Female	Non smoker	Housewife	Choking	123	127	255
25	67	Male	Non smoker	Construction	Heart failure	283	777	160
26	52	Male	Former smoker	Driver	Digestive haemorrhage	0	0	18
27	86	Male	Smoker	Welder	Heart failure	472	333	1.307
28	82	Male	Former smoker	Sales agent	Ischaemic heart disease	0	18	81
29	70	Male	Smoker	Computer technician	Traffic accident	87	42	154
30	76	Male	Unknown	Watchman	Ischaemic heart disease	78	77	64
31	42	Male	Smoker	Mechanic	Suicide	0	0	0
32	48	Male	Former smoker	Electrician	Sudden death	490	265	604
33	82	Female	Non smoker	Housewife	Pneumonia	21	0	22
34	65	Female	Non smoker	Administrative work	Sudden death	77	80	132
35	85	Female	Non smoker	Domestic worker	Heart failure	0	0	0

Exposure Assessment

After verifying that the individuals met the inclusion criteria, participation in the study was proposed. All interviews were carried out by one of the researchers. In all cases, informed consent was requested from the next of kin to carry out a study of asbestos in the lung tissue. A history of exposure to asbestos was

investigated through a structured and comprehensive questionnaire, which had been used in previous studies.[14]

Protocol for Obtaining Samples

In each necropsic procedure, 2cm^3 lung samples were obtained from three areas of the right lung: upper lobe, apical segment (zone 1), lower lobe, apical segment (zone 2) and base of lower lobe (zone 3). The samples were fixed in formol and sent to our hospital's laboratory for analysis.

All samples were studied by a pathologist from our centre. The presence of chronic pulmonary disease was ruled out in all subjects.

Preparation of Lung Samples

For each sample, two 0.5g fragments of lung tissue that did not contain pleura or vessels were weighed. Subsequently, one of the samples was frozen, lyophilised and weighed to determine the weight of dry tissue. Since there is international agreement regarding expressing the results of AB in relation to the grams of dry lung tissue, a study was conducted on the correlation between weight of wet tissue and weight of dry tissue, yielding a Spearman correlation coefficient of 0.803 (fig. 1). The lyophilised sample was subsequently discarded, since lyophilisation can cause changes in the concentration and size of the fibres.[7]

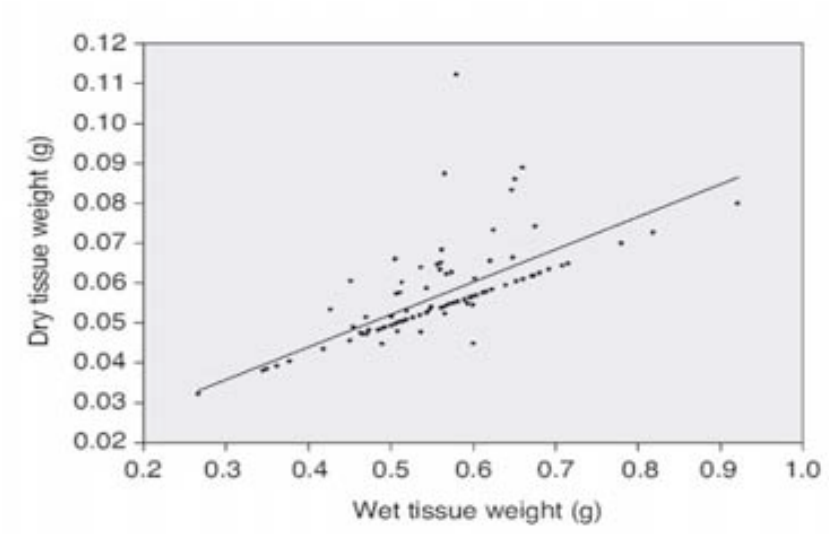

Figure 1. Correlation between weight of dry and wet tissue.

Then 30cc of previously filtered sodium hypochlorite was added to the non-lyophilised portion of the tissue, and was left to shake for 24 hours to facilitate tissue digestion and removal of organic matter. Subsequently, the sample was centrifuged at 3700 rpm for 20 minutes. After removing the sodium hypochlorite, the sample was resuspended in filtered distilled water. To dissolve the AB in the liquid, the sample was placed in an ultrasonic bath (UCI-50, 300W, 50/60Hz, Raypa S.L.) for 10". After further washing, the sample was resuspended in 20cc of filtered distilled water. The resulting solution was filtered using a 0.45µm diameter filter (Millipore Membrane filters HAWP02500). The filter was dried in an oven overnight at 37° C and transferred to a microscope slide for transparency by means of an acetone vaporiser (JS Holdings 240v/110v) for subsequent reading.

AB Analysis by Optical Microscopy

The analysis of each of the filters was performed using an optical microscope (OM) (Olympus CX21FS2, Olympus Life Science Europe GmbH, Hamburg, Germany) at 400x magnification. Pulmonary asbestos content for each individual was obtained for each of the 3 lung areas analyzed.

The OM examination helps identify ferruginous bodies. This study, which is in line with international standards[7], assumed that these bodies correspond to AB. In fact, although it is known that other minerals may have the same appearance as AB, Churg and Warnock showed that in lung tissue samples from individuals exposed to asbestos, the majority of ferruginous bodies correspond to ABs.[15]

The highest value for the 3 zones analyzed was considered to be the final value for each subject. Those levels that exceeded 1,000 AB per gram of dry tissue were considered to be potential causes of pathology, in accordance with criteria established by the *European Respiratory Society* (ERS) working group in 1998.[7]

Statistical Analysis

Data were expressed as mean and standard deviations (SD). The distribution of the AB values, analyzed by means of the Kolmogorov-Smirnov test, was normal, so the differences between the lung zones were analyzed by means of

the Student's t test. The Pearson correlation coefficient was calculated to establish the relationship between the various parameters analyzed. The statistical analysis was performed using SPSS software, version 12.0, for Windows (SPSS Inc, Chicago, IL, USA).

Results

Characteristics of the Study Population

Table 1 shows the main characteristics of the study population and the concentration levels of asbestos in each of the 3 lung areas analyzed in each individual.

The average age of the 35 patients was 64 years (range 35-87). Of the group, 22 were men (62.9%) and 13 were women (37.1%). In terms of occupation, the most significant were housewives, construction workers and those related to metallurgy. None of the families interviewed mentioned any history of workplace exposure to asbestos for the individuals studied. The principal causes of death were from heart disease and traffic accidents. The presence of chronic pulmonary disease was ruled out through interviews with the subject's next of kin. Furthermore, the histological examination performed in the study ruled out this condition.

Pulmonary Asbestos Content

The mean (SD) of the values obtained was 167 (280) AB/g of dry tissue. Only one individual (3%) had values higher than 1,000 AB/g. Four individuals (11%) had a concentration between 300-1,000 AB/g, while 30 (86%) had levels below 300 AB/g (Table 1). In 6 of the individuals of this last group (17%) no AB were found in the lung. There was no significant correlation observed between AB levels and age (correlation coefficient: 0.347).

The average concentration of asbestos in the group of men was 176 AB/g (range: 0-1307) and in the group of women was 151 AB/g (range: 0-667). There were no significant differences observed between the two groups. Information was available on the smoking habits of 33 of the patients, of which 20 were or had been smokers and the rest were non-smokers. There were no significant differences observed in the levels of AB/g of dry tissue between the 3 groups.

Distribution of Asbestos Content per Lung Area

The mean values of AB/g for each of the pulmonary lobes in the autopsied population studied were 72 (range: 0-490) for zone 1, 80 (0-777) in zone 2, and 141 (0-1307) in zone 3. In 17 individuals (48%), the asbestos deposit was greater in the lower pulmonary lobe (area 3) (fig. 2). However there were no significant differences observed in terms of AB deposit, taking neither into account the 3 areas studied nor in the possible comparisons between two zones. The Pearson correlation coefficient between the different lung zones was 0.6 between zones 1-2, 0.7 between zones 1-3, and 0.8 between zones 2-3.

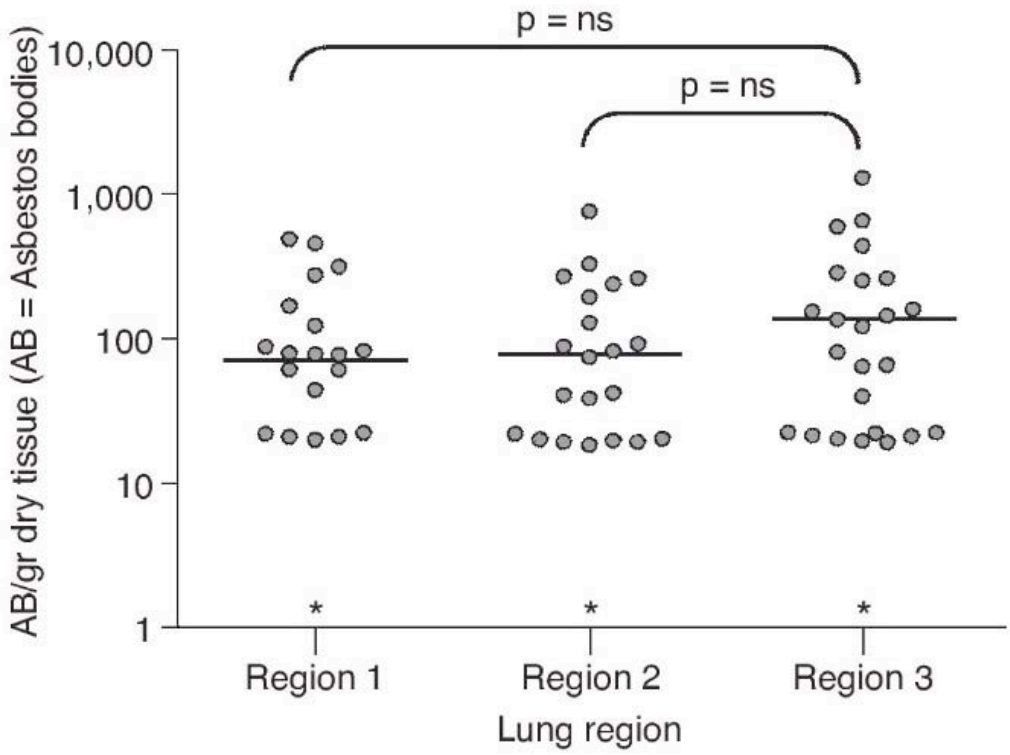

Figure 2. Pulmonary asbestos deposits in the different areas studied.

Discussion

This study provides the first data on the distribution of AB in the urban Spanish population. The results of this study show that there are no differences in the asbestos deposit in the analyzed lung areas.

The average concentration of asbestos in the lower pulmonary lobes was slightly higher than in the upper lobes, although this difference was not statistically significant. The homogeneity of the asbestos deposit was also shown by the good correlation of values in the different areas. As detailed in the introduction, several studies previously published in other countries have shown conflicting results in exposed individuals. Our population, however, had no occupational exposure to asbestos. The results seem to indicate that lower asbestos exposures do not result in differences in the deposit of AB between different lung areas. This fact is relevant when evaluating results obtained from clinical samples, which normally correspond to one area of the lung. Therefore, according to the results of this study, it can be confirmed that in these individuals the analysis of one single area of the lung is representative of the whole lung. However, despite the absence of statistical differences, we have observed a case in which the above must be questioned. The case in question is that of an 86-year-old man, a smoker, who had worked as a welder. The asbestos analysis showed values of 472.333 and 1,307 AB/g of dry tissue in the upper lobe and the middle and lower lobes, respectively. Therefore, in this case, according to the studied area, results were obtained above and below the threshold of 1,000 AB/g of dry tissue, which has been accepted internationally as an initiator of pulmonary and pleural pathology[7]. In our opinion, this case could involve occupational exposure that was not known to the family. In fact, working as a welder can be considered a risk for inhalation of asbestos and it is well known that the exposure questionnaires, obtained from family members, may suffer from a low diagnostic sensitivity[7]. With this exception, we believe there is high reliability in the diagnosis of pulmonary asbestos, even when analyzing only one area of the lung.

To date only one study has been published in Spain that provides values for pulmonary asbestos in the population that is not exposed to it in the workplace[8]. Ours is now the second study and has a greater number of cases relating to the urban population. The values measured in the study were lower than 300 AB/g of dry tissue in 86% of the study population. Compared to the Monsó et al study, the current study observed a lower percentage of cases with absence of asbestos (6/35, [17%]) compared to 8/18 (44%). Comparing the average of cases that showed presence of asbestos, Monsó et al found a mean value of 95.14 AB/g of dry tissue, while in the current study the value was 201 and an isolated case with a value greater than 1,000. These differences may be due to the fact that our study analyzed 3 lung areas and determined the maximum global value to be 3. We cannot rule out either the fact that there are differences in the degree of exposure among the population, even when that exposure is not due to the workplace. The presence of asbestos in the lungs of subjects that were believed not to be exposed can be explained by the ubiquitous presence of the silicate in the cities' air. In addition to environmental inhalation, which can be increased if the person has lived near industries that handle asbestos, there are other sources of exposure that often go unnoticed both at home and in the workplace.

Table 2
Asbestos bodies in lung tissue samples of non-exposed populations of different countries

Country or area	Population (n)	ABs/g of dry tissue	Reference
Barcelona (Spain)	8	0/8 > 100	8
Canada	81	9/81 > 750	16
Quebec (Canada)	49	24–471	17
Switzerland	137	4/137 > 500	18
Vancouver (Canada)	20	280 (average)	19
Tokyo (Japan)	390	0/90 > 40 5/300 > 400	20
Giessen (Germany)	41	3/41 > 3.000	21

With respect to series from other countries, it is noteworthy that the ranges of AB/g of dry tissue vary depending on the population but are comparable to those obtained in our study. In most of those series, the average values were lower than 500 AB/g of dry tissue[8,16-20], although individuals with values above 1,000 were encountered. In one German series, 3/41 non-exposed subjects

presented levels higher than 3,000 AB/g of dry tissue[21] (Table 2). This variability may be due in part to technical differences between laboratories, which confirm the need for reference values obtained for each population with each sample treatment protocol used. As for the extreme values above 1,000 AB/g of dry tissue, as was noted earlier, these reflect the lack of sensitivity that the case history can have in the detection of exposure to asbestos.

