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Study on the evolution over time of the risk of disease among former asbestos exposed subjects and genetic risk factors for pleural malignant mesothelioma

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Chapter 1

SUMMARY

Objective. This project was conducted in the framework of the "Asbestos project", funded by the Italian Minister of Health and coordinated by the Italian National Institute of Health (ISS) with the aim of studying epidemiology, diagnosis and treatment of asbestos-related diseases. Two lines of research are focused on increasing knowledge related to the risk of mesothelioma and other cancers among people working in facilities in which they have been exposed to asbestos, aiming in particular at studying time trends of cause-specific mortality. Another line is focused on the investigation of the role of genetic background in asbestos-related carcinogenesis. This line is developed starting from the case control study conducted in the area of Casale Monferrato, where the most important asbestos cement plant in Italy, the "Eternit plant", was active from 1907 to 1986.

Methods. A pool of 43 Italian asbestos cohorts (asbestos-cement, rolling-stock, shipbuilding and others industries) including 54436 subjects was established and mortality follow-up updated. Standardized mortality ratios (SMRs) were computed for the causes selected on the basis of a priori evidence of association with asbestos, considering time factors (duration of employment, time since first exposure, year since first exposure), using reference rates by region and calendar period. The mortality and mesothelioma incidence were also studied among 974 workers employed at the Balangero mine (Italy), the largest chrysotile mine in Western Europe, active from 1917 to 1985.

The association between pleural malignant mesothelioma (PMM) and asbestos cumulative exposure using individual assessment of occupational, environmental and domestic exposure was evaluated in a population-based case-control study that included cases diagnosed between January 2001 and June 2006 and a group of controls among residents in the Casale Monferrato Local Health Authority.

Biological samples of a group of cases affected by PMM and a group of controls were collected. The main hypothesis is that asbestos exposure and genetic factors interact in the causation of PMM, with asbestos as a necessary factor.

The gene-environment interaction analysis was performed including asbestos exposure and fifteen Single Nucleotide Polymorphisms (SNPs) previously identified through a genome-wide association study on Italian subjects based on 392 cases and 367 controls. Cases and controls were from four towns in Northern Italy: Casale Monferrato and Turin in the Piedmont Region, and Genoa and La Spezia in the Liguria Region. The role of germline BAP1 mutations among 40 families and 103 sporadic cases was also studied.

Results. The analysis of the pool of asbestos cohorts included 51,801 subjects (5,741 women) after the data quality control. At the end of follow up the 55.9% of subjects was alive, 42.6% had died (cause known for 95%), and 1.5% were lost to follow-up. Mortality increased for all causes (SMR: Men=1.05; Women=1.17), all malignancies (SMR among men=1.17 and 1.33 among women), pleural and peritoneal malignancies (Men: SMR=13.28 and 4.77; women: 28.44 and 6.75), lung (SMR men=1.26, women=1.43), all with $p<0.01$, and ovarian cancer (SMR=1.38; $p<0.05$). Pleural cancer rate increased during the first 40 years of time since first exposure and reached a plateau thereafter.

Among the cohort of workers of Balangero mine, mortality increased for all causes (SMR=1.28; CI95% 1.17-1.40), pleural cancer (SMR=4.30; CI95% 1.58-9.37) and asbestosis (SMR=375.06; CI95% 262.68-519.23). No statistically significant increase was found for lung cancer (SMR=1.14; CI95% 0.81-1.55) and peritoneal cancer (SMR=3.25; CI95% 0.39-11.75). An increase was observed in the incidence of MM (SIR=6.3;CI95% 2.3-13.7).

Among the case control study conducted in the area of Casale Monferrato, a trend in the risk of PMM was observed with increasing occupational and non occupational cumulative exposure. ORs increased with a cumulative exposure index ($p<0.0001$) from 4.4 (CI 95% 1.7 to 11.3) (for the class <1 f/mL-years) to 62.1 (CI 95% 22.2 to 173.2) (for the class ≥10 f/mL-years) when both occupational and non-occupational exposures were considered. Among subjects never occupationally exposed, corresponding ORs were 3.8 (CI 95% 1.3 to 11.1) and 23.3 (CI 95% 2.9 to 186.9) (reference: background level of asbestos

exposure). A significantly increased OR was observed when father or mother or spouse were occupationally exposed to asbestos. Having a garden or courtyard paved with asbestos cement tailings, an asbestos cement roof or buildings near home were also associated with a significant increase in the OR.

The gene-asbestos interaction studied among the 15 SNPs detected through the GWAS study showed an additional role of genetic modification on PMM susceptibility, in addition to the risk conferred by asbestos exposure.

As for the analyses of families, among the 40 probands with a family history of cancer, four *BAP1* germline variants and a recurrent pathogenic germline mutation in *CDKN2A* in three and one index cases, respectively were dectected. Two *BAP1* and one *CDKN2A* germline variants were found in families with both mesothelioma and melanoma. Two other *BAP1* germline variants were identified in a patient with multiple cutaneous amelanotic melanomas.

Conclusions. The results show that the hypothesis that PMM risk, rather than increasing indefinitely, reaches a plateau and even declines at long latency, coherently with asbestos clearance from the lungs is correct.

In the cohort of chrysotile asbestos miners, an increased mortality from pleural and peritoneal cancers and from asbestosis were detected, in confirmation of the carcinogenic risk of chrysotile exposure.

The case-control study underlined that, in addition to occupational exposures, environmental and familial/domestic exposures to asbestos also contribute to the occurrence of PMM in the population of Casale Monferrato with a dose-response effect.

The genetic study suggested that CDKN2A, in addition to BAP1, could be involved in the melanoma and mesothelioma susceptibility and these tumors share key steps that drive carcinogenesis. It also suggested that other genes may be involved in inherited predisposition to malignant mesothelioma.

CAPITOLO 1

SOMMARIO

Obiettivi. Questo progetto è incluso nel "Progetto Amianto" finanziato dal Ministero della Salute e coordinato dall'Istituto superiore di Sanità (ISS) riguardante lo studio dell'epidemiologia, diagnosi e trattamento delle patologie amianto associate.
Due linee di ricerca riguardano l'incremento della conoscenza sul rischio di mesotelioma e altri tumori tra le persone occupate in attività lavorative con esposizione ad amianto studiando in particolare i trend temporali della mortalità causa-specifica. Un'altra linea riguarda la valutazione del ruolo della genetica nella carcinogenesi in associazione all'esposizione ad amianto. Tale linea è sviluppata nell'ambito di uno studio caso-controllo condotto nell'area di Casale Monferrato, dove il più importante stabilimento di cemento amianto in Italia, lo stabilimento "Eternit", è stato attivo dal 1907 al 1986.
Metodi. Un pool di 43 coorti di ex esposti ad amianto (cemento-amianto, materiale rotabile, costruzione navale e altri settori) ha incluso 54436 soggetti con aggiornamento del follow up. I rapporti standardizzati di mortalità (SMRs) sono stati calcolati per le cause associate con l'esposizione ad amianto considerando differenti fattori temporali (durata lavorativa, tempo trascorso dalla prima esposizione, anno di prima esposizione), e utilizzando come tassi di riferimento i tassi per regione e periodo di calendario. La mortalità e l'incidenza di mesotelioma sono stati studiate tra 974 lavoratori della miniera di Balangero (Italia), la più grande miniera di crisotilo in Europa occidentale, attiva dal 1917 al 1985.
L'associazione tra il mesotelioma maligno della pleura (MMP) e l'esposizione cumulativa ad amianto tramite valutazione individuale di esposizione lavorativa, ambientale e domestica è stata studiata in uno studio caso-controllo su base di popolazione che ha incluso i casi diagnosticati tra il gennaio 2001 e giugno 2006 e un gruppo di controlli tra i residenti nell'area di Casale Monferrato.

Sono stati raccolti campioni biologici di un gruppo di casi affetti da MMP e un gruppo di controlli. L'ipotesi principale è che l'esposizione all'amianto e fattori genetici interagiscano nell'insorgenza di MMP, con l'amianto fattore principale.

L'analisi di interazione gene-ambiente è stata eseguita considerando l'esposizione ad amianto e 15 polimorfismi (SNPs) precedentemente identificati attraverso uno studio di associazione genome-wide su 392 casi e 367 controlli. I casi e controlli sono provenienti da quattro città del Nord Italia: Casale Monferrato e Torino nella Regione Piemonte, e Genova e La Spezia nella regione Liguria. È stato inoltre studiato il ruolo delle mutazioni della linea germinale BAP1 in 40 famiglie e 103 casi sporadici.

Risultati. L'analisi delle coorti pooled ha incluso 51801 soggetti (5741 donne) dopo il controllo di qualità dei dati. Al termine del follow-up, 55,9% soggetti erano vivi, 42,6% deceduti (causa nota per il 95% dei soggetti), e 1,5% erano persi al follow-up. La mortalità è aumentata per tutte le cause (SMR: uomini = 1,05; donne = 1,17), tutti i tumori maligni (SMR tra gli uomini = 1,17 e 1,33 tra le donne), tumori maligni della pleura e del peritoneo (Men: SMR = 13,28 e 4,77; donne: 28,44 e 6,75) , del polmone (SMR = 1,26 gli uomini, le donne = 1,43), tutti con $p<0,01$, e tumore ovarico (SMR = 1,38; $p <0,05$). La mortalità per tumore pleurico è aumentata nel corso dei primi 40 anni dalla prima esposizione raggiungendo in seguito un plateau.

Nella coorte dei lavoratori della miniera di Balangero, la mortalità è aumentata per tutte le cause (SMR = 1,28; IC95% 1,17-1,40), tumore della pleura (SMR = 4,30; IC95% 1,58-9,37) e asbestosi (SMR = 375,06; IC95% 262,68-519,23). Nessun incremento statisticamente significativo è stato individuato per tumore polmonare (SMR = 1,14; IC95% 0,81-1,55) e del peritoneo (SMR = 3,25; IC95% 0,39-11,75). È stato osservato un aumento dell'incidenza di MM (SIR = 6,3; IC95% 2,3-13,7).

Nello studio caso-controllo di Casale Monferrato, un aumento di rischio di MMP è stato osservato con l'aumentare dell'esposizione cumulativa considerando sia l'esposizione professionale che extra-professionale. L'OR è aumentato con l'esposizione cumulativa ($p<0,0001$) da 4,4 (IC 95% 1,7-11,3) (per la classe <1 f/mL-anni) a 62,1 (IC 95% 22,2-173,2) (per la classe ≥10 f/mL-anni) considerando entrambe le tipologie di esposizione. Tra i soggetti non professionalmente esposti, gli OR corrispondenti erano 3,8 (IC 95% 1,3-

11,1) e 23,3 (IC 95% 2,9-186,9) (riferimento: livello background di esposizione ad amianto). Un aumento significativo del rischio è stato osservato quando il padre o la madre o il coniuge erano stati professionalmente esposti all'amianto. Un aumento dell'odds ratio è stato osservato tra i soggetti che hanno avuto un giardino o un cortile pavimentato con parti in cemento amianto, un tetto di cemento-amianto o edifici vicino a casa in cemento-amianto.

L'interazione geni-amianto è stata studiata tra i 15 SNPs rilevati attraverso uno studio GWAS evidenziando un ruolo aggiuntivo sullo sviluppo di MMP oltre al rischio associato all'esposizione ad amianto.

In riferimento alle analisi sulle famiglie, tra i 40 probandi con una storia familiare di cancro, sono state rilevate quattro mutazioni germline in BAP1 e un mutazione in CDKN2A in tre e un caso indice, rispettivamente.

Due mutazioni germline in BAP1 e una in CDKN2A sono state trovate in famiglie con mesotelioma e melanoma. Altre due mutazioni in BAP1 sono state identificate in un paziente con più melanomi cutanei amelanotici.

Conclusioni. I risultati ottenuti confermano l'ipotesi che il rischio di MMP piuttosto che aumentare indefinitamente, raggiunga un plateau o diminuisca dopo un lungo periodo di latenza, in accordo con l'ipotesi della clearance dell'amianto dai polmoni.

Nella coorte di minatori di crisotilo, è stato rilevato un aumento della mortalità per tumori della pleura, del peritoneo e asbestosi confermando il rischio carcinogenico dell'esposizione a crisotilo.

Lo studio caso-controllo ha evidenziato che, oltre a esposizioni professionali, anche le esposizioni ambientali e familiari contribuiscano all'insorgenza di MMP nella popolazione di Casale Monferrato con un effetto dose-risposta.

Lo studio genetico suggerisce che CDKN2A, oltre a BAP1, siano coinvolti nella suscettibilità di melanoma e mesotelioma e che questi tumori condividano pattern di carcinogenesi. Tale studio ha anche evidenziato che altri geni possano essere coinvolti nella predisposizione ereditaria al mesotelioma maligno.

Chapter 2

INTRODUCTION

2.1 Asbestos: properties

Asbestos is a term applied to several mineral species (clinochrysotile, orthochrysotile, parachrysotile, riebeckite, grunerite, cummingtonite, gedrite, anthophyllite, tremolite, actinolite, richterite, winchite, ferriwinchite) when they occur in a fibrous form (asbestiform). When the mineral species are asbestiform, they have physical characteristics such as large aspect ratio of fibers, flexibility, separability and weavability of fibers, and chemical and physical durability, that are associated to the definition of asbestos.

Asbestos is made of thin but very dense fibers, which make it a material highly resistant from a mechanical point of view, but at the same time flexible. It is resistant to chemical and biological agents, abrasion and wear. Finally it has a satisfactory behavior in both thermal and mechanical wear, even at very high temperatures. All asbestos types have been widely used as fire-proofing (e.g. on steel structural beams and soffits) and insulation materials (e.g. on boilers, ovens, kilns, steam pipes and hot water pipes).

However, in addition to those common properties, each asbestos mineral species has unique chemical and physical properties that distinguishes it from others.

The primary mineral groups (minerals with similar composition and structures) for asbestos are amphibole and serpentine and the main mineral species include asbestiform serpentine (chrysotile) and varieties of amphiboles such as tremolite, actinolite, anthophyllite, grunerite, crocidolite, winchite and richterite [Institute of Medicine, 2006]. Chrysotile has long relatively flexible fibers, whereas amphiboles are characterized by shorter, rigid fibers. Fiber types sometimes occur in combination, e.g., chrysotile from Quebec, Canada, typically contains approximately 1% of tremolite, an amphibole [Osinubi et al., 2000].

Chrysotile is sometimes known as white asbestos, although it can also be amber, gray, or greenish in color. Most chrysotile fibers are about 6.4-12.7 mm long. Amosite, sometimes called brown asbestos, often has a light brown tinge, but is also found in dark colors, as well as white and it has coarse fibers that are about 3.0-152.0 mm long.
Crocidolite has a bluish tinge, and its fibers are about 0.12-3.0 in (3.0-76.0 mm) long. The other types of asbestos have no significant commercial applications and are rarely mined [www.madehow.com].