On the other hand, the possibility that an AB analysis underestimates the exposure should not be ruled out. Indeed, it should be noted that asbestos, after being inhaled, goes through a drainage process that alters its final deposit. Chrysotile, for instance, is known to undergo considerable drainage after inhalation, which means that the chrysotile deposit detected years afterwards may not reflect the intensity of the exposure.[22]

This has special significance in our environment, since it has been reported that the majority of asbestos imported in recent years corresponds to chrysotile.[2] In short, one must contemplate the possibility that, as in all cases that analyze AB content, said deposit does not fully reflect the actual amount of asbestos a population has been exposed to.

The AB content of our population was analyzed in relation to certain variables of interest. In regards to smoking, the Selikoff et al[23] study showed increased deposits of asbestos in smokers with lung cancer. In our study, an increased deposit of AB was not detected among smokers as compared to non-smokers. Our results are similar to those obtained by Monsó et al[7], which suggest that smoking does not change the deposit of asbestos in the lung for low levels of exposure. In terms of age, there was no significant correlation with AB values in the lungs in our population. Previously, a Canadian study observed a tendency for the average value of pulmonary asbestos to rise as the population aged, although a statistical difference was not demonstrated[16]. Although one might think *a priori* that the deposit of AB should be proportional with the number of years lived in an urban setting, the clearing effect produced in the case of chrysotile[22, 24], one of the most common fibres employed in our environment, has already been discussed. However, we believe that this issue is open to further analysis in series with greater numbers of subjects.

A limitation of this study may be possible bias due to the indirect collection of work history. In this respect, De Vuyst el al[7] affirm that studies carried out in

reference population groups (autopsic) allow for optimum sampling, while lung cancer groups make it is possible to perform a direct anamnesis, although with risk of bias towards a greater load of asbestos, and therefore an optimum correlation between antecedents and pulmonary asbestos. In our case, since the objective was to analyze the possible existence of AB deposits in the urban population of Barcelona that had no respiratory pathologies, it was necessary to opt for an autopsy population when sampling, assuming that the indirect medical history may not be accurate.

In conclusion, the results of this study show that the majority of the urban population in our area has levels of asbestos in the lung, with values falling below 300 AB/g of dry tissue. As a result, we believe that the threshold of 1000 AB/g is fully applicable for differentiating asbestos contents with pathological values. In the analysis of the lung areas studied, there were no differences detected in terms of asbestos deposits, with good correlations between values.

Conflict of Interest

The authors affirm that they have no conflicts of interest.

Acknowledgements

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Chapter 2

Reproducibility of asbestos body counts in digests of autopsy and surgical lung tissue

María Isabel Velasco García, María Jesús Cruz, Laura Ruano, María Ángeles Montero, Asunción Freixa and Jaume Ferrer

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Abstract

Background

Asbestos body counting by a single observer is the most commonly used objective technique to demonstrate asbestos deposition in the lung. In this study, the accuracy and reliability of this procedure is analyzed by evaluating the degree of agreement between two experienced readers.

Methods

Lung tissue specimens from 66 individuals, most of who had not been exposed to asbestos, were studied: 35 were obtained in postmortem studies (upper, middle, and lower lung) and 31 were from patients who underwent surgery for lung cancer. Overall, 167 samples were analyzed. Lung tissue sections weighing 0.5 g were obtained prospectively and processed, and the inorganic residue was analyzed by light microscopy (LM) at 400x magnification by two experienced readers. Results were expressed as AB/g of dry lung tissue. Interobserver variability was analyzed using the Spearman correlation coefficient and agreement was evaluated by the Bland-Altman method and kappa index.

Results

The interobserver correlation was 0.8975: 0.8029 for autopsy samples and 0.9592 for biopsy samples. Bland-Altman plots showed that most values were grouped around the 95% limits of agreement. The kappa index was 0.87 for all samples, and 0.79, 0.65, and 0.54 for upper, middle, and lower lung specimens, respectively.

Conclusion

Asbestos body counting by a single reader is a reliable method, especially at low concentrations of asbestos bodies in lung. Double reading may be indicated in borderline cases with asbestos body levels close to levels of 1000 AB/g.

Introduction

Asbestos is a natural fibrous silicate mineral that has been widely used for industrial purposes[1]. Inhalation of asbestos fibres increase the risk of developing several respiratory diseases, including pleural plaques, fibrosis, and effusion, as well as more severe diseases, such as malignant mesothelioma, lung cancer, and asbestosis[2]. Asbestos-related conditions occupy the second position in the voluntary registry of occupational diseases in Catalonia[3].

Diagnosing asbestos-related diseases may be difficult, because the clinical picture is confusing or because there is a lack of information regarding the patient's exposure status. The problem in these cases is not only clinical, but also legal, since economic compensation is frequently involved[4]. In several countries, management of these difficult cases requires demonstration of asbestos deposition in the lung. The finding of elevated levels of asbestos in lung tissue is definite proof of exposure and provides powerful evidence favoring the diagnosis of asbestos-related disease[5,6].

AB counting using light microscopy is the simplest way to determine the lung asbestos content[7]. The threshold of 1000 AB/g dry tissue has been proposed as an indicator of significant asbestos lung burden. There is some agreement that above this threshold, there is a risk for the development of asbestosis[6]. Nevertheless, in the case of mesothelioma, slight exposures and AB counting below this threshold have been reported[5]. As such, the precision of AB counting is important, particularly in cases with AB values close to the threshold. In these cases, even small errors in the reading could imply a difference when attributing a specific disease to asbestos.

International guidelines have pointed out that sample preparations for this purpose vary considerably and have advised individual laboratories to standardize their reference values[6]. However, little attention has been focused on determining the reliability of AB reading by a single observer. AB evaluation includes detection and counting. As in many other measurement systems performed by humans, it depends on the personal experience of the observer and implies a certain margin of error. Considering that the reader is an inherent source of variation in AB counting[8], it is important to know the interobserver

reproducibility of the lung AB results to determine the number of readers needed to accurately carry out the technique.

The aim of this study is to determine the reliability of the currently used method for AB counting, based on sample examination by a single observer.

Material and methods

Sixty-six individuals were included in the study, distributed in two groups: 35 cadavers of persons with no medical record of respiratory disease undergoing post-mortem examination (group A) and 31 patients with lung cancer undergoing surgery (group B) (Table I).

TABLE I. Asbestos Body Values in the Study Population

	N samples	Reader 1 AB/g dry tissue[a]	Reader 2 AB/g dry tissue[a]	K	95% CI
Total (groups A and B)	169	43 (0–32146)	42 (0–30875)	0.869	0.766–0.972
Group A					
Total	105	21 (0–1308)	19 (0–1212)	0.645	0.342–0.949
Zone 1	35	21 (0–490)	0 (0–429)	0.785	0.370–1.200
Zone 2	35	20 (0–778)	0 (0–489)	0.653	0.016–1.323
Zone 3	35	22 (0–1308)	21 (0–1212)	0.535	0.033–1.038
Group B					
Total	64	102 (0–32146)	72 (0–30875)	0.930	0.833–1.026

[a]Data expressed as median (range).

A specific questionnaire was administered in all cases to evaluate asbestos exposure[9]. The questionnaire was completed by the patients, themselves, in group B and by close relatives in group A. The study was approved by Ethic Committee of the Vall d'Hebron Hospital in Barcelona. All patients in Group B and the relatives in Group A gave their written consent.

Sample Collection Protocol

Post-mortem examinations were performed at the *Instituto Anatómico-Forense de Barcelona* (Forensic Anatomy Institute of Barcelona). At group A, lung tissue specimens 2 cm^3 in size were obtained from three regions of the right lung: apex of the upper lobe (zone 1), apex of the lower lobe (zone 2) and base of the lower lobe (zone 3). The 105 samples obtained were fixed in formol and sent to our hospital laboratory for analysis.

The surgical specimens (group B) were obtained from the Pathology Department of Hospital Vall d'Hebron. The size of the specimen was 2 cm^3 whenever possible. The region of the lung sampled depended on the size where the tumour had developed: specimens came from the right lung in 14 patients and from the left lung in the remaining 17. Sixty-four samples were obtained in this group, with more than one from some patients.

All lung specimens were examined by a pathologist from our hospital. The presence of chronic pulmonary disease was ruled out in all cases.

Preparation of Lung Samples

Two 0.5 g fragments of lung tissue that did not contain pleura or vessels were obtained from each specimen. One of these fragments was frozen, lyophilized, and weighed to determine the dry tissue weight, in keeping with an international agreement to express the AB results in terms of grams of dry lung tissue. Once the dry weight was known, the lyophilized sample was discarded, because this technique can cause changes in the concentration and size of the fibers[6].

A 30 cc amount of filtered sodium hypochlorite was added to the non-lyophilized tissue section, and the sample was shaken for 24 h to facilitate digestion of the tissue and elimination of other organic material. The sample was then centrifuged at 3700 rpm for 20 min, sodium hypochlorite was eliminated, and the sample was re-suspended in filtered distilled water. To dissolve the AB present in this liquid medium, the sample was sonicated for 10 min using an ultrasonic water bath (UCI-50 Raypa SL; 300 W, 50/60 Hz), and was then washed and re-suspended in 20 cc of filtered distilled water. The solution obtained was passed through a 0.45 µm pore diameter filter (Millipore membrane filters, HAWP02500). The filter was dried overnight at 37ºC, placed on a microscope slide and made transparent using acetone vapor (JS Holdings vaporizer; 240 v/110 v) for subsequent reading. In all the samples, we obtained an adequate filter loading for counting, and it was not necessary to dilute the sample.

Asbestos Body Counting by Optic Microscopy

The filters were viewed with an optic microscope (CX21FS2; Olympus Life Science Europe GMBH, Hamburg, Germany) at 400x magnification. All filters were examined by two experienced readers according to a protocol previously described by our group[9].

All the samples obtained for each individual were assessed: in group A, the values obtained for each of the three lung zones were evaluated, and in group

B, all the zones from which a sample could be obtained. In accordance with the criteria established by the working group of the European Respiratory Society in 1998, AB levels exceeding 1000 AB/g dry tissue were considered significant[6].

Statistical Analysis

The data are expressed as the median and the range (r). On analysis with the Kolmogorov-Smirnov test, AB values did not follow a normal distribution; hence, the Wilcoxon test was used to determine the differences between groups. The Spearman correlation coefficient was calculated to establish the relationships between the parameters analyzed. The statistical analysis was carried out with GraphPad software (2002-2005).

Interobserver variability in AB counts was compared using Bland-Altman difference plots[10]. Logarithmic transformation of the data was then used to correct the relationship between the ratio and the average. The 95% confidence interval for the difference was used to assess the differences between the two readers.

The kappa coefficient (κ), corresponding to the percentage of agreements over the total of observations, excluding agreements attributed to chance, was used to determine the degree of interobserver variability. For this purpose, the AB counts obtained were divided into three groups: (1) 0-300 AB/g, (2) 301-1000 AB/g, and (3) >1000 AB/g. As was reported in a previous study[9], the majority of the urban population in our setting has an AB lung content of less than 300 AB/g.

Results

Study Population

Individuals in group A had a mean age at death of 64 years (r: 35-87); 63% were men, 36% had been smokers and 24% ex-smokers. The most common cause of death was heart failure. Patients in group B had a mean age of 62.5 years; 87% were men, 6% were smokers and 84% ex-smokers, and all had lung cancer.

Lung Content of Asbestos

The median (range) of the values obtained by the two readers was 43 (0-32.146) AB/g for reader 1 and 42 (0-30.875) AB/g for reader 2 (Table I). Only six individuals (3.6%) presented AB values higher than 1000 AB/g of dry tissue (one from group A and five from group B). The cause of death in patient in group A with high levels of AB was cardiovascular disease. In group B, five patients had lung tumours, including three with adenocarcinoma and two with epidermoide carcinoma.

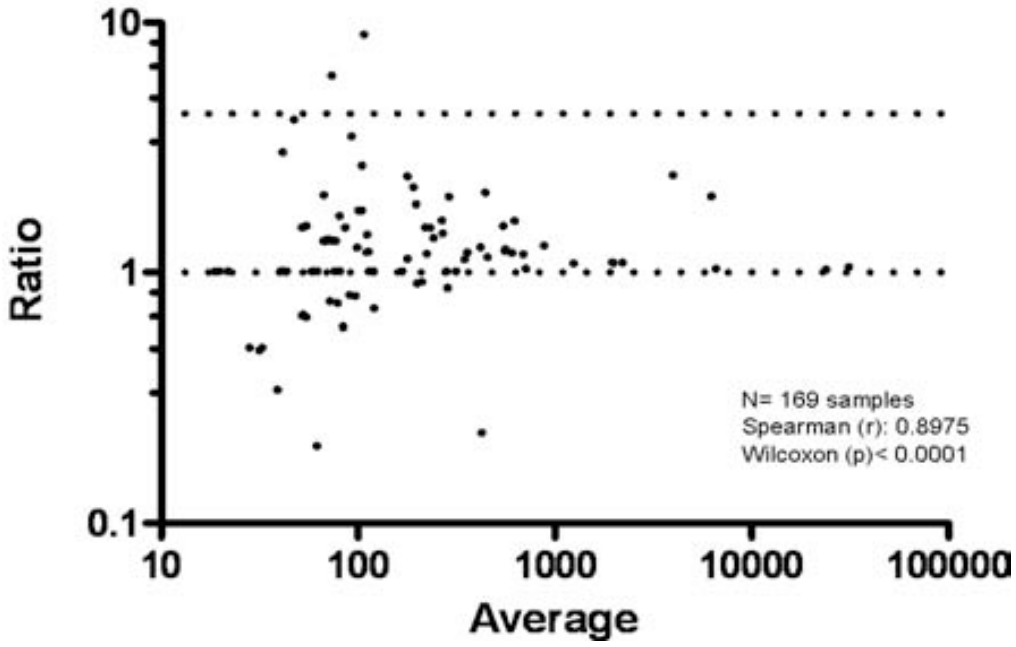

Figure 1. Bland-Altman plot of all 66 individuals (169 samples; Dashed lines: 95% confidence interval for the difference).