2.2 Asbestos: uses

Asbestos has been used since ancient times but its consumption markedly increased from the 1920s until the 1980s. Asbestos was used in the manufacture of products including textiles, building materials, insulation and brake linings. At a global scale, the highest level of asbestos consumption occurred in 1977, when approximately 4.7 million tons were reached. Then, asbestos health risks triggered country-wide bans and stringent regulations, which resulted in a worldwide asbestos consumption decline until the late 1990s, when it leveled at two million tons, a consumption level that has been maintained since then with some minor fluctuations [Valenzuela et al., 2016].
In 2006, the World Health Organization [WHO, 2006] called for the elimination of asbestos-related diseases taking the position that the most efficient way to eliminate them is to cease using all types of asbestos. The 2014 update of this statement, which was attached to the WHO document “Chrysotile Asbestos” published in response to the continuing widespread production and use of chrysotile, emphasized that all forms of asbestos, including chrysotile, are causally associated with an increased risk of cancer of the lung, larynx, and ovary, mesothelioma and asbestosis [Takahashi et al., 2016]. These observations are in line with the recent evaluation by the International Agency for Research on Cancer. Positive associations have also been observed between asbestos and cancer of the pharynx, stomach and colorectum [IARC, 2012].

All forms of asbestos are now banned in 52 countries [International Ban Asbestos Secretariat, 2010], including all European Union member countries. Nonetheless, these 52 countries make up less than one-third of WHO member countries. A much larger number of WHO member countries still use, import, and export asbestos and asbestos-containing products [LaDou et al., 2010]. About 90% of world asbestos comes from four countries: Russia, China, Brazil and Kazakhstan. Approximately fifty percent of world asbestos is used by two countries, China and India, followed by Brazil, Indonesia and Russia [Marsili et al., 2016]. Chrysotile is the most frequently used type of asbestos (about 95% of world production and use) [Kanarek, 2011].

Production of chrysotile asbestos in 2012 was around 2 kilotonne a year with the main producers being Russia (50%), China (22%), Brazil (15%), Kazakhstan (12%) [USGS, 2013]. Increased usage has been most prominent in the Asia-Pacific region [WHO, 2014]. In particular in China, the demand for asbestos has increased over time, with total production increasing from 310,000 tons in 2001 to 450,000 tons in 2009 [Wang et al., 2013].

Rates of asbestos-related diseases are expected to decrease in Western developed countries as the results of bans. By contrast, the rates will rise in countries where asbestos use continues and most of these are low-and middle-income countries [Frank et al., 2014].

2.3 Asbestos-related diseases and their relation with asbestos type

Currently, about 125 million people in the world are exposed to asbestos at the workplace. According to global estimates, at least 107 000 people die each year from asbestos-related lung cancer, mesothelioma and asbestosis resulting from occupational exposures [WHO, 2014]. Moreover, asbestos may not only affect workers, but also their families, through the exposure of fibres brought home on shoes, clothes, skin and hair [Nymark, 2010].

Mesothelioma is commonly recognized as the primary asbestos-related cancer type. Malignant mesothelioma is an aggressive, diffuse, fatal, asbestos-associated disease

originating from the lining cells (mesothelium) of the pleural (65–70%), peritoneal (30%) or, rarely, pericardial (1–2%) cavities around the lung tissue, abdominal cavity and heart.
The definitive epidemiological study linking mesothelioma to asbestos came from South Africa, published in 1960 by Wagner et al. [Wagner et al., 1960] showing that mesothelioma was frequent in people living or working in the crocidolite asbestos mine area. Epidemiological data suggest that the amphibole, crocidolite, is associated with the highest risk of mesothelioma and that the serpentine fibre, chrysotile, has the lowest [IARC, 2012; Moore et al., 2008].
An evaluation of the relative potency of the different fibre types of asbestos has been considered in the meta-analyses by Hodgson & Darnton [2000; 2010] and Berman & Crump [2008]. Hodgson & Darnton stated that amosite and crocidolite were on the order of 100 and 500 times more potent than chrysotile for causing mesothelioma. Berman and Crump [2008] concluded that potency of chrysotile is at least six-fold less compared with amphiboles and that chrysotile fibers longer than 10 μm are more carcinogenic than the shorter fibers. Biopersistence in the lung is lower for chrysotile and short fibers [Pinto et al., 2013]. Predominantly amphiboles were detected in the lungs of patients living around asbestos-cement factory of Casale Monferrato and Bari, due probably to the lower lung clearance of amphiboles [Barbieri et al., 2012]. Few studies were conducted to investigate cancer risks in exclusive chrysotile asbestos workers [Dement et al., 1994; Liddell et al., 1997; Wang et al., 2012]. The results of these studies suggested excessive cause-specific mortality in particular from lung cancer and respiratory diseases.
Debate on potency difference is not yet resolved but it is claimed as of public health importance to stop chrysotile use, common in many countries around the world [Kanarek, 2011].

2.4 Descriptive Epidemiology of Malignant Mesothelioma

Malignant mesothelioma is a rare malignancy that is mainly localized to the pleura. The diffuse malignant mesothelioma of the pleura is characterized by the tendency to spread within the entire pleural cavity. In the early stages, the lesions appear as small multiple

nodules at the level of visceral pleura or, more frequently, parietal pleura. Afterwards, tumor nodules extend along the pleural surface, forming a pleural thickening that compresses the underlying lung. The pleural cavity can be mostly completely obliterated or, at other times, arranged as two separate sheets for the presence of a hemorrhagic effusion.

With the advance of growth, the tumor invades the lung parenchyma, the intercostal and subcutaneous tissue and can metastasize to regional lymph nodes, contralateral lung, liver, brain, bone and other venues.

Worldwide malignant mesothelioma incidence has been rising since the mid 20th century. Analysis of mesothelioma mortality recorded in the WHO mortality database between 1994 and 2008 yielded an age-adjusted mortality rate of 4.9 per million, a mean age at death of 70 years and male to female ratio of 3.6:1 [Robinson, 2012].

The highest mesothelioma incidence rates are reported from some countries in Europe (UK, The Netherlands, Malta, Belgium) and in Oceania (Australia, New Zealand). Countries with intermediate incidence rates include a large part of Europe (Germany, France, Iceland, Sweden, Norway, Finland, Denmark, Austria, Croatia, Italy), and the United States. Low incidence/mortality rates are reported from various countries of Central Europe, Ireland, Spain, and from several countries of Asia [Bianchi et al., 2014].

In Italy, in 2011, the pleural malignant mesothelioma (PMM) incidence was 3.49 and 1.25 cases per 100,000 person/years in men and women respectively with 1428 (1035 in men and 393 in women) reported as incident cases [Marinaccio et al., 2015].

Currently, the incidence of the disease is still increasing in most countries around the world, and only in countries in which asbestos control measures were taken during the 1970s, such as Sweden and the UK, such increase has levelled off. In countries which produce and/or use asbestos, such as China, India, Russia, Zambia, Colombia and Kazakhstan, a sharp rise in incidence is expected [Røe et al., 2015].

2.5 Lung cancer and asbestos exposure

The first epidemiological study that demonstrated an excess of lung cancer in relation to exposure to asbestos were reported in the 1950s. In a historical cohort study that represented an important advance in epidemiological methods, in addition to providing important new data about asbestos, Doll reported a more than 10-fold excess of lung cancer among British asbestos textile workers [Doll, 1955].

Since 1955, numerous cohort and case-control studies have identified an association between lung cancer and occupational exposure to asbestos. There are controversies on how the risk could vary by exposure to different fibre types and sizes [IARC, 2012].

Asbestos elevates the risk of contracting lung cancer in non-smokers, but the risk seems to increase even more significantly in smokers, indicating that tobacco smoke and asbestos act as co-carcinogens in a synergistic manner. Some investigators have observed a synergistic multiplicative effect between tobacco smoking and asbestos exposure in the development of lung cancer [Harris, 2015]. Results of recent studies show that smoking cessation is associated with a substantial reduction of lung cancer risk among individuals exposed to asbestos [Barone Adesi et al., 2016]. A study by Frost et al. [2011] reported that the earlier asbestos workers stopped smoking, the greater is the benefit.

It is generally agreed that the presence of asbestosis, a type of pleural fibrosis, greatly increases the risk of lung cancer [Nymark, 2010]. A study of insulator workers exposed to asbestos showed that asbestosis increases lung cancer risk and, if considered jointly with smoking, has a supra-additive effect [Markowitz et al., 2013].

2.6 Evaluation of risk for different types of asbestos exposure

Traditionally, occupational exposures have been documented to be the most hazardous, and asbestos workers develop the greatest amount of asbestos-related diseases but risk affects also other categories of exposure. The causal relationship between occupational exposure and pleural mesothelioma was well established and it is estimated that about 80% of male cases may be attributed to occupational asbestos exposure [Lacourt et al., 2014].

Occupational exposure to asbestos causes an estimated 107,000 deaths each year worldwide [Collegium Ramazzini, 2016]. Family members are also at risk because workers carry asbestos home on their clothing and evidence of pleural mesotelioma in relation to domestic exposure to asbestos has been presented in several case reports and case–control studies and two cohort studies [Ferrante et al., 2007]. Living near a facility that uses asbestos, such as a mine or a manufacturing plant, can put people at risk [Frank et al., 2014].

The area of Casale Monferrato in Italy showed an extremely high incidence of PMM. The average annual incidence (definite diagnosis) in the 2009-2013 period was 51.2 (per 100,000) among men and 20.2 (per 100,000) among women. An asbestos-cement (AC) factory (Eternit) was active in the town of Casale Monferrato for 80 years, until 1986, and was the major source of asbestos pollution. Several studies on the effects of asbestos exposure in the area were performed.

The environmental concentration of asbestos was evaluated before the closure of the factory and in later times but not in the 50s to 70s, during the periods of major activity of the factory. Available data on the environmental concentration of asbestos fibers in Casale showed a high level of environmental exposure [Marconi et al., 1989; Chiappino et al., 1991].

The previous reports considered the association of PMM with asbestos exposure separately for occupational, environmental and familial/domestic routes of exposures, and using qualitative exposure assessment [Magnani et al., 2000, Magnani et al., 2001, Magnani et al., 2008]. This project includes a more refined assessment of asbestos exposures taking into consideration the frequency, duration and intensity of exposure for all subjects and all types of asbestos exposure, and a cumulative exposure index was computed. Estimates of PMM risk by this cumulative exposure index with consideration of all sources of exposure have been computed.

2.7 Genetic risk factors for pleural malignant mesothelioma

The molecular mechanisms underlying pathogenicity of the asbestos fibers have not yet been fully understood. The scientific community is unanimous in considering fibrous form (long and thin fibers), surface reactivity (release of radicals and reactive oxygen species, ROS) and high biopersistance (low fragmentation, poor solubilization and slowed clearance) the three characteristics determining the pathogenicity of asbestos [IARC, 2012].

Airborne asbestos fibres tend to accumulate in the alveoli adjacent to small airways and can lodge and penetrate the alveolar wall. Once *in situ*, fibres are resistant to destruction, persist in the lung and cause low-grade inflammation which results in fibrosis. Fibres gradually migrate into the lung tissue between the alveoli causing an extension of the low-grade inflammation and interstitial fibrosis develops and extends to present as asbestosis [Sen et al., 2015].

Asbestos fibers retained in the lung and pleura may be carcinogenic, either through direct mechanical or biochemical effects, or through the activation of inflammatory cells. Persistent inflammation can induce chronic oxidative stress, genotoxic lesions, chromosomal aberrations and epigenetic alterations. Asbestos fibers may also interfere with chromosome segregation and mitosis [Matullo et al., 2013]. Some studies have proposed a role of genetic polymorphisms of DNA repair and oxidative metabolism enzymes. These studies showed that genetic factors could indeed have a role in the pathogenesis of PMM, though much lower than that brought by asbestos [Betti et al., 2009; Betti et al., 2011; Dianzani et al., 2006].

The role of genetic factors is assumed when only a part of the individuals exposed to a carcinogen develops the disease and/or when there are families in which the disease is present in greater proportion then the general population.

Genetic risk factors are represented by variants of a gene, or a portion of the genome, able to increase the risk of developing the disease. The biologically effect can produce a change in the biological function of the gene that contains the variation or adjacent to it.

Such variants are divided into three groups: low risk (RR <1.5), intermediate risk (RR 1.5-5) and high risk (RR>5) variants [Cazier et al., 2010]. Most of the genetic risk factors

associated with types of sporadic cancer (that is, non-familial) provide low or intermediate risk. Asbestos exposure represents the main risk factor for PMM increasing the risk of mesothelioma 10-20 times compared to non-exposed populations [Magnani et al., 2001]. This shows the important role of environmental factors. However only 5-17% of individuals exposed to asbestos develop PMM [Matullo et al., 2013] and familial clustering of PMM suggests that genetic factors (predisposition) may be involved in the development of the disease [Ascoli et al., 2007; Bianchi et al., 2004].

A genome-wide association study on 392 cases and 379 controls from three towns in Northern Italy (Casale Monferrato, Turin, Genoa) genotyped for more than 370.000 SNPs was performed showing several SNPs associated with PMM ($p=10^{-5}/10^{-7}$). Most of these SNPs were located in regions reported to harbor aberrant alterations in MM (SLC7A14, THRB, CEBP350, ADAMTS2, ETV1, PVT1 and MMP14 genes), causing at the most a 2–3-fold increase in PMM risk [Matullo et al., 2013]. The Australian study published by Cadby et al. [2013] on 428 cases and 1269 controls confirmed the genomic regions where there are some of the identified variants as risk factors in the Italian study. These studies are useful in order to study low risk genetic factors.

Examples of genetic factors able to confer a high risk are the inactivating mutations in the tumor suppressor genes, which are inherited in an autosomal dominant mechanism and responsible for rare syndromes with a high risk of cancer.

BAP1 tumor predisposition syndrome (BAP1-TPDS) is associated with an increased risk for the specific skin lesion – atypical Spitz tumors – and the following cancers, in descending order of frequency: uveal (eye) melanoma (UM), malignant mesothelioma (MM), cutaneous melanoma (CM), clear cell renal cell carcinoma (ccRCC), and basal cell carcinoma (BCC). Affected individuals can have more than one type of primary cancer [Pilarski et al., 2016]. This co-occurrence suggests that these tumors share a common stepwise carcinogenic pathway. This tumor predisposition syndrome is inherited in an autosomal dominant pattern. BAP1-mutated MM is associated with female predominance, earlier age of onset, epithelioid differentiation and longer survival time [Wang et al., 2016].

Chapter 3

AIM OF THE PROJECT

This project is part of the "Asbestos" project, funded by the Italian Minister of Health and coordinated by the "Istituto Superiore di Sanità" (ISS), (Italian National Institute of Health) that represents the first implementation of the National Plan for Asbestos [http://www.salute.gov.it/imgs/C_17_pubblicazioni_1945_allegato.pdf, last accessed 15 february 2017].