Interobserver Variability

Bland-Altman plots were generated for the entire population analyzed (Fig. 1), for each of the groups (A and B) separately (Fig. 2), and for each of the 3 lung zones in group A (Fig. 3). In these plots, the x-axis is the Average between the determinations of reader 1 and reader 2, and the y-axis is the Ratio of the 2 determinations.

An acceptable correlation between the two readings was found for the entire study population (r = 0.8975; Fig. 1) Reader 1 reported a 20% higher level of AB/g on average that reader 2. Following classification of AB values into groups, the kappa index was 0.869; the differences between the ratios were 0.766 and 0.972 in approximately 95% of cases.

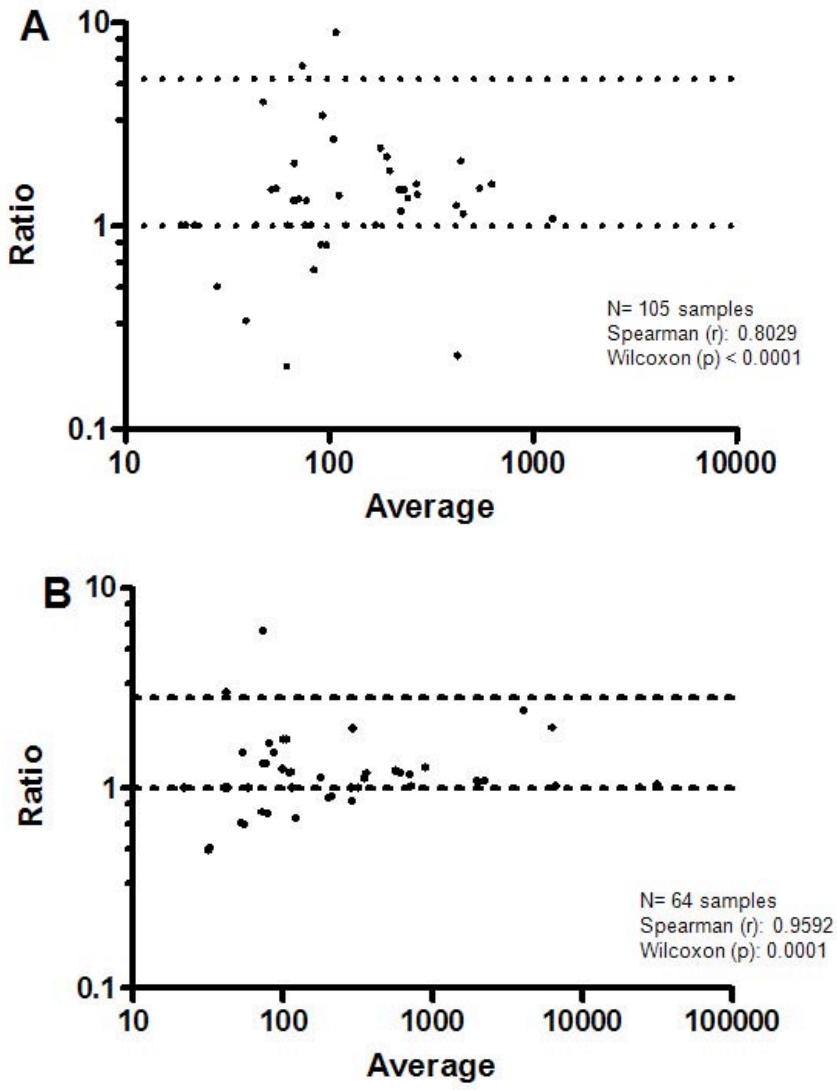

Figure 2. Bland-Altman plot. (**A**) Group A: 35 autopsy studies (105 samples), (**B**) Group B: 31 biopsies (64 samples) (Dashed lines: 95% confidence interval for the difference).

As is shown in Table I, the median count in group A was 21 AB/g for reader 1 and 19 AB/g for reader 2, yielding a Spearman correlation coefficient of 0.8029. In group B, the median count was 102 AB/g for reader 1 and 72 AB/g for reader, with a correlation coefficient of 0.9592. Kappa indices were 0.645 and 0.930 for groups A and B, respectively. Differences between the ratios were 0.833 and 1.026 for group B in approximately 95% of cases.

In group A, AB counts for the three lung regions studied were as follows: in zone 1, the median was 21 AB/g for reader 1 and 0 AB/g for reader 2, with a correlation coefficient of 0.9496 (κ = 0.785); for zone 2, the median was 20 AB/g for reader 1 and 0 AB/g reader 2, with a correlation coefficient of 0.7246 (κ = 0.653); for zone 3, the median was 22 AB/g for reader 1 and 21 AB/g for reader 2, with a correlation coefficient of 0.7270 (κ = 0.535).

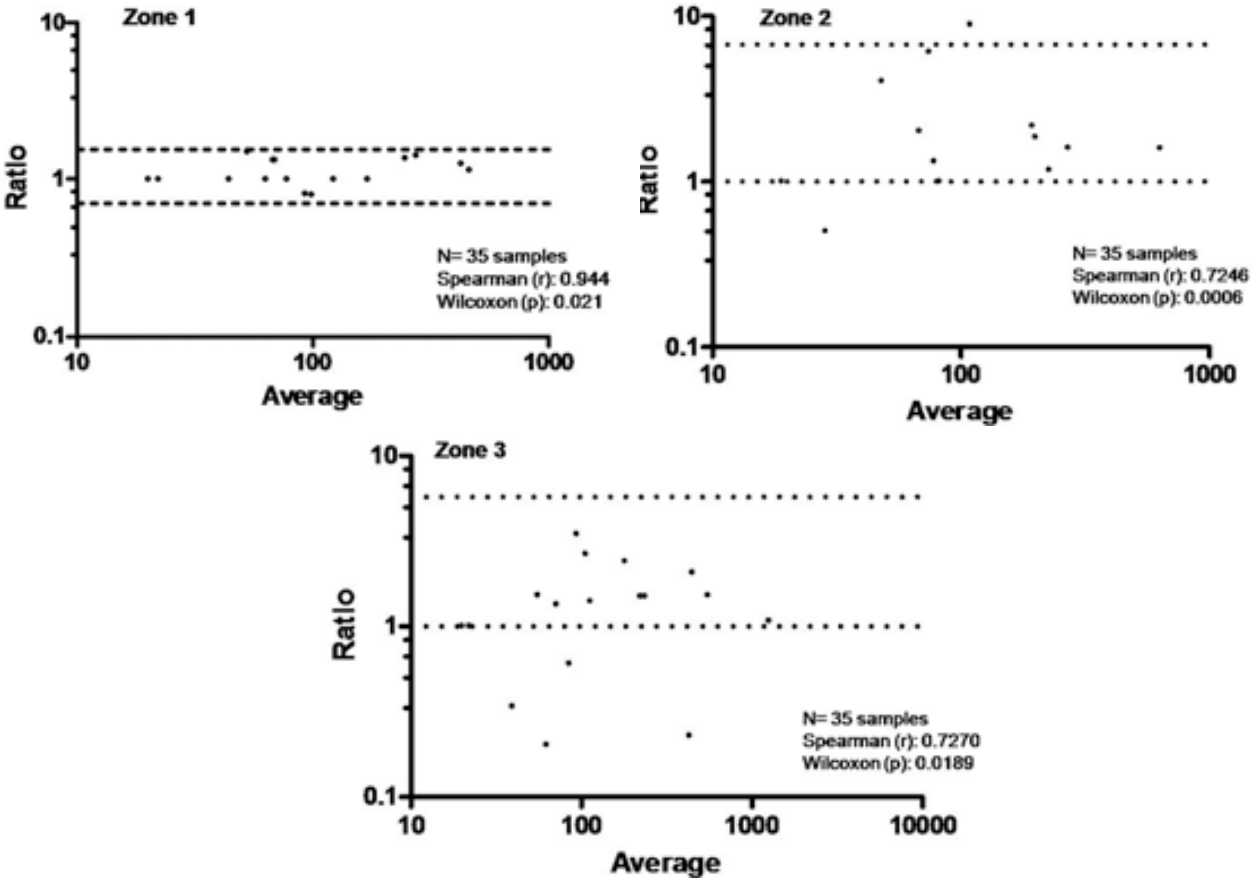

Figure 3. Bland-Altman plot of group A. (**a**) zone 1, (**b**) zone 2, and (**c**) zone 3 (Dashed lines: 95% confidence interval for the difference).

Both readers reported AB values exceeding 1000 AB/g in five specimens, and in one other case, reader 1 reported a value above 1000 AB/g whereas reader 2 reported a value below this cut-off. The median in these six cases was 2091 AB/g for reader 1 and 1932 AB/g for reader 2, with a correlation coefficient of 0.9725. The degree of interobserver variation for AB count in samples with counts greater than 1000 AB/g was 17%; therefore, interobserver agreement when determining counts greater than 1000 AB/g was 83%.

Discussion

There is an increasing need for lung asbestos measurement in the diagnosis of asbestos-related diseases for both medical and legal reasons[11]. For many years, the presence of AB in tissue sections has been an aid in the diagnosis of asbestosis. For other asbestos-related diseases, evaluation of asbestos lung burden requires AB or fibre counting in lung or bronchoalveolar lavage fluid[6].

Asbestos body count in digested lung samples by light microscopy is the most widespread method to evaluate asbestos exposure. Despite the usefulness of this method, however, AB counting depends on the ability of the observer to detect these bodies in samples extracts. These and other factors expose the technique to a certain degree of variability. Some effort has been dedicated to determining the variability implicated in sample preparation[12], and this had led to the recommendation that reference values be determined in each laboratory to define pathologic levels. One important related aspect that has received little attention is interobserver variability in detecting and counting AB in lung preparations. The results of this study demonstrate a good correlation between the findings of the two experienced readers, thus indicating that AB counts determined by a single observer are reliable.

Among the methods used to determine interobserver agreement in this study, the best indicator was the Bland-Altman test, which showed that values were grouped around the 95% limits of agreement. Considering the data as a whole, these limits are acceptable for high and low values. The high concordance between counts was confirmed by the correlation index and the kappa index. The latter is has been widely used to evaluate subjective measurements between observers. The kappa index obtained in our study was 0.869, which indicates very high agreement between observers. This figure is higher than the acceptable kappa indexes obtained in radiologic studies of lung cancer patients, which range from 0.58 to 0.64[13].

Our results were obtained in a population with relatively low lung AB content. Since most of the individuals studied have not been occupationally exposed to asbestos, further studies are needed in order to know if the results obtained are applicable to more highly exposed individuals. Our literature review retrieved

two articles evaluating the reproducibility of lung AB counting in individuals who had been exposed to asbestos[14-15]. Roberts et al. reported an interobserver kappa index of 0.60, although the semiquantitative method used to measure AB (none, few, and many) is probably less accurate than the method described in the present study. Wright et al. compared reproducibility between three observers using surgically resected lung, and they found an adequate agreement between the three readers' counts of AB in the samples analyzed.

The degree of agreement between readers was high when all samples were considered as a group. Nonetheless, the variability according to the lung zone analyzed is also a relevant issue. In clinical practice, lung samples are taken from a specific zone, but the available data on the distribution of asbestos in the lung are inconclusive. Whereas significant variability between lobes has been described in exposed individuals[16-20], the distribution in a reference population seemed to be homogeneous[9]. In the present study, a good level of agreement between readers was evident for the three zones examined. The poorest kappa index corresponded to results for the lower lobe, although the kappa value of 0.54 is considered a reasonably good level of agreement. Findings from the present study provide the first evidence that AB counting in clinical practice is a reliable measure regardless of the lung area sampled.

Although the analysis including all samples showed high concordance, there were some discrepancies between readers when AB content in each subject was considered separately. In one biopsy, the results reported by the two readers were above and below 1000 AB/g, respectively, and in two other biopsies, the counts were situated on both sides of a 5000 AB/g level. These discrepancies may be relevant, because 1000 AB/g is the cut-off commonly used to consider that a patient's lung deposition can give rise to asbestosis. Thus, although our results indicate that lung asbestos count carried out by one observer is generally reliable, we believe that caution should be used in borderline cases. In cases yielding AB values close to a specific threshold, examination by two readers should be carried out whenever possible. This method would be especially useful in individuals in whom the values reported are slightly below the 1000 AB/g level, because the risk of underestimation is higher at these levels and errors in this regard could lead to misdiagnosis and unfairly refused legal and economic compensation.

As mentioned, AB examination by light microscopy is a simple, cheap and reproducible method. However, light microscopy has a limited ability to detect uncoated fibres, thus underestimating total lung asbestos burden. Moreover, Chrysotile is known to from asbestos bodies poorly, limiting the utility of light microscopy to measure chrysotile asbestos lung burden.

In conclusion, AB counting in lung specimens showed good reproducibility, especially at low concentrations of asbestos bodies in lung tissue, as evidenced by an excellent degree of agreement when counting was carried out by experienced readers. Thus, this commonly used technique to determine asbestos deposition can be considered accurate and fair. Double reading may be indicated in cases in which the lung AB count is close to 1000 AB/g or higher.

Acknowledgements

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Chapter 3

Asbestos pulmonary content in workers of Ferrol shipyards, Spain

Carmen Diego, María Isabel Velasco García, María Jesús Cruz, María Dolores Untoria, Ferran Morell and Jaume Ferrer

Med Clin (Barc). 2013 Feb 16;140(4):152-6

Abstract

Background

Deceased shipyard workers from El Ferrol, Spain, exposed to asbestos during their working lives have been studied in a research project designed to diagnose exposure-related diseases. The objective of the present study was to report the asbestos content of lung tissue in this population.

Methods

Autopsy pulmonary samples were obtained from individuals who had worked in the shipyards of El Ferrol, Spain. In most cases, samples from both lungs were analyzed. After removal of organic matter with sodium hypochlorite, the inorganic residue was analyzed using optical microscopy. Results were expressed as asbestos bodies per gram of dry tissue. Levels above 1000 AB/g were considered to be causative of disease.

Results

The sample comprised 30 male subjects with a mean age at time of death of 67 years (range 56-89 years). Twenty-six were smokers or former smokers, and four had never smoked. All had pulmonary, pleural or peritoneal disease related to asbestos exposure (16 lung cancer, six mesothelioma, and 25 benign pleural disease). Only six out of the 16 lung cancer subjects presented concomitant asbestosis. The median (interval) AB count was 6,171 (249-4.660.059) AB/g. Ninety-seven per cent had levels above 1000 AB/g. A correlation was found between AB and age (r=.5676; P=.0011).

Conclusion

These ex-shipyard workers from El Ferrol, Spain presented high levels of asbestos in the lung. Asbestos should be considered as a potential cause of pulmonary disease in shipyard workers.

Introduction

Asbestos is a fibre which, if inhaled, carries a high risk of respiratory disease. Exposed individuals may develop pleural plaques, fibrosis and pleural effusion, malignant pleural mesothelioma, lung cancer and asbestosis[1]. In Spain, between 1970 and 1990 more than two million tons of asbestos were imported for use in industry[2]. The use and commerce of all types of asbestos was not totally prohibited until June 2002, and so a large number of workers were exposed over this period. Several reports have suggested that of all occupational diseases asbestos-related diseases are the second most frequent[3-6].

Although most studies have shown a direct link between exposure to asbestos and cancer, the relations between the dose and the response vary substantially in the populations analyzed. The risk of developing cancer due to asbestos is related with accumulated exposure, the type of industry and the type of fibre. Moreover, several studies have demonstrated a higher risk in subjects with high exposure and also a substantial synergy with smoking[7, 8].

In the 1950s areas like El Ferrol, in north-western Spain, developed flourishing shipbuilding industries. Asbestos was used to provide thermal insulation for ships, and as a result many shipyard workers were exposed to high environmental concentrations of the mineral during their working day. According to the Galician Association of Asbestos Victims (AGAVIDA), in El Ferrol alone 3,000 workers have presented asbestos-related diseases. Navantia, the shipyard owner, recently provided the public heath services with a list of 6,007 workers who had had some degree of contact with asbestos during their working lives[9] and who, in accordance with the Spanish health ministry's inspection protocol and the legislation passed in 2006, were advised to attend regular preventive medical examinations[10].

For several years now a study has been conducted of the shipyard workers of El Ferrol in order to establish the diagnosis of the diseases deriving from their

exposure to asbestos. In many cases, the diagnosis is based on the knowledge of the exposure associated with a compatible clinical picture, in the absence of distinctive signs of other potential causative diseases. One of the basic tests in this process is the identification and counting of asbestos bodies in the lung[7,11-12]. A recent study in Spain published reference levels for AB in the lung in the non-exposed population[12], and found that the international threshold of 1000 AB[13] for classifying values potentially causative of disease was applicable in our environment[13]. Of course, it should be borne in mind that the identification and quantification of asbestos bodies is a sign of exposure rather than disease.

The asbestos content in lungs of workers exposed to asbestos in Spain has not been assessed to date. The aim of the present study was to analyze the lung asbestos content of deceased shipyard workers in El Ferrol in whom autopsy could be performed.

Material and methods

Study population

The study was performed in 30 lung autopsy samples from shipyard workers from the city of El Ferrol, recorded between 2008 and 2011 at the Pathology Service of the Hospital Arquitecto Marcide (Table 1). Information regarding the subject's possible exposure was obtained from an interview with a close family member. Criteria for inclusion were a history of work-related exposure to asbestos and a suspicion of disease related to this exposure.

Tabla 1
Características de la población de estudio

Id	Edad (años)	Hábito tabáquico	Años de exposición a amianto	CA/g	Tipo de trabajo en los astilleros	Diagnóstico
1	57	Ex fumador	NR	2.310	NR	Adenocarcinoma pulmonar, fibrosis pleural
2	60	Fumador	NR	2.767	Máquinas	Carcinoma epidermoide pulmonar
3	73	Fumador	33	23.576	Calderería	Adenocarcinoma pulmonar, placas y fibrosis pleural
4	60	Fumador	30	4.378	Armador	Adenocarcinoma pulmonar, fibrosis pleural
5	86	Ex fumador	44	107.493	Electricista	Adenocarcinoma pulmonar, asbestosis, fibrosis pleural
6	68	Fumador	40	20.992	Tubero	Mesotelioma pleural maligno, placas pleurales
7	67	Fumador	NR	5.326	Tubero	Mesotelioma pleural maligno, placas pleurales
8	89	Fumador	30	7.282	Armador	Adenocarcinoma pulmonar, asbestosis, placas pleurales
9	63	Fumador	20	6.150	Ajustador-montador	Carcinoma vesical, placas pleurales
10	77	No fumador	27	47.991	Monturas a flote	Metástasis pulmonares de adenocarcinoma de sigma, placas pleurales
11	69	Fumador	21	5.744	Calafate	Asbestosis
12	56	Fumador	36	3.330	Armador	Adenocarcinoma pulmonar, asbestosis, placas pleurales
13	61	Ex fumador	NR	39.212	Tornero	Adenocarcinoma pulmonar, placas pleurales
14	68	Fumador	30	110.246	Tubero	Carcinoma epidermoide pulmonar, placas pleurales
15	63	No fumador	NR	2.358	Mecánico	Adenocarcinoma pulmonar
16	64	Ex fumador	NR	8.249	Delineante	Adenocarcinoma pulmonar
17	62	Fumador	26	1.011	Electricista-soldador	Carcinoma epidermoide pulmonar, placas pleurales
18	58	Fumador	30	249	Soldador	Carcinoma indiferenciado pulmonar, placas pleurales
19	66	Ex fumador	19	30.557	Calderería	Fibrosis y placas pleurales
20	76	No fumador	15	4.660.059	Mecánico	Asbestosis, derrame y placas pleurales, atelectasia redonda
21	77	NR	15	4.354	NR	Mesotelioma maligno
22	72	Fumador	30	1.327	Soldador	Asbestosis, placas pleurales
23	70	Fumador	44	16.322	Soldador	Mesotelioma maligno
24	58	No fumador	13	3.358	Tubero	Placas pleurales
25	64	Ex fumador	NR	1.908	Soldador	Carcinoma epidermoide pulmonar
26	59	Ex fumador	13	6.191	Mecánico	Adenocarcinoma pulmonar, fibrosis pleural
27	64	Fumador	20	45.100	Soldador	Adenocarcinoma pulmonar, placas y fibrosis pleural
28	80	Fumador	NR	13.323	NR	Mesotelioma peritoneal maligno
29	60	Ex fumador	10	5.125	Calderería	Carcinoma epidermoide de esófago, placas pleurales
30	77	Ex fumador	20	385.196	NR	Mesotelioma maligno

CA/g: cuerpos de amianto por gramo de tejido seco; NR: no recogido en la historia clínica.

The presence of asbestosis or any other histological alteration was determined from the autopsy reports produced by the Pathology Service.

Protocol for obtaining samples

In each autopsy two lung samples measuring 2 cm^3 were obtained (whenever possible, one from each lung). The samples were fixed in formol and were sent to the Hospital Vall d'Hebron for analysis. The study was approved by the hospital's Ethical Committee. Family members of the deceased gave informed written consent for the analyses to be performed.

Preparation of the lung samples

Of each sample two fragments of lung tissue weighing 0.5 grams were obtained, not containing either pleura or vessels. One of the samples was frozen, lyophilized and weighed to determine the weight of the dry tissue, in order to be able to express the AB results in accordance with the international conventions. The lyophilized sample was later discarded, as lyophilization may modify the concentration and size of the fibres[13].

Thirty cm^3 of previously filtered sodium hypochlorite was added to the non-lyophilized portion of tissue and shaken for 24 hours to aid the digestion of the tissue and the elimination of the organic matter. Subsequently, the sample was centrifuged at 3700 rpm for 20 minutes. After discarding the sodium hypochlorite, the sample was resuspended in filtered distilled water. To dissolve the AB in the liquid, the sample was submerged in an ultrasound bath for 10" in a sonicator (UCI-50. 300 W, 50/60 Hz, Raypa SL). After washing again, the sample was resuspended in 20 cm^3 of filtered distilled water. The solution obtained was filtered with a 0.45µm diameter filter (Millipore, Membrane filters HAWP02500). The filtrate was dried in an oven overnight at 37ºC and was then transferred to a slide using an acetone vaporizer (JS Holdings 240v/110v).

AB analysis using optical microscopy

The samples were analyzed using optical microscopy (Olympus CX21FS2, Olympus Life Science Europe GMBH, Hamburg, Germany, x 400). All the filtrates were examined by an expert examiner in accordance with the protocol previously described by the group[12]. Levels over 1000 AB/g dry tissue were considered to be potentially causative of disease, in accordance with the criteria established by the working group of the European Respiratory Society in 1998[13].

Statistical analysis

The data were expressed as medians and intervals. The Kolmogorov-Smirnov test showed that the distribution of AB values was not normal. Spearman's

correlation coefficient was calculated to establish the relation between the different parameters analyzed, and the Kruskal-Wallis test was applied to determine the differences between groups. The statistical analysis was performed with the program GraphPad (Analyze, graph and organize your data. GraphPad Software. 2002-2005).

Results

Characteristics of the study population

Table 1 shows the main characteristics of the study population and the values of AB concentration in each individual. All 30 subjects were male (mean age 67 years, interval, r: 56-89 years), ex-shipyard workers in El Ferrol, Spain, and all had records of work-related exposure to asbestos in the clinical history. Sixteen subjects were smokers, nine ex-smokers, four subjects were non-smokers, and in one case data on smoking were not available. The mean time (interval) of exposure to asbestos was 26 (10 - 44) years.

All subjects presented pulmonary disease and/or pleural or peritoneal diseases related to the exposure to asbestos (Table 1). In 23 cases the condition was malignant. In six cases the diagnosis was malignant mesothelioma (five pleural and one peritoneal); the strain was epithelial in five cases and fibrous in one. Only five of the 16 cases of lung cancer presented concomitant asbestosis. In 25 of the 30 cases there was some type of pleural involvement. The cause of death was the asbestos-related disease in 27 cases, while in the other three it was bladder cancer, pulmonary metastasis of sigmoid colon cancer and oesophageal cancer respectively.

Lung asbestos content

A median of 6,171 AB/g dry tissue was obtained was ((interval: 249 – 4,660,059). Only one individual presented values below 1,000 AB/g. A significant correlation was observed between the levels of AB/g dry tissue and the age of subject ($r = 0.5676$; $p = 0.0011$) (Figure 1). However, no correlation was observed between the levels of AB/g dry tissue and the years of asbestos exposure.

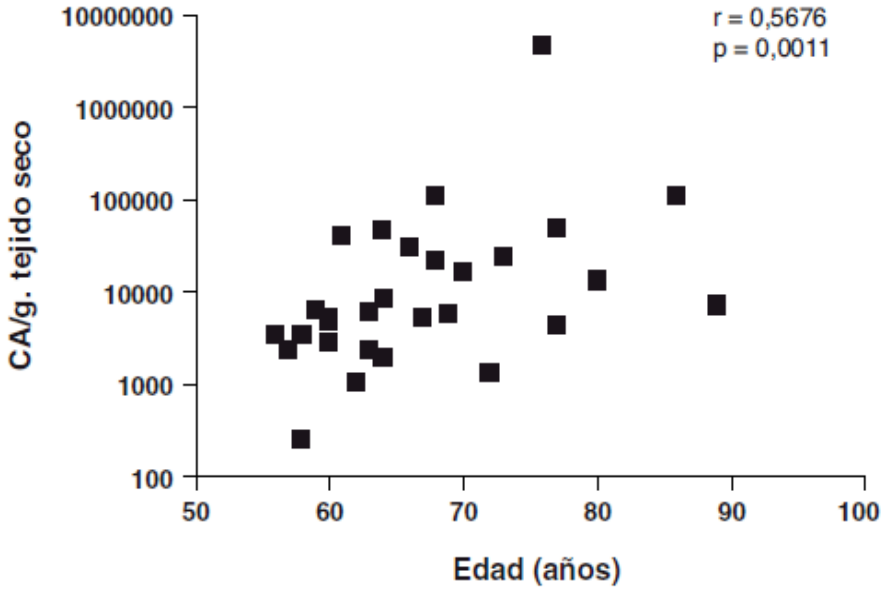

Figura 1. Correlación entre la concentración de CA/g de tejido seco y la edad de los pacientes estudiados.
CA: cuerpos de amianto.

As for the distribution of asbestos in different areas of the lung, in 20 cases in which fragments of both lungs were analyzed, medians (intervals) of 2,849 (99 – 40,902) and 4,099 (199 -45,100) AB/g dry tissue were obtained for the left and right lung respectively. No significant differences were found between the two groups.

The median (interval) of AB/g dry tissue was 5,947 (249 - 110,246), 8,249 (1,908 - 385,196) and 25,674 (2,358 - 4,660,059) for smokers, ex-smokers and non-smokers respectively. No significant differences were observed in the concentration de AB/g dry tissue with regard to smoking.

The median (interval) concentration of AB/g dry tissue was 4,378 (249 - 110.246), 18,919 (1.327 - 4.660.059), 14,823 (4,354 - 385,196) and 27,070 (6,150 - 47,991) for subjects with bronchopulmonary neoplasia, asbestosis, mesothelioma and pleural plaques respectively. No significant differences were observed in the concentration of AB/g dry tissue according to diagnosis (Figure 2).