The general objective of the "Asbestos" project was to provide answers to the open issues concerning epidemiology, diagnosis and treatment of asbestos-related diseases. In particular the activities were focused on: the risk related to environmental exposure after the asbestos ban, methods for health and epidemiological surveillance, innovative methods of diagnostics and treatment of the diseases related to exposure to asbestos.

The project involved four research units including the ISS in Rome, the University of Eastern Piedmont in Novara, the University of Turin and "Regina Elena" Cancer Institute in Rome.

Our unit was involved in the research regarding new knowledge about the risk of mesothelioma and other type of cancers among subjects occupationally exposed to asbestos: the risk after the long latency in a pool of Italian asbestos-exposed cohorts, the risk among the "Balangero" mine worker cohort (Balangero mine was the biggest open air chrysotile asbestos mine in Europe), and the evaluation of individual factors that could be relevant in detecting the individual susceptibility to asbestos exposure.

The Casale case-control study was supported by a grant from Regione Piemonte and the analyses were supported by the ISS.

Chapter 4

MATERIAL & METHODS

4.1 Italian pool of former asbestos exposed subjects

The first part of the project consisted in a pool of 43 Italian cohorts of asbestos exposed subjects of different Italian regions: Campania, Emilia Romagna, Lazio, Lombardia, Marche, Piemonte, Puglia, Toscana, Veneto. The main production sectors are: asbestos cement (AC), rolling stock construction (RSC) and rolling stock maintenance and reparation (RSM), Shipyards (construction and reparation) (SY). The cohorts selected had already been the subject of epidemiological studies.

The follow-up of the subjects in each cohort, including the ascertainment of the causes of death, was carried on separately by each research unit (one in each region). The Registrar's Offices of the town of residence were accessed to obtain the information on vital status. The causes of death for decedents after 1985 were provided by the Local Health Authorities or the Regional Registries of Causes of Death. For earlier deaths, the cause was provided by the Registrar Office of the municipality where it occurred. The underlying cause of death was coded according to the International Classification of Disease, 8th, 9th, and 10th Revisions, according to the date of death. The date of follow-up varied depending on the most recent available update of files but it was required to be at least 31/12/2010.

After the completion of follow-up, each research unit forwarded to the study coordination the anonymized list of workers in each cohort, with sex, date of birth, starting and ending date of the working period, vital status, date of the end of follow up, cause and date of death if the subject was deceased.

A dataset including each cohort was set up and the individuals were 54436. The subjects could have been working in multiple cohorts. Quality control led to the exclusion of 2453 records (4.5% of the initial 54436) because of: incomplete working periods, conflicting

dates or impossible hiring or retirement age (n=737), first employment after the asbestos ban, set on 1.1.1993 - midpoint date between approval and enforcement of the law (n=594). Because of follow-up incompleteness, two cohorts, namely the asbestos cement factories of Eternit-Bagnoli and of Fibronit-Broni, were limited to the workers hired after 1.1.1950 (n=1122).

Workers employed in different cohorts were identified according to gender and birth date, with enquiries to the study coordinators, and were annotated, for a total of 178 workers (of which 4 with 3 records) and their work histories were merged in the pooled analyses.

Finally, the subjects included in the analysis were 51801. The table below provides information on location, use of asbestos and number of workers in the study.

Statistical analyses were based on computation of the number of person years (p-years) and standardised mortality ratios (SMR) (the ratio of observed to expected deaths using indirect standardization) [Breslow & Day, 1987]. Workers in the cohort contributed until their most recent date of observation.

Mortality rates of the regions where the cohorts were located were used. The set of rates was prepared by the National Institute of Health, using mortality and population figures provided by the National Institute of Statistics - ISTAT (Rome, Italy) and available from 1970 [Pirastu et al., 2016]. Correspondingly, analyses were restricted to p-years and events occurring after January 1st 1970. Therefore, deaths and p-years before 1970 were excluded from the analyses. The SMRs for the major causes of death, including accidents and violence were computed. The analyses included the causes selected on the basis of a priori evidence of association with asbestos [IARC, 2012] and causes associated with the healthy worker effect (HWE) [Radon et al., 2002]. HWE reflects that an individual must be relatively healthy in order to be employable and both mortality and morbidity rates within the workforce are usually lower than in general population [Li et al., 1999].

Duration of employment was computed by summing up the duration of all employment periods in the cohort. Latency was computed from the date of first employment. Statistical significance was set at 5%, however alpha errors (p-value) at 1% or lower were highlighted. Ninety five percent confidence intervals (95%CI) were estimated assuming the Poisson distribution of the number of observed cases [Breslow and Day, 1987].

Analyses were carried out using OCMAP PLUS v 3.10 (University of Pittsburgh, PA, USA), SAS v8 and STATA 11 (Stata, College Station, TX, USA).

N	Industrial activity	Company or cohort name	Town	Period of activity	Asbestos type used	Asbestos used from:	N. in the study
1	AC	**Sacelit**	Senigallia	1948-1984	Chrysotile, crocidolite, amosite	1948	589
2	AC	**Eternit**	Casale Monferrato	1907 - 1986	Chrysotile, crocidolite	1907	3366
3	AC	**SACA**	Cavagnolo	1948 - 1982	Chrysotile, crocidolite, amosite	1948	812
4	AC	**Fibronit**	Broni	1932 - 1993 #	Chrysotile, crocidolite, amosite	1932	1333
5	AC	**Edilit**	Vigodarzere	1961 - active	Chrysotile, crocidolite	1947	539
6	AC	**Baraclit**	Bibbiena	1943 - active	Chrysotile from 1945; Mixed from 1963	1945	725
7	AC	**Fibronit**	Avenza	1940-1983	Mixed fibres	1935	226
8	AC	**Veronit**	Livorno	1930-1985	Mixed fibres	1950	157
9	AC	**Cemamit**	Ferentino	1963-1984	Chrysotile, crocidolite	1963	81
10	AC	**Artclit**	Cadelbosco Sopra	1965-1987	Chrysotile, crocidolite	1965	50
11	AC	**Cemental**	Correggio	1952-1989	Chrysotile, crocidolite	1952	548
12	AC	**Cemiant**	Cadelbosco Sopra	1968 - 1991	Chrysotile	1968	103
13	AC	**ICAR Eternit**	Rubiera	1961 - 1992	Chrysotile, crocidolite	1961	553
14	AC	**Fibrotubi**	Bagnolo in Piano	1957 - 1993	Chrysotile, crocidolite	1957	284
15	AC	**Itamiant**	Castelnovo Sotto	1955 - 1993	Chrysotile, crocidolite	1955	1187
16	AC	**Maranit**	Poggio Renatico	1962- 1993	Chrysotile, crocidolite	1962	201
17	AC	**Sidercam**	Boretto	1969 - 1993	Chrysotile, crocidolite	1969	128
18	AC	**Superlit**	Novi di Modena	1954 - 1993	Chrysotile, crocidolite	1954	175
19	AC	**Uprocem**	Boretto	1973 - 1993	Chrysotile, crocidolite	1973	58
20	AC	**Fibronit**	Bari	1934 - 1989	Chrysotile, crocidolite, amosite	1934	413
21	AC	**Eternit**	Napoli	1939 – 1986 #	Chrysotile, crocidolite	1939	1447
22	SY (merchant ships)	**Fincantieri**	Venezia	1917 - active	Mixed fibres	1950	4378
23	SY	**Cantieri Navali Apuania**	Massa	1945 - active	Mixed fibres	1945	742
24	RSM	**Officina Grandi Riparazioni FS**	Vicenza	1914 - active	Crocidolite	1960	1628
25	RSM	**Officine di Cittadella**	Cittadella	1946 - 2003	Crocidolite	1964	1216
26	RSM	**Officine Meccaniche della Stanga**	Padova	1920- 2003	Crocidolite	1946	2016
27	RSM and construction	**FERVET**	Castelfranco Veneto	1908 – 2013	Crocidolite	1960	947
28	RSM	**Officina Grandi Riparazioni FS**	Firenze	1930-2005	Crocidolite	1957	998
29	RSM	**FERVET**	Viareggio	1930-1989	Chrysotile	1956	841
30	RSM	**Italian Railways**	Lazio region	Early 1900 - Active	Mixed fibres		8395
31	RSM	**Casaralta**	Bologna	1919-1998	Mixed fibres	1960	1843
32	RSC	**Breda**	Pistoia	1930 - active	Mixed fibres	1956	3535
33	RSC	**SACFEM**	Arezzo	1945-1983	Crocidolite	1956	681
34	RSC	**Officine Gallinari**	Reggio Emilia	1957-1991/1992	Chrysotile, crocidolite	1957	1680
35	Harbour	**Compagnia lavoratori Portuali**	Venezia	1929 - active	Mixed fibres	1966	1939
36	Ship furniture	**Signani**	Aulla	1945-1994	Amosite	1945	1170
37	Heatproofing	**Siri**	Sesto Fiorentino	1951-1989	Chrysotile, crocidolite, amosite	1951	205
38	Glassworks	**Saivo**	Firenze	1936-1995	Chrysotile	1936	1317
39	Glassworks	**Borma**	Livorno	1940-1992	Mixed fibres	1940	2410
40	Industrial ovens construction	**Santa Lucia**	Pistoia	1962 - active	Chrysotile, crocidolite	1962	217
41	Asphalt rolls	**Derbit**	Bologna	1964-1997	Chrysotile	1964	413
42	Italian miners in Wittenoom	**Australian Blue Asbestos (ABA) Italian workers returned to Italy**	Wittenoom (Australia)	1943-1966	Crocidolite	1943	300
43	Wives of AC workers	**Wives of Eternit workers**	Casale Monferrato	1907 - 1986	Chrysotile, crocidolite	1907	1777
	Workers in multiple cohorts						178