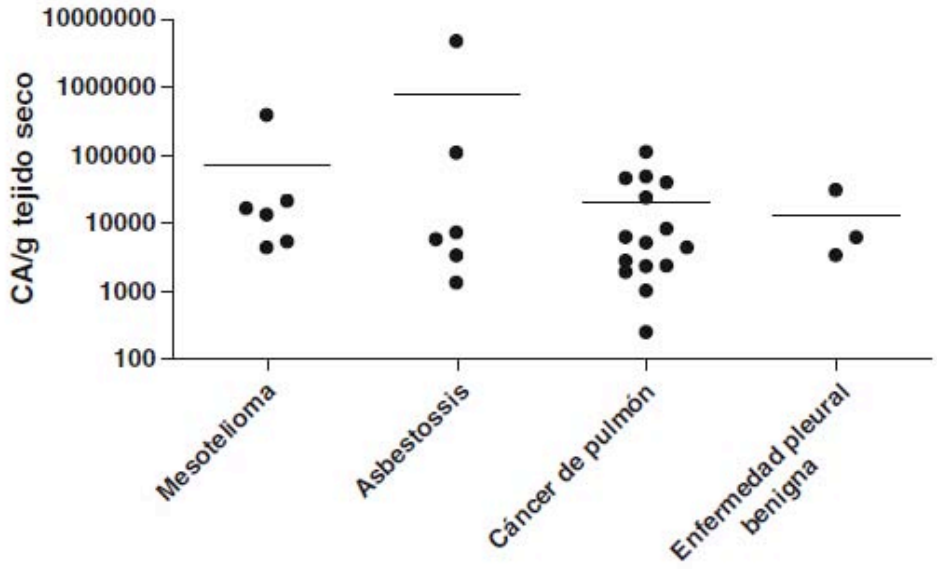

Figura 2. Valores de CA/g de tejido seco en función del diagnóstico de los pacientes estudiados.
CA: cuerpos de amianto.

Discussion

The present study provides the first data on the lung asbestos content in shipyard workers diagnosed with pulmonary disease in Spain. The results show that the asbestos concentration in the lung in the vast majority of these workers was above the internationally established safety limits for this mineral.

The municipality of El Ferrol in Galicia was one of Spain's main shipbuilding cities. Given that asbestos was widely used in the construction and repair of ships, it was estimated that a large number of workers in these shipyards had been exposed to the mineral and would therefore have developed associated diseases. This study analyzed pulmonary autopsy specimens of subjects who had worked in the shipyards and had died of respiratory diseases. The results show that the levels of asbestos detected might have been cause of the diseases they contracted. However, as no data were available on the duration and the intensity of smoking or the presence of other risk factors such as the exposure to radon, it is not possible to attribute this relation exclusively to asbestos. With respect to the series published in shipyard workers in other countries, we stress that the intervals of AB/g dry tissue vary according to the populations studied, but are comparable to those obtained in our study[14-16]. In a study of naval installations in the north of Italy, Biachi and cols.[17] detected high exposure to asbestos in 893 workers, 97% of whom presented AB and 64% had levels above 1,000 AB/g. Analyzing the subgroup of 430 individuals who had worked specifically in shipyards, 79% presented levels above 1,000 AB/g of lung tissue.

The high levels of AB in the workers from El Ferrol confirm that employment in the shipyards has been an important source of exposure to asbestos until very recently. In effect, the high levels found in the present study (a median of 6,171 AB/g dry tissue) coincide with the results of other studies. For example, Karjalainen et al.[18] found that Finnish shipyard workers presented levels of up to 11 AB/ml of bronchoalveolar lavage, a figure far above the safety threshold of 1 AB/ml.

With the exception of pleural plaques, most asbestos-related diseases are recognized as occupational diseases and may therefore be the subject of litigation between workers, government and firms. Only in the case of malignant mesothelioma is diagnosis alone sufficient for the disease to be conclusively defined as work-related, and thus to qualify for a possible economic compensation. In the rest of the diseases it is crucial to establish whether the exposure to asbestos met specific characteristics that make the cause-effect relation plausible.

At present, lung cancer is the condition in which it is most difficult to identify asbestos exposure as the cause of the disease. Lung cancer is the most frequently detected disease (16/30) in our series. Although tobacco smoking is well known to be a pulmonary carcinogen, exposure to asbestos is also acknowledged as a risk factor[11, 19]. Indeed, several authors have argued for the existence of synergies between smoking and cancer. It is estimated that the risk of lung cancer is ten times greater in smokers, and between three and four times greater in people exposed to asbestos; in smokers exposed to asbestos, however, the risk rises between 30 and 50 times[20], and may persist up to twenty years after smoking cessation[21].

The results of the present study provide important data on the role of asbestos exposure in the development of lung cancers. The role is clear enough in non-smokers, but as the series presented here shows, the proportion of smokers in these professions is extremely high. In smokers, the implication of exposure to asbestos in the development of cancer depends on its intensity and duration. In general it is accepted that intense, prolonged exposure causes lung cancer, although no precise criteria have been defined in our country. The data obtained in our study support this hypothesis, since the mean length of exposure in these subjects was 26 years. With regard to the intensity of exposure, and in spite of the fact that we do not have data on the asbestos load in the air breathed, it seems fair to assume that shipyard air contains a high asbestos content. The findings in the lung confirm this supposition, since the

median values of AB were well above the threshold of 1,000 AB/g mentioned above.

Interestingly, only six of the 16 subjects with lung cancer presented pulmonary asbestosis. In early studies, as most subjects exposed to asbestos with lung cancer also had asbestosis, it was assumed that the presence of lung fibrosis was necessary for the development of asbestos-related cancer [11]. However, recent epidemiological and experimental evidence has disproved this hypothesis: in a longitudinal study of more than 4,000 workers with high exposure to asbestos over 9-17 years, Cullen and col.[24] found that although the highest risk of developing cancer was associated with the presence of asbestosis, the risk also remained high (relative risk of up to 5 for more than 40 years of exposure) in subjects with chest X-rays that showed no signs of the condition. Our findings also suggest that the two conditions are independent.

In accordance with the results of previous studies in other countries, such as the one by Kurumatani and col.[23] in 249 Japanese shipyard workers, the deceased subjects in our series presented a characteristic variety of respiratory diseases caused by inhalation of asbestos, As in other series, the most frequently found disease in this study was benign pleural disease, with 20 cases, followed by lung cancer, asbestosis and malignant mesothelioma. No statistical differences were detected in the lung asbestos content in subjects with the different diseases, who had all been exposed for similar periods of time. In this regard, although a correlation was found between the lung asbestos deposit and subjects' age, there was no correlation with years of exposure. This result is in agreement with those of the study of Japanese shipyard workers mentioned above[23], but not with other series[18]. One possible explanation of this apparent paradox is that the data recorded regarding exposure may include very different activities. More detailed work-related information in the population of the present study might have allowed us to analyze this point in greater detail. As for the relation between the quantity of asbestos and smoking, the data from our study suggest that accumulated exposure to tobacco does not influence the retention of asbestos in the lung.

In conclusion, this sample of shipyard workers from El Ferrol, Spain had high levels of asbestos in the lung, and these levels were directly correlated with age. Although the use of asbestos has been reduced or completely eliminated in the majority of the world's shipyards, a large number of workers have been exposed in the past and many others working in old facilities that still use asbestos remain exposed today. Clinicians should maintain a high suspicion of this mineral as a potential cause of pulmonary disease in this population. Systematized questionnaires should be used to test its involvement in the development of disease and, whenever possible, asbestos levels in the lung should be determined.

Acknowledgements

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Chapter 4

First identification of pulmonary asbestos fibers in a Spanish population

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Abstract

Introduction

This study aimed to characterize, for the first time in Spain, the type of asbestos fibres (AF) in the lungs of exposed and non-exposed populations.

Material and methods

Lung samples from 38 subjects were studied, divided into three groups: Group A - five subjects without known respiratory disease; Group B - 20 ex-shipyard workers from El Ferrol, Spain; Group C - 13 patients with lung cancer.
After eliminating the organic material, the inorganic residue was analysed using electronic microscopy (EM). To identify the type of fibre, the samples were analysed by scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX).

Results

All the fibres identified corresponded to amphiboles (crocidolite 45%, anthophyllite 22%, tremolite 16%, amosite 15% and actinolite 3%). In 14 patients (37%) a single type of asbestos was found in the lungs (two amosite, one actinolite, four anthophyllite, five crocidolite and two tremolite). Forty-six per cent of the AF analysed had a length >5 μm and a diameter <0.2 μm.

Conclusion

The results of this study provide the first data on the type of asbestos retained in lung of Spanish population. Particularly striking is the exclusive retention of amphibole, suggesting elimination after inhalation of chrysotile.

Introduction

Asbestos is the generic name given to a group of silicate minerals. The most common are amphiboles: amosite, crocidolite, tremolite, actinolite and anthophyllite, and the serpentine chrysotile. These forms differ in terms of their chemical structure, their biopersistence in human and their toxicity. Inhaled asbestos fibres are deposited in the respiratory system where they interact with epithelial cells and alveolar macrophages to produce an immune response. As described extensively in the scientific literature, exposure to asbestos has been associated with asbestosis, mesothelioma, lung cancer and benign pleural lesions[1-3].

In Spain, asbestos was widely used in industry for many years. Its use in this country reached its peak between 1970 and 1990. In 1992 Spain was the second largest European importer with 25428 tons[4], and total ban on the use of the mineral did not come until 2002. This means that a large number of workers have been exposed to asbestos and will remain exposed in the future, given the incorporation in a great many structures and buildings[5]. According to the voluntary registry of respiratory diseases in Catalonia, diseases resulting from exposure to asbestos fibres are the second largest group[6].

The diagnosis of diseases caused by the inhalation of asbestos is based on three factors: knowledge of exposure, a compatible clinical picture, and exclusion of other diseases. In some cases, there is a mismatch between the exposure and the clinical picture, which makes diagnosis difficult and often poses medical-legal problems. In these cases it is necessary to establish the amount of asbestos in lung tissue [7-8]. Detection of asbestos fibres in lung tissue requires observation and counting by optical or electron microscopy. Light microscopy allows the detection of low asbestos concentrations, but it does not identify the type of asbestos. Asbestos identification is carried out by electron microscopy equipped with devices like EDS (Energy-dispersive X-ray spectroscopy), which are able to determine the chemical composition and the crystal structure of the fibre.

In Spain, only three studies have provided data concerning lung deposition of asbestos in the population: the paper by Monsó et al.[9], and two studies by our group[10-11]. None of these studies identified the most prevalent type of asbestos or the dimensions of inhaled fibres in exposed individuals, although several authors have drawn attention to the importance of this information[12-13].

The aim of this study was to characterize, for the first time in Spain, the type and dimensions (length and diameter) of asbestos fibers in exposed and unexposed populations.

Materials and methods

Study population

We studied lung samples from 38 subjects, divided into three groups: five residing in the city of Barcelona without known respiratory disease (Group A), 20 who had worked in the Ferrol shipyards and had been exposed to asbestos (Group B) and 13 lung cancer patients living in the city of Barcelona (Group C).

Sampling criteria

Lung samples from patients in Group A were collected prospectively from June 2004 to June 2005 at the Institute of Legal Medicine of Catalonia. In this group, the following sampling criteria were applied: absence of pulmonary disease and residence in the city of Barcelona for at least 10 years. Lung samples from Ferrol shipyard workers (Group B) were consecutively sent to our laboratory between 2008 to 2010 from the Pathology Department of Hospital Arquitecto Marcide in El Ferrol. The lung cancer group (Group C) comprised 13 patients diagnosed with lung cancer and operated consecutively at the Vall d'Hebron Hospital, from whom resected specimens were obtained.

Exposure determination

Information on the potential exposure of the patient was obtained by interview with the next of kin in autopsy cases (Groups A and B) and through a personal interview with the patient in cases of pulmonary resection (Group C).

After verifying that they met the inclusion criteria, patients were invited to participate in the study. In the case of autopsies, the proposal was made to the next of kin. All interviews were conducted by two of the researchers, one in each of the cities. In all cases, informed consent was requested. Consent forms were signed by the affected individual or by a close relative, depending on whether the samples had been obtained by resection or autopsy. The local Ethics Committee approved the study.

Protocol for obtaining samples

In Group A, lung tissue specimens of 2 cm^3 in size were obtained from three regions of the right lung: apex of the upper lobe (zone 1), apex of the lower lobe (zone 2), and base of the lower lobe (zone 3). In Group B, in each autopsy lung tissue specimens of 2 cm^3 in size were obtained from an area in the right lung and another area in the left lung whenever possible. As regards the resections in Group C, samples were obtained from the lung area where the cancer had appeared and whenever possible from more than one area.

All samples obtained were fixed in formol and sent to our hospital laboratory for analysis. All the lung specimens were examined by a pathologist from our hospital. The presence of chronic pulmonary disease was ruled out in all cases.

Preparation of lung samples

Two 0.5 g fragments of lung tissue that did not contain pleura or vessels were obtained from each specimen. One of these fragments was frozen, lyophilized, and weighed to determine the dry tissue weight, in keeping with an international agreement to express asbestos body (AB) results in terms of grams of dry lung tissue. Once the dry weight was known, the lyophilized sample was discarded, because this technique may cause changes in the concentration and size of the fibers[8]. Thirty cc of filtered sodium hypochlorite was added to the non-lyophilized tissue section, and the sample was shaken for 24 h to facilitate digestion of the tissue and to eliminate other organic material. The sample was then centrifuged at 3 700 rpm for 20 min, the sodium hypochlorite was eliminated, and the sample was re-suspended in filtered distilled water. To dissolve the AB present in this liquid medium, the sample was sonicated for 10 min using an ultrasonic water bath (UCI-50 Raypa SL; 300 W, 50/60 Hz), and was then washed and re-suspended in 20 cc of filtered distilled water. The solution obtained was passed through a 0.45-mm pore diameter filter (Millipore membrane filters, HAWP02500). The filter was dried overnight at 378C, placed on a microscope slide and made transparent using acetone vapour (JS Holdings vaporizer; 240 v/110 v) for subsequent reading. In all the samples, an

adequate filter loading for counting was obtained, and it was not necessary to dilute the sample.

Asbestos body counting by optic microscopy

The filters were viewed with an optic microscope (CX21FS2; Olympus Life Science Europe GMBH, Hamburg, Germany) at x400 magnification. All filters were examined by a single experienced reader in accordance with a protocol previously described by our group[10]. All the samples obtained for each individual were assessed: in Group A, the values obtained for each of the three lung zones were evaluated, and in Groups B and C all the zones from which a sample could be obtained. Applying the criteria established by the working group of the European Respiratory Society in 1998, AB levels exceeding 1000 AB/g dry tissue were considered significant[8]. For the analysis, the highest value of the different samples analyzed in each patient was considered.

Asbestos fibre identification by electron microscopy

After completing this reading in the optic microscope, the filter was cleaned by immersion in serial baths of ethylene glycol and carbon tetrachloride. The filter was degassed overnight and was then covered again with carbon (Emitech CC7650, Quorum Technologies; Sussex, UK). Once the carbon layer was deposited on the filter, the asbestos was determined using scanning electron microscopy (SEM) (Quanta-200, FEI, Hillsboro, Oregon, USA). The type of AF in each subject was assessed at an accelerating voltage of 15Kv, screen magnification of x2.000 and a scan rate of 10 seconds per frame. Chemical analysis of the fibres observed was performed with electron microscope including electron diffraction and energy-dispersive X-ray spectral analysis (EDX). Fibre analysis was performed at a magnification of x20.000.

Statistical analysis

The data are expressed as the median and the range unless otherwise stated. Analyzed with the Kolmogorov-Smirnov test, the values obtained did not follow a normal distribution; therefore, the Wilcoxon test was used to determine the

differences between groups. The statistical analysis was carried out with GraphPad software (2002–2005).

Results

Clinical characteristics of the study population

The baseline characteristics of the 38 individuals included in the study are shown in Table 1. In the five patients in Group A the main causes of death were heart disease and traffic accidents. In the 20 patients of group B the diagnosis was lung cancer in eight patients, mesothelioma in four, pleural plaques in four, asbestosis in three and pulmonary thromboembolism in one. The 13 patients in Group C were all diagnosed with lung cancer.

Table 1: Baseline characteristics of the 38 individuals included in the study.

	Group A n = 5	**Group B n = 20**	**Group C n = 13**
Age, yrs*	66 (48-78)	69 (56-86)	66 (51-77)
Sex	4M / 1F	20M	12M / 1F
Smoking habit, n (%)		**	
Smoker	1 (20%)	7 (44%)	2 (15%)
Ex-smoker	2 (40%)	6 (37%)	10 (78%)
Non smoker	2 (40%)	3 (19%)	1 (7%)
Exposed / Non exposed (%)	0 / 100	100 / 0	77 / 23
Years of exposure*	-	27 (13-44)	ND
Disease, n			
No respiratory disease	5	-	-
Asbestosis	-	3	-
Lung cancer	-	8	13
Mesothelioma	-	4	-
Pleural plaques	-	4	-
Pulmonary embolism	-	1	-

M – Male; F – Female
* Data expressed as median (range)
ND – No data recorded in the clinical history
** In four patients smoking habit was not specified in the medical record

Types and dimensions of asbestos fibres

Figure 1A summarizes the types of asbestos found in the different samples. Amphibole fibres accounted for 100% of fibres recovered in lung samples. No

chrysotile fibres were found in the samples analyzed. Crocidolite was the most common type of fibre observed (45% of samples analyzed). In 14 patients (37%) a single type of asbestos was found in the lungs (two amosite, one actinolite, 4 anthophyllite, five crocidolite and two tremolite).

Patients in Group A had a higher percentage of actinolite than their Group B and C peers (p = 0.012 and 0.023 respectively). Patients in Group B had a higher percentage of amosite than those in groups A and C (p = 0.022 and 0.031, respectively). Finally, Group C patients had a higher percentage of tremolite than those in Groups A and B (p = 0.011 and 0.017 respectively) (Figure 1B).

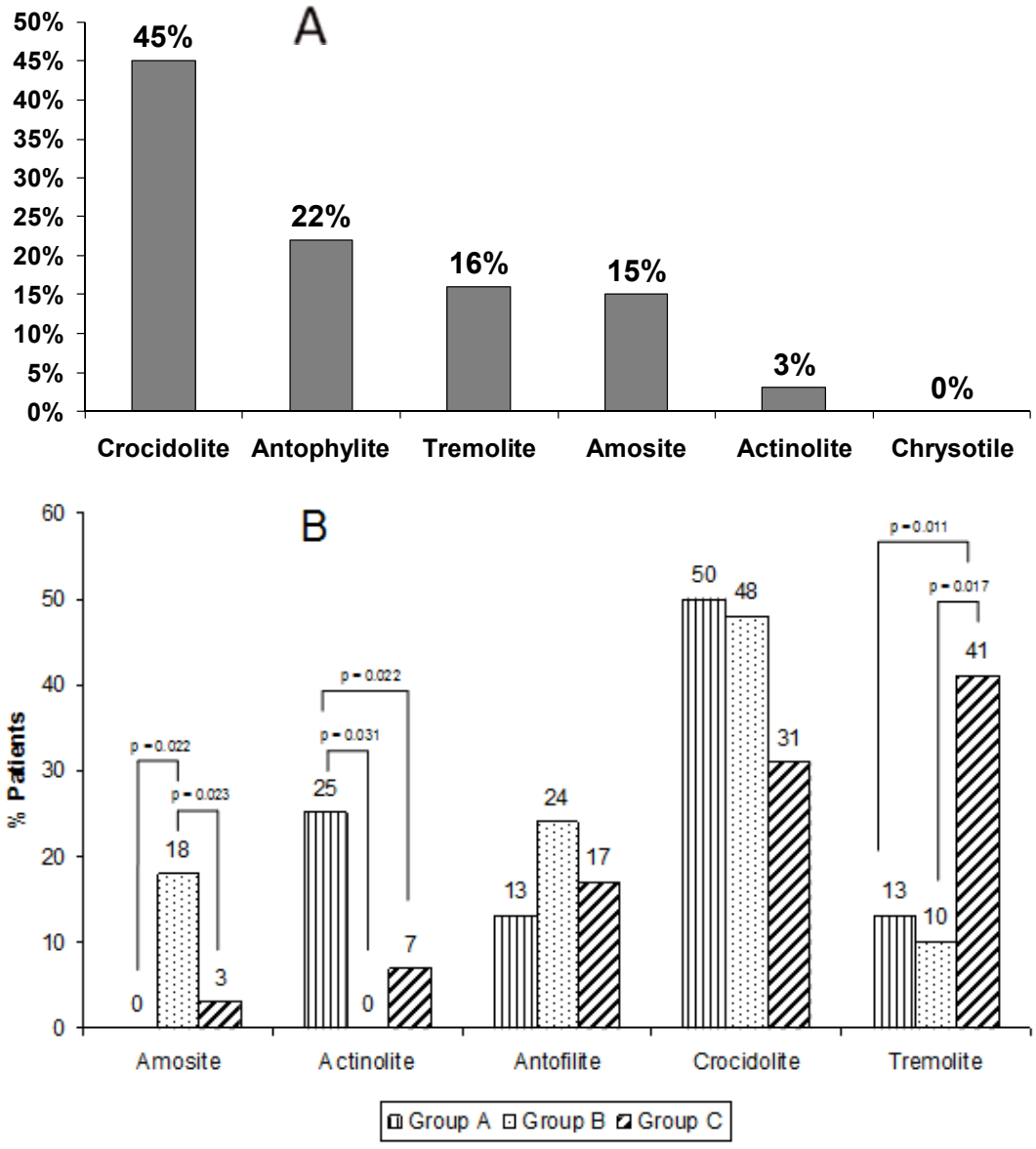

Figure 1. Types of asbestos fibres found in the different samples analyzed.

Table 2 shows the levels of asbestos bodies measured by optical microscopy and the mean dimensions and length distribution of the fibres obtained from the different samples. Group B, had higher levels than Group A and C (p = 0.001 and 0.02 respectively). Group C also had higher levels of AB than Group A (p = 0.032). The length of the fibres exceeded 5 microns in over 80% of the samples analysed in the three groups. In Group B patients, 49% of the fibres tested had a length > 5µM and a diameter < 0.2 (p = 0.001). In all groups, the fibres with a length > 5µM and a diameter < 0.2 um corresponded to amosite in 97% of cases.

Table 2: Length (L) and diameter (ø) of the asbestos fibres analysed by SEM (Scanning Electron Microscope).

	Group A n = 5	**Group B n = 20**	**Group C n = 13**
Number of AB*	0 (0 – 599)	14647** ¶ (1327 – 4660059)	326& (0 – 26280)
AB>1000; n (% patients)	0 (0%)	20 (100%)	6 (46%)
L>5µm	90%	88%	84%
Ø<0,2µm	10%	56% ***	21%
L>5µm y ø<0,2µm	9%	49% ***	18%

* Data expressed as median (range)
AB – Asbestos bodies measured by optical microscopy
** p<0.001 compared with group A
¶ p=0.02 compared with group C
*** p<0.001 compared with groups A and C
& p=0.032 compared with group A

Table 2 shows the mean dimensions and length distribution of the fibres obtained in the different samples. The length of the fibres exceeded 5 microns in over 80% of the samples analyzed in the three groups. In patients of group B, 49% of the fibres had a length >5 µM and a diameter <0.2. In all groups, the fibres with a length> 5 µM and a diameter <0.2 um corresponded to amosite in 97% of cases.

Discussion

This study is the first to characterize the type of asbestos retained in the lung in a Spanish population. We found an exclusive presence of amphiboles, suggesting clearance of inhaled chrysotile. Moreover, the physical characteristics of the fibres detected in the samples of workers in the shipyards of Ferrol differed from those of our other two study populations. The shipyard workers presented the longest and thinnest fibres, which are considered the most likely to cause asbestos-related diseases.

Electronic microscopy analysis showed that the most common fibre found in our study population was crocidolite, despite the fact that it has been prohibited since 1984. Furthermore, 72% of individuals in the Ferrol shipyard group predominantly presented crocidolite in their lung samples. Similarly, crocidolite was the most common fibre type in reference population. Explanations for this high content may be exposure previous to 1984 and subsequent exposures occurring during repair activities carried out in recent years in asbestos-containing buildings and structures.

One remarkable result was the absolute predominance of amphiboles and the absence of chrysotile in the samples analyzed. It occurs with previous data showing that although chrysotile is the most commonly used type of asbestos, crocidolite is the fibre found most often in the lungs of patients with mesothelioma. Indeed, the 1974 study by Desbordes and Fondimare[14] already suggested this idea: those authors reported that in the lung tissue of patients with high exposure to both amphiboles and chrysotile, the fibres present were almost exclusively large amphiboles. This is thought to be due to the fact that, after inhalation, asbestos undergoes a drainage process that alters its final deposit. It is known that large proportions of chrysotile are removed in this process, which means that the chrysotile levels detected years afterwards may not reflect the intensity of the previous exposure[15]. Contrarily, amphibole clearance is much lower. Churg and Vedal[16], in a series of 144 shipyard

workers, found that time since last exposure was correlated with decreasing amosite concentration, and calculated a clearance half-time of about 20 years. The idea of a higher lung persistence of amphibles over chrysotile is supported by animal studies. Thus, in animal studies, pulmonary accumulation of crocidolite has been shown be three or four times greater than that of chrysotile[17]. In contrast, some authors argue that the difficulty of observing chrysotile is due to the technical procedure. In their study of asbestos in 110 cases, Roggli et al[18] observed a loss of a substantial proportion of small chrysotile fibres during the centrifugation step in the ethanol-chloroform interface.

Interestingly, 16% of asbestos corresponded to tremolite, a percentage that raised to 41% in lung cancer patients. Despite tremolite is associated with environmental asbestos-related diseases in the Mediterranean area (in Turkey, Greece, and Corsica, for instance) its commercial importance is relatively low and it is a frequent natural contaminant (as a geological component) of other minerals as chrysotile or talc. In American shipyard workers, tremolite and chrysotile concentrations were significantly correlated, indicating that the tremolite originated from chrysotile products. Therefore, one possible explanation of our results, particularly among cancer patients, is that the presence of tremolite may be an indicator of chrysotile[19].

With regard to the other types of fibres found, amosite was only found in Groups B and C. The most notorious amosite burden was observed in workers in the shipyards of Ferrol. These results reflect the widespread use of amosite in shipyards as an effective pipe insulator. In this sense, Kishimoto and coworkers[20], analysing the characteristics of 32 patients exposed to asbestos in a former Japanese naval shipyard, found that 14 patients had been exposed to crocidolite and ten to amosite. Langer and coworkers[21] found that amosite was present in all the lungs of the insulation workers studied, most of whom were shipyard workers, and that its highest concentrations were found in this exposure category. Probably the type of fibres retained in the lung of the workers in Spain reflects the type of work performed in shipyards in repair and construction, which was probably not very different from the work carried out in

Japanese or American shipyards. Unfortunately, no data on the types of asbestos imported are available in Spain, either in general or in shipyards such as El Ferrol.

Regarding the physical characteristics of the fibres detected, 46% of the AF analyzed had a length >5 microns and a diameter <0.2 μm, and these fibres were mainly found in the samples analyzed in the shipyard workers. There is evidence that these long, thin fibres are the most likely to cause asbestos-related diseases. Fibres below 5 microns in length show greater penetration into the respiratory system, but have a shorter retention as they are easily removed. In a case control study of patients with mesothelioma, McDonald and coworkers[22] found that the concentration of amphiboles (amosite, crocidolite, tremolite) longer than 8μm was the best predictor of mesothelioma, and that shorter fibres did not contribute to its development. Nevertheless, the results of experimental and epidemiological studies suggest that the toxicity of small asbestos fibres cannot be ignored. Recent data in humans[23-28] indicate that exposure to longer fibres was associated with higher rates of lung cancer, but no definite conclusions can be ascertained for the other size classes. Nevertheless, the authors of these studies noted that exposure to short, thin fibres was associated with lung cancer risk, and that these fibres represented the majority of those counted in the lung samples of the patients. We cannot yet determine whether the association of these short fibres with lung cancer is a spurious effect due to correlations between fibre-size categories, or evidence that short fibres do indeed play a specific role in carcinogenesis.

In conclusion, the results of this study provide the first data on the type of asbestos retained in the lungs of Spanish populations. Particularly striking is the exclusive retention of amphiboles, which appear to be responsible for the asbestos-related diseases observed in exposed populations.