#Restricted to workers first employed from 1950.

4.2 The "Balangero" mine cohort

The second part of the project regards the cohort of miners and millers of "Balangero", the largest chrysotile mine in Western Europe.

Chrysotile from Balangero mine in Italy, active from 1917 to 1985, was tremolite-free and contained trace of balangeroite (0.2%-0.5% of the total mass of samples of chrysotile), a non asbestos fibrous mineral similar in shape to amphiboles [Piolatto et al., 1990]. In light of current knowledge, balangeroite cannot be considered a carcinogen, nor it can be implied to cause mesotheliomas instead of chrysotile [Mirabelli et al., 2008].

The first measurements of airborne fibre concentration in Balangero were performed in the late 1960s: from 1967 to 1970 four measurement campaigns were carried out by industrial hygienists from the Occupational Health Department of the University of Milan. Fibre concentrations were estimated by area. A limited number of air samples were examined from 1970 and 1974, and in 1975 systematic monitoring by the health and safety office of the mine began.

The occurrence of mesotheliomas in individuals with exposure to Balangero chrysotile is important because no tremolite has been detected in it [Mirabelli et al., 2008]. Instead, this mineral was present in the Quebec Chrysotile mines [Liddell et al., 1998].

The cohort was established by Rubino et al. [1979] and studied further by Piolatto et al. [1990] and Pira et al. [2009]. The Unit of Cancer Epidemiology of Turin re-established the cohort of employees using the factory rosters available in the State Archives of Turin.

The cohort included 974 male workers employed for at least 6 months and active at the Balangero mine on January 1st, 1946 or hired subsequently until the cessation of activity.

The list of cohort members and their working periods and job assignments were abstracted from the factory rosters, kept since the closure of the mine at the Turin section of the Italian State Archives.

Vital status information was obtained either at the municipality of last known residence or through the records of the National social security and welfare institute in Italy (INPS), along with the date and place of death for subjects who died. The causes of death were provided by the Register Office of the place of death and were coded by us to the

International Classification of Diseases - ICD classification (9th revision). For the present study, the follow-up was extended up to 31.5.2013.

Standardized mortality ratios (SMRs) were computed using as reference the mortality rates of the Piedmont population provided by the Italian National Institute of Health (ISS). Reference rates were available from 1970 to 2012, in five year periods. Person-years (and, thus, expected deaths and SMRs) were calculated from 1965 to 2013. The rates of 1970-1974 was extended to 1965-1969 and those of 2010-2012 to 2013. Ninety five percent confidence intervals (95%CI) were estimated assuming the Poisson distribution of the number of observed cases [Breslow and Day, 1987].

Duration of exposure was computed as the sum of all working periods for workers who left and re-entered the mine. Latency equals the time elapsed since first employment to the most recent observation and corresponds to follow-up duration. Time since last exposure is the duration from cessation to the most recent observation.

The diagnosis of mesothelioma among the subjects in the cohort could be checked by the Registry of Malignant Mesotheliomas of Piedmont (RMM) [Mirabelli et al., 2007]. SIRs for PMM were calculated using the indirect standardization for the period 1990-2012.

Analyses were carried out using the OCMAP PLUS v.3.10 (University of Pittsburgh, PA, USA), STATA 11 (STATA, College Station, TX, USA) and SAS 8.02 programs.

4.3 Case control study in the area of Casale Monferrato

The study included the incident cases of pleural malignant mesothelioma (PMM) diagnosed from 1/1/2001 to 30/6/2006 to residents in the area of Casale Monferrato.
Casale Monferrato (North West Italy) is an area with an exceptionally high incidence of mesothelioma caused by asbestos contamination at work and in the general environment from the asbestos cement Eternit plant. "Eternit" was one of the major plant for the production of corrugated and plain sheets, tubes and high-pressure pipes in asbestos-cement in Italy active between 1907-1986 [Magnani et al., 2008].
Incident cases in the period January 2001- June 2006 were detected in the hospitals of the area. The controls were a random sample of residents matched to cases by sex and date of birth. Cases and controls were matched by date of birth (±18 months) and gender. To increase power in the younger age classes, when occurrence of PMM is lower, the case-control ratio was 1:2 for cases 60 years and older and 1:4 for younger cases.
Cases and controls were interviewed with a standardized questionnaire including sections on demographic characteristics, lifelong occupational and residential histories, selected leisure time activities and characteristics of the home environment possibly relevant for asbestos exposure. Two hundred cases and 348 controls were included in the study.
The evaluation of asbestos exposure (occupational, environmental, domestic) was conducted blindly to the case/control status by an experienced rater.
As individuals often had multiple exposure circumstances, work and not work-related, exposure assessment took into account their whole exposure history and computed a single exposure index, reflecting the contribution of all sources. Exposures unrelated with work included living in proximity to industrial or natural sources of airborne asbestos (environmental exposures), sharing home with individuals occupationally exposed to asbestos (familial exposure), having asbestos-containing materials installed at home or handling such materials during home repairs or leisure-time activities (domestic exposures).
For occupational exposures, exposure dose was computed by multiplying the indexes of intensity, and frequency of each pattern/job, taking into account the logarithmic nature of the intensity index scale, by multiplying again the score obtained times the duration of the

pattern/job and by summing up all the doses accumulated during the biologically relevant life span.