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Competing Interest

None to declare

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General Discussion

This thesis provides the first data on the distribution of asbestos bodies (AB) in the Spanish urban population, showing that the majority present values of lung deposition below 300 AB/gr. dry tissue[1]. It also demonstrates that the method used to establish these reference values – light microscopy – shows good reproducibility, as evidenced by an excellent degree of agreement when the counting was carried out by experienced readers[2]. Moreover, the study provides the first data on the lung asbestos burden in shipyard workers diagnosed with pulmonary disease in Spain. The results show that the asbestos concentration in the lung in the vast majority of these workers is above the internationally established safety threshold for this mineral[3]. The study also presents the first characterization of the type of asbestos retained in the lungs of the Spanish population: the exclusive presence of amphiboles suggests that chrysotile is eliminated after inhalation[4].

It is estimated that in industrialized countries at least 30,000 people will die each year of cancer related to asbestos exposure[5]. This ominous forecast is due mainly to the long latency period between asbestos exposure and diagnosis – more than 20 years – meaning that the detected diseases are due to exposures that occurred 30 or more years previously. It should also be borne in mind that a significant number of buildings and structures in our country contain asbestos, and so a large number of workers are currently exposed to these materials during their work activities[6-7]. Therefore, although its industrial use is now prohibited the harmful impact of asbestos in our population remains and will remain in the future; as a result, the diagnosis of diseases caused by asbestos is a priority for our health system.

From 1962, when asbestosis was defined as a compensable occupational disease in Spain, until 1974, only 44 cases were acknowledged by the Spanish Compensatory Fund[8] and only 815 up to 2010[9]. This figure is far below what the most conservative estimates suggest and is not reflected in the findings of more realistic studies. In the mid-seventies, the first estimates on underreported asbestos pathology estimated that at least 500 to 600 individuals were suffering from undiagnosed asbestosis. A retrospective case study conducted in risk areas in Spain has confirmed that the number of people affected by asbestosis

and other fibrosing lung diseases of occupational origin was far higher than the official figures[10].

Data from other European countries support the notion that asbestos-related diseases are under-diagnosed in Spain. Although the comparison of statistics between countries is difficult because of the diversity of the social security systems in place, the report by Eurogip based on the survey conducted in 13 European countries in 2005 showed incidences of asbestosis ranging from 0.15 per 10^5 insured people in Spain to 5.23 per 10^5 insured people in Germany. Moreover, Spain was the European country with the lowest level of occupational asbestosis recorded: 35 times less than Germany, 21 times less than Belgium, and 15 times less than France and Italy[11].

The reason for this under-diagnosis is probably due in part to the difficulty in determining the history of exposure to asbestos on the basis of clinical interviews. In a large percentage of cases, diagnosis can be established through a proper anamnesis, a compatible clinical and radiological picture and, if required, a conventional histological examination. The establishment of a reliable link between asbestos exposure and disease constitutes a challenge for the clinician. If the patient reports definite exposure, or if the disease has a specific causal association with asbestos (for instance, malignant mesothelioma), the notion of exposure does not represent a problem for establishing diagnosis. Quite frequently, however, the doubt regarding a history of exposure or a clinical or legal imperative makes it necessary to confirm conclusively that the patient's disease is caused by asbestos. In these cases, the detection of asbestos in lung tissue is relevant. Examination of pulmonary samples using light microscopy allows the detection of asbestos bodies (AB), which are the result of macrophage phagocytosis of larger asbestos fibres which are covered with a ferruginous layer[12]. Although only a small part (%) of the total asbestos fiber burden is transformed into asbestos bodies, several studies have demonstrated an acceptable correlation between the number of AB and fibers in the lung[13].

The detection of lung AB is especially helpful in the attribution of lung cancer to asbestos in exposed subjects. Attribution is crucial in these cases, since lung cancer due to asbestos exposure is recognized as a professional disease in Spain and therefore has economic consequences for the patient. In spite of well-documented exposure, attribution is simpler in non-smokers. However, this is an infrequent scenario, as was seen in the series of shipyard workers of Ferrol included in this thesis[3], since most patients exposed to asbestos are also smokers, the plausibility of asbestos as a causative factor depends on the intensity and duration of exposure. It is generally accepted that neoplastic lung disease occurs after intense exposures to asbestos over a certain period of time, although in Spain there are no specific criteria for attribution.

The legal implications involved in the diagnosis of asbestos-related diseases highlight the importance of having access to reliable analytical methods that are both sensitive and reproducible. The analysis of asbestos in biological samples requires a reference laboratory and trained personnel to prepare the samples and perform the asbestos count. In addition, each laboratory must establish its reference values according to the population in its environment. Therefore, it seems logical that the lung content of asbestos should be determined in specialized centers.

At the time work began on this thesis, there was only one study in Spain that provided data on the pulmonary asbestos content in population not exposed to the substance in the workplace[14]. The present study is therefore only the second one on the subject and provides data from a larger number of cases considered as urban populations. Determination of the concentration of AB in lung tissue was performed following the recommendations of the European Respiratory Society (ERS) Guidelines[15]. Interestingly, in 86% of the study population AB counts were lower than 300 AB/g of dry tissue. Taking into account that the ERS Guidelines proposes 1000 AB/g of dry tissue as the cut-off above which asbestos burden is potentially causative of disease; we believe that this recommendation is fully applicable in our population. In the present study, as well as in others performed in different countries. The presence of asbestos in the lungs of non-occupationally exposed individuals can be

explained by the ubiquitous presence of the silicate in the cities' air. In addition to environmental inhalation, which may be increased if the person has lived near industries that handle asbestos, there are other sources of exposure that often go unnoticed both at home and in the workplace.

With regard to reference population series from other countries, the ranges of AB/g of dry tissue vary depending on the population but are comparable to those obtained in our study. In most of these series, the average values were lower than 500 AB/g of dry tissue[14,16-20], although some individuals with values above 1000 AB/g were also reported. In our reference population, one individual had an AB burden over 1000 AB/g. This variability may be due in part to technical differences between laboratories, a situation that confirms the need to obtain reference values for each population according to the specific sample treatment protocol used. In any case, the extreme values above 1000 AB/g of dry tissue in the reference population reflect the low sensitivity of the case history approach in certain individuals in which exposure to asbestos may remain unknown.

In addition to the need to establish baseline levels of AB in each population, other aspects of the standardization of the method should also be taken into account. The accuracy of the results mainly relies on the ability of the observer to detect these bodies in sample extracts. As has been noted, detection and counting of asbestos bodies in lung tissue is currently restricted to specialized centers and is carried out by a single observer. Of course, the dependence on an observer introduces a certain degree of variability. As in many other measurement systems performed by humans, accuracy depends on the observer’s personal experience and implies a certain margin of error, given the unavoidable variation in AB counting[21]. So it is mandatory to determine the inter-observer reproducibility of the lung AB results in order to establish the number of readers needed to accurately administer the technique. The results of this study demonstrated a good correlation between the findings of the two experienced readers, thus indicating that AB counts determined by a single observer are reliable[2]. Nevertheless, in spite of the high concordance detected, there were some discrepancies between observers in some individual cases. In

one sample, the results reported by the two readers were above and below 1000 AB/g, respectively, and in two other samples, the counts were situated on both sides of the 5000 AB/g threshold. These discrepancies may be relevant, because 1000 AB/g is the cut-off commonly used to consider that a patient's lung deposition can give rise to respiratory disease. The cut-off of 5000 AB/g was established in order to define the asbestos burden needed to develop certain diseases such as lung cancer and asbestosis[22]. Thus, although our results indicate that lung asbestos counts carried out by a single observer are generally reliable, we believe that caution is required in borderline cases. In cases yielding AB values close to a specific threshold, examination by two readers should be carried out whenever possible. Double counting is especially useful in individuals whose reported values are slightly below the threshold, because the risk of underestimation is higher at these levels and mistakes in this regard could lead to misdiagnosis, meaning that legal and economic compensation may be unfairly refused.

Another important aspect in the standardization of the method is the election of the lung area undergoing analysis. In clinical practice, lung samples are taken from a single zone, but the available data on the distribution of asbestos in the lung are inconclusive. Whereas significant variability between lobes has been reported in exposed individuals[23-27], the distribution in a reference population seemed to be homogeneous[1]. In the present study, a good level of agreement between readers was evident for the three areas examined. Our findings provide the first evidence in our country that AB counting in clinical practice is a reliable measure regardless of the lung area sampled.

The method standardized in this thesis was used to analyze the lung asbestos content of deceased shipyard workers in El Ferrol for whom autopsy studies were available[3]. In the 1950s, several areas in north-western Spain developed flourishing shipbuilding industries. The municipality of El Ferrol in Galicia was one of Spain's main shipbuilding cities. Given that asbestos was used in the construction and repair of ships all around the world, it was estimated that a large number of workers in El Ferrol shipyards had been exposed to the mineral and would therefore have developed associated diseases. Asbestos was used

to provide thermal insulation for ships, and as a result many shipyard workers were exposed to high environmental concentrations of the mineral during their working day. According to the Galician Association of Asbestos Victims (AGAVIDA), in El Ferrol alone 3,000 workers have presented asbestos-related diseases. Navantia, the shipyard company, recently published a list of 6007 workers who had had some degree of contact with asbestos during their working lives and who, in accordance with the Spanish health ministry's inspection protocol and the legislation passed in 2006, were advised to attend regular preventive medical examinations[28].

The El Ferrol study provides the first data on the lung asbestos content in shipyard workers diagnosed with pulmonary disease in Spain. The results show that the asbestos concentration in the lung in the vast majority of these workers was above the internationally established safety limits for this mineral. The high levels of AB in the workers from El Ferrol confirm that employment in the shipyards was an important source of exposure to asbestos until very recently.

Moreover, the results of the present study provide important data on the role of asbestos exposure in the development of lung cancers. The role is clear enough in non-smokers, but as the series presented here from the El Ferrol shipyards shows, the proportion of smokers among these workers is extremely high. The data obtained in our study support the idea that long asbestos exposures are needed to develop lung cancer, since the mean length of exposure in these subjects was 26 years. With regard to the intensity of exposure, although no data on asbestos load in the air breathed were available it seems fair to assume that shipyard air contains a high asbestos content. Our findings confirm this supposition, since the median values of AB were well above the threshold of 1000 AB/g mentioned above.

As previously mentioned, AB examination by light microscopy is a simple, cheap and reproducible method. However, it has the limitation that it does not identify the type of asbestos fiber. For this reason, the last aim of the present thesis was to characterize, for the first time in Spain, the asbestos fibers in exposed and unexposed populations[4]. Characterization of these fibers requires

the use of electron microscopy. Electron microscopes are usually equipped with additional devices like EDS or EDX (Energy-dispersive X-ray spectroscopy), which allow determination of the chemical composition and the crystal structure of the fiber.

Using electronic microscopy analysis, we observed that all the fibers detected were amphiboles, and that the most common type was crocidolite, despite the fact that this type of asbestos has been prohibited since 1984. Furthermore, 72% of individuals in the group from the El Ferrol shipyard analyzed in this study presented mainly crocidolite in their lung samples. Explanations for this burden may be exposure previous to 1984 or posterior exposure occurring during repair activities carried out in recent years.

The predominance of amphiboles and the absence of chrysotile in the samples analyzed is probably due to clearance of the inhaled chrysotile. Asbestos, after being inhaled, undergoes a drainage process that alters its final deposit. It is known that large proportions of chrysotile are removed in this process, which means that the chrysotile detected years afterwards may not reflect the intensity of the original exposure[29]. Indeed, in 1974, Desbordes and Fondimare[30] already provided support for this idea by reporting that the lung tissue of patients with high exposure to both amphiboles and chrysotile presented almost exclusively large amphibole fibers. In a series of 144 shipyard workers Churg and Vedal[31] found that time since last exposure was correlated with decreasing amosite concentration, and calculated a clearance half-time of about 20 years. In contrast, some authors argue that the difficulty of observing chrysotile is due to the technical procedure. In their study of asbestos in 110 cases, Roggli et al[32] observed a loss of a substantial proportion of small chrysotile fibers during the centrifugation step in the ethanol-chloroform interface.

Furthermore, animal studies have shown that persistence in the lungs is higher for crocidolite than for chrysotile. Although chrysotile is the most commonly used type of asbestos, the fiber most often found in the lungs of patients with mesothelioma is crocidolite. Again, this is due to the fact that chrysotile is soluble in organic liquids, a property that facilitates its removal and

fragmentation over time. Thus, in animal studies, levels of pulmonary accumulation of crocidolite have been found to be three or four times higher than chrysotile[33].

Moreover, the proportion of tremolite in the analyzed samples amounted to 16%, with cancer patients (Group C) presenting the highest pulmonary retention of this mineral (41%). Although tremolite has been associated with environmental asbestos-related diseases in the Mediterranean area (in Turkey, Greece, and Corsica, for instance) it is of relatively little commercial importance and it is a frequent natural contaminant (as a geological component) of other minerals like chrysotile or talc. Therefore, one possible explanation of our results, particularly in the group of cancer patients, is that the presence of tremolite is in fact a surrogate for chrysotile[34].

With regard to other types of fibers found, workers in the shipyards of El Ferrol also had higher amosite deposits in the lung than patients in Groups A and C. These results are consistent with previous reports. Analysing the characteristics of 32 patients exposed to asbestos in a former Japanese naval shipyard, Kishimoto T and coworkers[35] found that 14 patients were exposed to crocidolite and ten to amosite. Langer and coworkers[36] found that amosite was present in all the lungs of the insulation workers studied, most of whom were shipyard workers, and that its highest concentrations were found in this exposure category. Probably the type of fibers retained in the lung of the workers in Spain reflects the type of work performed in shipyards in repair and construction, which was probably not very different from that carried out in Japanese or American shipyards. Our results confirm the risk of amosite for inducing lung and pleural diseases.

Regarding the physical characteristics of the fibers detected, 46% had a length >5 μm and a diameter <0.2 μm, and were mainly found in the samples analyzed in the shipyard workers. There is evidence suggesting that these long, thin fibers are the most likely to cause asbestos-related diseases. Fibers below 5 microns in length show greater penetration into the respiratory system but have a shorter retention as they are easily removed. In a case control study of

mesothelioma, McDonald and coworkers[37] found that the concentration of amphiboles (amosite, crocidolite, tremolite) longer than 8 μm was the best predictor of mesothelioma, and that shorter fibers did not contribute to its development. Nevertheless, in view of the results of experimental and epidemiological studies, the toxicity of small asbestos fibers cannot be ruled out. Recent data in humans[38-43] suggest an association between exposure to longer fibers and higher rates of lung cancer, but no definite conclusion can be ascertained for the other size classes. Nevertheless, the authors of these studies noted that exposure to short, thin fibers was associated with lung cancer risk, and these fibers represented the majority of the ones counted in patients' lung samples. It cannot yet be determined whether the association of these short fibers with lung cancer is a spurious effect due to correlations between fiber-size categories, or whether it represents evidence that short fibers do indeed play a specific role in carcinogenesis. It should be mentioned that the predominant fiber type varied between the populations studied, possibly due to variations in the type of asbestos imported in different Spanish areas; unfortunately, however, data on types of imported asbestos are not available in Spain.

Lung samples from elsewhere in Spain are currently sent to our laboratory for asbestos counting, especially samples from lungs excised due to lung tumors. These samples are analyzed to confirm previous asbestos exposure. Asbestos reports are used at present as objective proof of exposure in affected workers. Determination of asbestos bodies is routinely used for diagnostic purposes, particularly in cases in which exposure is doubtful. The hypothetical interest of lung asbestos counting to guide follow-up protocol in each exposed subject should be investigated in further studies.

In conclusion, this thesis provides new data on asbestos lung deposition in different Spanish populations. Our results show that Spanish workers retain mainly amphibole fibres, a considerable percentage of which are particularly dangerous. The availability of reference values and the standardization of sampling and analytical methods have established our laboratory as a reference centre for asbestos analysis in Spain.

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Conclusions

The results of this thesis show that:

1. The majority of the urban population in our area has levels of asbestos in the lung, with values below 300 AB/g of dry tissue. The threshold of 1,000 AB/g is fully applicable for identifying pathological asbestos content.

2. This study, performed following the recommendations of the ERS Guidelines, verifies for the first time that Spanish population has asbestos levels in lung comparable to other European populations (as established in the ERS Guidelines).

3. Asbestos body counting by a single reader is a reliable method, especially at low concentrations of asbestos bodies in lung. Double reading may be indicated in cases in which the lung AB count is close to the commonly applied thresholds of 1000 and 5000 AB/g, particularly if the value is slightly lower.

4. Shipyard workers from El Ferrol, Spain presented high levels of asbestos in the lung. Exposure to asbestos should be considered as a cause of pulmonary disease in this group.

5. Whenever possible, the determination of asbestos in lung is essential to prove causation of asbestos.

6. The results of this thesis provide the first data on the type of asbestos retained in the lungs of Spanish populations. Particularly striking is the exclusive retention of amphiboles, which appear to be responsible for the asbestos-related diseases observed in exposed populations.

Summary

The idea for this thesis emerged from the results of a previous study with a small patient sample which assessed the deposition of asbestos in the lung in residents of the city of Barcelona. Using light microscopy (LM), we also aimed to establish accurate, reproducible reference levels of asbestos in the lung in the Spanish population, in order to allow routine analyses of asbestos bodies (AB). After standardizing the technique in our laboratory, we aimed to assess whether certain respiratory diseases had a higher lung deposition of AB. To do so, we studied patients with lung cancer and asbestosis and compared their results with those of healthy, unexposed individuals. Finally, after performing studies in patients from different Spanish hospitals, we aimed to identify the most prevalent type of asbestos deposited in the lung in the Spanish population.

The initial studies carried out as part of this thesis established that the prevalence of asbestos in the Spanish urban population was between 0 and 300 AB/g dry lung tissue. Levels over 1000 AB/g are regarded internationally as potential causes of pathology. Studying the distribution of asbestos in various areas of the lung, no significant differences were observed, although the highest concentrations were found in the lower lobe in 48% of cases.

Thanks to our team's development of a reproducible method for the analysis of lung AB, our laboratory is now established as a reference centre in Spain. We demonstrated that a single observer's reading of AB was sufficient, and that a second reading was only necessary in patients with levels around 1000 AB/g dry tissue.

The research project aimed to studying asbestos-related disease in workers exposed to the mineral in the course of their occupational activity. We were able to study the relationship between years of asbestos exposure and smoking. We found that the shipyard workers of El Ferrol had high levels of asbestos in the lung, and concluded that asbestos should be considered as a potential cause of lung disease in this population.

In our characterization of the various types of asbestos fiber found in the lungs of Spanish population with and without exposure to asbestos, crocidolite was

the most frequently identified. Chrysotile was not recorded in the samples analyzed, suggesting that this form of the mineral is eliminated after inhalation.

Resumen

Las motivaciones para iniciar el desarrollo de esta tesis se basaron en un estudio previo, con pocos pacientes, que exponía que los individuos residentes en la ciudad de Barcelona presentan un determinado depósito pulmonar de amianto. Quisimos también establecer los niveles de cuantificación de amianto en pulmón, en población española, mediante microscopia óptica (MO) de manera que fueran precisos y reproducibles con tal de poder realizar análisis de cuerpos de amianto (CA) de forma rutinaria. Después estandarizar la técnica en nuestro laboratorio quisimos estudiar si unas patologías respiratorias presentaban un mayor depósito de CA pulmonares; así que estudiamos pacientes con carcinoma pulmonar y asbestosis, y comparamos sus resultados con los de individuos sanos no expuestos. Finalmente, y tras haber realizado estudios en pacientes de distintos hospitales españoles, quisimos profundizar más y estudiamos el tipo de amianto pulmonar más prevalente en población española.

Las primeras investigaciones llevadas a cabo en el desarrollo de esta tesis nos han permitido determinar que la prevalencia de este mineral en población urbana española es de entre 0 y 300 CA/g tejido pulmonar seco, teniendo en cuenta que niveles superiores a 1000 CA/g se consideran internacionalmente como potencialmente causantes de patrología. Al estudiar la distribución de amianto en las distintas zonas pulmonares, no encontramos diferencias significativas; aunque pudimos observar que las concentraciones más elevadas se encontraron en el lóbulo inferior en el 48% de los casos.

Al establecer un método reproducible de análisis de CA en pulmón, nos posicionamos como laboratorio de referencia en España. Demostramos que la lectura de los CA por un único observador era suficiente de manera sistemática, y que únicamente en aquellos casos con niveles alrededor de los 1000 CA/g tejido seco era necesaria doble lectura.

El proyecto de investigación destinado a estudiar las enfermedades relacionadas con el amianto en trabajadores expuestos al amianto en el desarrollo de su trabajo nos permitió estudiar la relación entre los años de exposición a este mineral y el hábito tabáquico. Observamos que los

trabajadores de los astilleros del Ferrol tenían unos elevados niveles de amianto pulmonar; y concluimos que el amianto debería considerarse como causa potencial de enfermedad pulmonar en trabajadores de los astilleros.

Al caracterizar las distintas fibras de amianto encontradas en los pulmones de población española, con exposición al amianto y sin ella, pudimos ver que la crocidolita era el tipo de amianto más frecuente. Observamos la ausencia de crisotilo en las muestras analizadas y ello nos llevó a sugerir que se producía una eliminación de las fibras de crisotilo después de su inhalación.

Scientific production and financial support

List of publications

Velasco-García MI, Recuero R, Cruz MJ, Panades R, Martí G, Ferrer J. Prevalencia y distribución del depósito pulmonar de amianto en población urbana española. Arch Bronconeumol. 2010 Apr;46(4):176-181

Velasco-García MI, Cruz MJ, Ruano L, Montero MA, Freixa A, Ferrer J. Reproducibility of asbestos body counts in digests of autopsy and surgical lung tissue. Am J Ind Med 2011 Aug;54(8):597-602.

Diego C, **Velasco-García MI**, Cruz MJ, Untoria MD, Morell F, Ferrer J. Asbestos pulmonary content in workers of Ferrol shipyards, Spain. Med Clin (Barc). 2013;140(4):152-6.

List of national and international presentations

The results obtained in this thesis have been presented in partial form in the following conferences:

- Ruano L, Cruz MJ, Recuero R, Freixa A, Majó J, **Velasco-García M**, Morell F, Ferrer J. Variabilitat entre observadors en l'anàlisi de cossos d'amiant en mostres de pulmó. XXV Diada Pneumològica. Barcelona, 23-24 de Marzo de 2007. (poster presentation)

- "Cicle de sessions docents i de recerca" del Servicio de Neumologia del Hospital Vall d'Hebron. "Microscopia electrónica para la determinación de fibras de amianto". Barcelona, 18 de Abril de 2007. (oral presentation)

- Ruano L, Cruz MJ, Recuero R, Freixa A, Majó J, **Velasco-García M**, Morell F, Ferrer J. Variabilidad entre observadores en el análisis de cuerpos de amianto en muestras de pulmón. 40º Congreso Nacional SEPAR. Barcelona, 1-4 de Junio de 2007. (poster presentation)

- "XI Curso de Patología Ocupacional Respiratoria", organized by Servicio de Neumología del Hospital Vall d'Hebron. "Determinación de cuerpos y fibras de amianto en muestras de tejido pulmonar y lavado broncoalveolar". Barcelona, 12-14 de Noviembre de 2007. (oral presentation)

- **Velasco MI**, Cruz MJ, Untoria MD, Lopez I, Montero MA, Rodríguez E, Hernández S, Panadés R, Calleja A, Ferrer J. "Prevalença del dipòsit valorable d'amiant a pacients amb càncer de pulmó al nostre medi". XXVI Diada pneumològica SOCAP. Igualada, 11-12 de Abril de 2008. (poster presentation)

- **Velasco MI**, Cruz MJ, Untoria MD, Lopez I, Montero MA, Rodríguez E, Hernández S, Panadés R, Calleja A, Ferrer J. "Prevalencia del depósito valorable de amianto en pacientes con cáncer de pulmón en nuestro medio. "

41º Congreso Nacional de la SEPAR". Tenerife, 30 de Mayo – 2 de Junio 2008. (poster presentation)

- **Velasco MI**, Cruz MJ, Untoria MD, Lopez I, Montero MA, Rodríguez E, Hernández S, Ferrer J. "Prevalence of assessable asbestos deposit in patients with lung cancer". European Respiratory Society 18th Annual Congress. Berlín (Alemania), 4-8 de Octubre de 2008. (poster presentation)

- "XII Curso de Patología Ocupacional Respiratoria", organized by Servicio de Neumología del Hospital Vall d'Hebron. "Determinación de cuerpos y fibras de amianto en muestras de tejido pulmonar y lavado broncoalveolar". Barcelona, 3-5 de Noviembre de 2008. (oral presentation)

- **Velasco MI**, Cruz MJ, Untoria MD, Montero MA, Morell F, Ferrer J. Prevalencia del depósito valorable de amianto en pacientes con cáncer de pulmón en nuestro medio. 1as Jornadas de Formación del Ciberes. Bunyola (Mallorca), 13-14 de Noviembre de 2008. (poster presentation)

- "II Jornada Científica IR-HUVH", organized by Unitat de Gestió del Coneixement del Institut Recerca de l'Hospital Universitari Vall d'Hebron. "Estudio del contenido pulmonar de amianto en población urbana española y en pacientes con cáncer de pulmón". Barcelona, 10 de Diciembre de 2008. (oral presentation)

- "XXIII Curso de Avances en Neumología Vall d'Hebron", organized by Servicio de Neumología del Hospital Vall d'Hebron. "Utilidad de la cuantificación de amianto en el pulmón". Barcelona, 18-20 de Febrero de 2009. (oral presentation)

- CM Diego Riza, R de los Reyes Cruz, MJ Mejuto Martí, J Moreno Barragán, A Souto Alonso, **MI Velasco García**, MJ Cruz Carmona, J Ferrer Sancho. "Determinación de la concentración intrapulmonar de asbesto en trabajadores postexpuestos al amianto". 42º Congreso Nacional SEPAR. Santander, 5-8 de Junio de 2009. (poster presentation)

- "XIII Curso de Patología Ocupacional Respiratoria", organized by Servicio de Neumología del Hospital Vall d'Hebron. "Determinación de cuerpos y fibras de amianto en muestras de tejido pulmonar y lavado broncoalveolar". Barcelona, 2-3 de Noviembre de 2009. (oral presentation)

- "XXIV Curso de Avances en Neumología Vall d'Hebron", organized by Servicio de Neumología del Hospital Vall d'Hebron. "Patología pleuropulmonar por amianto: utilidad de la dosificación de amianto en el pulmón". Barcelona, 24-26 de Febrero de 2010. (oral presentation)

- "4a Jornada Científica VHIR", organized by Unidad de Comunicación e Imagen del Vall d'Hebron Institut Recerca (VHIR). "Contenido pulmonar de amianto". Barcelona, 2 y 3 de Diciembre de 2010. (oral presentation)

- **Velasco-Garcia MI**, Cruz MJ, Diego C, Montero MA, Morell F, Ferrer J. "Primeres dades sobre el tipus d'amiant pulmonar a la población urbana española". XXIX Diada pneumològica SOCAP. Badalona (España), 7-9 de Abril de 2011. (poster presentation).

- **Velasco-Garcia MI**, Diego C, Cruz MJ, Untoria MD, Morell F, Ferrer J. "Determinació del contingut pulmonar d'amiant en treballadors de les drassanes del Ferrol". XXIX Diada pneumològica SOCAP. Badalona (España), 7-9 de Abril de 2011. (poster presentation).

- **Velasco-García MI**, Cruz MJ, Diego C, Montero MA, Ojanguren I, Morell F, Ferrer J. "Primeros datos sobre el tipo de amianto pulmonar en población urbana española". 44º Congreso Nacional SEPAR. Oviedo (Spain), 17-20 de Junio de 2011. (poster presentation).

- Ferrer J, Diego C, **Velasco-García MI**, Cruz MJ. Determinación de la concentración intrapulmonar de asbesto en trabajadores postexpuestos al amianto. 44º Congreso Nacional SEPAR. Oviedo (Spain), 17-20 de Junio de

2011. (oral presentation). This presentation received the award for best communication of the EROM area.

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- Estudio del contenido pulmonar de asbesto en población urbana española. Beca FUCAP 2003.

- Prevalencia del depósito valorable de amianto. Beca FUCAP 2007.

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