Exposure frequency was defined as the percent proportion of working time spent at a certain exposure level and duration of exposure for a given period was computed as the difference between the year of start and year of end, or 6 months if both occurred in the same year. The frequency was assigned according to the job description provided at interview and if no direct estimate could be obtained, information provided by other interviewees engaged in similar work activities and the personal experience of the rater were considered.

In order to assess the intensity of exposure, quantitative data on exposure levels are rarely available in a population based case control study and when available they have often serious limitations in validity (because of limited time and/or job representativeness). Accordingly, a quantitative approach is not proposed for use in intensity assessment. Rather, an intensity level scale might prove useful, with the following level ranks, especially developed and adjusted for asbestos fibres:

Semiquantitative scale for intensity assessment

Rank code	Description	Concentration range	Criteria
Fibres / litre range			
0	Concentrations typical of very clean areas, without anthropogenic as well as natural sources	< 0.3 ff/l	No known asbestos fibres sources: no traffic, no industries, no quarries, no widespread serpentinous rocks
1	Concentrations typical of areas with natural sources, or involved by slight pollution	0.3 - 3 ff/l	Areas with light sources, such as widespread serpentinous rocks, light traffic
2	Concentrations typical of heavily polluted areas, and of extremely clean industrial practices.	3 - 30 ff/l	Areas interested by heavy traffic, widespread asbestos cem. roofing in poor conditions, far field from asbestos industries/quarries
3	Concentrations typical of very heavily polluted areas, and of well protected industrial jobs/tasks	30 - 300 ff/l	Areas in the near field from asbestos industries. Good confinement/capture devices during direct handling. Indirect, by-stander exposures.
Fibres / milliliter range			
4	Concentrations typical of poorly protected/unprotected jobs/tasks, without powerful sources	0.3 - 3 ff/cc	Attainable without confinement/capture, sophisticated control devices.
5	Concentrations typical of poorly protected/unprotected jobs/tasks, with powerful sources	3 - 30 ff/cc	Reached in the absence of any confinement and control device, and in presence of powerful sources of pollution

Rank code	Description	Concentration range	Criteria
Fibres / milliliter range			
6	Concentrations typical of unprotected jobs/tasks, with very powerful sources.	30 - 300 ff/cc	Reached in presence of very powerful sources, such as major, and/or high speed emitting sources, without any confinement and control device
7	Concentrations typical of jobs/tasks as before, plus further critical factors	300-3000 ff/cc	Like 6, but in presence of highly confined work-places and/or very pure asbestos-based materials, and/or incorrect cleaning practices ...

The probability of occupational exposure was also assessed and classified as definite, probable, possible and unlikely. Exposure was considered definite when reported at interview or known to the rater. It was classified as unlikely, when it had not been reported at interview and the rater had no knowledge of its occurrence under the specific circumstance being evaluated or other plausibly similar circumstances. Intermediate conditions, that is, exposure neither reported nor denied at interview but known to the rater to have occurred under the same or similar circumstances, could be classified as probable (if prevalence of exposure in the specific job, industry and period was estimated to be high) or possible (if prevalence was estimated to be low). Self-report of exposure was decisive for the assessment of exposures due to presence or use of asbestos-containing materials at home.

All of the above concepts were applied to a large number of conditions entailing exposure either in the general environment, or at home. It was originally developed for occupational exposures assessment, and work takes usually 40 hours a week, i.e. 40 out of 168. This means that a continuous, non-occupational exposure lasts three to four times a similar, continuous occupational exposure. Environmental and domestic exposures were typically assigned a standard 300% frequency index as reported in the guidelines for risk assessment US-EPA [U.S. EPA, 1998].

The data analysis was based on unconditional logistic regression adjusting all models for gender, age at diagnosis and type of interview (subjects vs with proxy).

4.4 Genetic risk studies

4.4.1 Gene–asbestos interaction in pleural malignant mesothelioma susceptibility

In order to study the interactions between genetics and asbestos exposure, and their effects in modulating pleural malignant mesothelioma risk in Italian population, for 15 of the SNPs with highest significance levels detected by Matullo et al. [2013], the interaction between the genotype and asbestos exposure levels was evaluated. The study sample included pleural malignant mesothelioma (PMM) cases and controls from cities located in Northern Italy: Casale Monferrato and Turin in the Piedmont Region, and Genoa and La Spezia in the Liguria Region. Casale Monferrato sample was a population based case-control study [Dianzani et al., 2006], and included 241 PMM patients and 252 population controls. Turin sample was a hospital-based case-control study [Betti et al., 2011], and consisted of 91 PMM patients and 56 controls (non-neoplastic and non-respiratory conditions). The hospital-based study in Genoa and La Spezia included 75 incident PMM cases and 81 controls (healthy subjects or patients hospitalized for non-neoplastic and non-respiratory conditions) [Ugolini et al., 2008].

Asbestos exposure was carefully assessed in both cases and controls. For statistical analysis, a binary classification was used both for asbestos exposure (exposed versus unexposed) and for genotypes (homozygous for major allele versus one or two copies of the minor allele). Subjects with exposure coded as “no/unlikely exposure” were considered as unexposed, while subjects coded as “low” or “high” were considered as exposed.

The interaction was evaluated using an additive model and a multiplicative model. The deviation from the additive model was investigated using the risk of excess index (RERI) and synergy index (SI) and the deviation from the multiplicative model was assessed by multivariable logistic regression.

Under the null hypothesis of no interaction under the additive model, RERI is not significantly different from 0, whereas SI is not significantly different from 1. RERI > 0 indicates positive interaction and RERI < 0 negative interaction. SI > 1 means positive interaction and SI < 1 negative interaction. Synergistic interaction (positive interaction)

implies that the combined action observed between two factors in an additive model is greater than the sum of individual effects. On the contrary, antagonistic interaction means that in the presence of two factors in an additive model, the action of one exposure variable reduces the effects of the other [Rothman et al., 1998]. Deviations from a multiplicative model were explored by multivariable logistic regression models including: asbestos exposure, one SNP at time and the corresponding interaction term (SNP × exposure). Logistic regression models were adjusted for age, gender, PCA cluster and center of recruitment. P values for multiplicative interaction were calculated by comparing the full model including a multiplicative interaction term to a reduced model without it, using the likelihood ratio test.

4.4.2 BAP1 Mutations: Analysis of familial and sporadic mesothelioma

In order to study the contribution of BAP1 mutations in familial MM aggregations, five Italian families that showed malignant tumor predisposition and multiple patients with MM were studied. One hundred and three cases of PMM were sequentially selected from the list of cases, with histologically diagnosed MM and resident of the Local Health Authority of Casale Monferrato, included in a population based, case-control study, whose detailed study design was previously reported [Betti et al., 2011]. No selection was applied for this study, except for the availability of DNA. The sample size was chosen to estimate a possible prevalence of 7.7% (the value reported by Testa et al., 2011) with a confidence interval width of 65.0% (i.e., a precision equal to 65% of the point estimate). Subsequently, the contribution of BAP1 mutations in familial MM aggregations was studied among 40 Italian families classified into three groups were identified: 6 families with both mesothelioma and melanoma, 23 families with melanoma (without mesothelioma) and with familiarity for BAP1 associated tumors and 11 families with mesothelioma (without melanoma) and with familiarity for BAP1 associated tumors.

BAP1 gene was sequenced in a representative subject of each family and the same index cases were also studied for the most common melanoma predisposition genes (i.e.

CDKN2A, CDK4, TERT, MITF, POT1) [Hill et al., 2013] to investigate if these genes may also confer susceptibility to mesothelioma.

All the probands were Caucasian of Italian ancestry and signed an informed consent to participate in the research project.

Blood collection and storage was performed according to "consensus" protocols derived from the standard protocols already used among the Units involved [Matullo et al., 2013], in agreement with Ethical guidelines.

Chapter 5

RESULTS

5.1 Italian pool of former asbestos exposed subjects

Pooled cohorts analysis included 51801 subjects (46,060 men and 5,741 women). Table 5.1 shows the subjects on the basis of the working activities. At the end of follow-up, the 55.9% of the subjects were alive, 42.6% had died, and 1.5% were lost to follow-up or had moved abroad (Table 5.2). The cause of death was unknown for the 5% of deceased subjects (1092 subjects: 960 men and 132 women). Considering the follow up period after 1970, the cohorts contributed altogether 1,430,713 person-years for men and 184,940 py for women. The 1,172 deceased subjects (1,024 men and 148 women), 32 moved abroad (25 men and 7 women) and 230 lost to follow up (211 men and 19 women) before 1970 were excluded from the analysis.

Table 5.1. Industrial activities of the subjects included in the cohort

	Men		**Women**		**Total**		
	N	**%**	**N**	**%**	**N**	**%**	**p-y**
Asbestos-cement	10,714	23.3	2,362	41.1	13,076	25.2	388,915
Rolling stock construction and maintenance	23,099	50.1	711	12.4	23,810	46.0	755,034
Shipyards	5,099	11.1	21	0.4	5,120	9.9	172,583
Glassworks	2,966	6.4	761	13.2	3,727	7.2	105,446
Heat proofing	205	0.4	-	-	205	0.4	6,482
Ship furniture	1,150	2.5	20	0.3	1,170	2.3	36,957
Harbour	1,938	4.2	1	0.02	1,939	3.7	62,102
Asphalt rolls	341	0.7	72	1.2	413	0.8	14,429
Industrial ovens construction	202	0.4	15	0.3	217	0.4	7,107
Miners	299	0.6	1	0.02	300	0.6	9,314
Domestic exposure	-	-	1,777	30.9	1,777	3.4	55,658
Workers in multiple sectors	47	0.1	-	-	47	0.1	1,626
Total	**46060**	**100**	**5741**	**100**	**51801**	**100**	**1,615,653**

Table 5.2. Status at follow up

	Men		**Women**		**Total**	
	N	%	N	%	N	%
alive	25,977	56.4	3,010	52.4	28,987	55.9
deceased	19,394	42.1	2,651	46.2	22,045	42.6
emigrated	172	0.4	31	0.5	203	0.4
Lost to follow-up	517	1.1	49	0.9	566	1.1
Total	**46,060**	**100**	**5,741**	**100**	**51,801**	**100**

Before 1970: 1,172 deaths (1,024 men and 148 women), 32 emigrated (25 men and 7 women), 230 lost to follow-up (211 men and 19 women)

Table 5.3 presents the SMRs for the main causes of death. The table includes causes selected on the basis of a priori evidence of association with asbestos, causes associated with the healthy worker effect and also causes that showed a statistically significant SMR in exploratory analyses. Both genders showed increased mortality for all causes, all malignancies, pleural and peritoneal malignancies, lung cancer, respiratory diseases and asbestosis (all $p<0.01$). An excess of mortality was also observed for bladder cancer ($p<0.05$ among men and $p<0.01$ among women); in women, ovarian malignancies were more frequent than expected ($p<0.05$). No statistically significant increase was found for laryngeal cancer in either genders. The number of deaths due to cardiovascular diseases was lower than expected in both genders, which is indicative of HWE.

Table 5.4 presents SMR analyses by latency for selected causes of death. A large proportion (19%) of the deaths in the cohort occurred after 50 years of latency. A statistically significant increase in SMRs by latency was observed in both genders for all causes and all malignant neoplasm (χ^2 trend: $p<0.0001$). Among men, SMRs for lung cancer increased with maximums at 30–39. A statistically significant increase in mortality for pleural neoplasm was observed beginning in the class 10–19 years of latency for both sexes. Mortality for pleural cancer increased during the first 40 years of time since first exposure and reached a plateau thereafter. Regarding peritoneal neoplasm, SMRs become statistically significant after 20 years of latency among men and after 40 years in women. The number of deaths from "all causes" and "cardiovascular diseases" was lower than expected in men in the first 10 years of latency, suggesting a marked HWE.

The analyses by duration of exposure (table 5.5) showed a statistically significant increase in SMR for a period of exposure less than 10 years for pleural and lung cancer and for asbestosis in both sexes, and among men for peritoneal cancer.

In men, SMRs for pleural, peritoneal malignancies and for asbestosis increased with duration of the work period (χ^2 trend: $p<0.01$), while no clear trend was observed for lung cancer. Cardiovascular and digestive diseases showed a moderate downward trend. Women showed more unstable SMRs. However, an increasing trend with duration was observed for deaths from malignant neoplasms, pleural and peritoneal malignancies and asbestosis (χ^2 trend: $p<0.01$).

Table 5.6 shows mortality figures considering both time since first exposure and duration of employment, using the same time classes showed in Table 5.3, 5.4, 5.5. Results were presented only for pleural and lung cancer. The mortality for pleural cancer seemed to increase with increasing time since first exposure within the same class of duration of exposure, and a decline in SMRs after 30-39 years since first exposure was observed.

The analysis by "Year of first employment", defined as the calendar year when the worker was first hired to work in the factory, showed that in men there was a statistically significant excess in mortality from "all causes" for periods up to 1969, while it was not different from expected for later periods. Lung cancer was in excess in all periods, with lower SMRs for more recent ones. SMRs for pleural neoplasms were statistically increased for all periods, with the highest risk for first exposure in 1950-9 and 1960-9. SMRs for asbestosis and peritoneal neoplasms for the most recent periods were relatively lower than in earlier periods. However, one death from asbestosis was observed among workers first employed in 1980-89. Among women, SMRs for pleural neoplasms were statistically increased for all periods, and the SMRs for peritoneal neoplasms for the most recent periods were lower than in earlier periods (Table 5.7).

Table 5.3. Number of observed deaths, standardized mortality ratio (SMR) and 95% confidence interval (CI95%)

	Men					Women				
	OBS	EXP	SMR	CI95%		OBS	EXP	SMR	CI95%	
All causes	18370	17551.8	1.05**	1.03	1.06	2503	2138.0	1.17**	1.12	1.22
Malignant neoplasm (MN)	7361	6293.7	1.17**	1.14	1.20	818	612.7	1.33**	1.24	1.43
MN lip, oral cavity and pharynx	149	191.5	0.78**	0.66	0.91	9	6.6	1.37	0.62	2.59
MN digestive organs (incl peritoneum)	2198	2194.5	1.00	0.96	1.04	262	226.9	1.16*	1.02	1.30
MN stomach	523	575.2	0.91*	0.83	0.99	44	47.9	0.92	0.67	1.23
MN small intestine	14	10.8	1.30	0.71	2.18	1	1.2	0.84	0.02	4.68
MN colon	408	413.2	0.99	0.89	1.09	62	52.8	1.17	0.90	1.50
MN rectum	173	180.4	0.96	0.82	1.11	22	20.3	1.08	0.68	1.64
MN of liver and intrahepatic bile ducts	378	380.4	0.99	0.90	1.10	25	28.9	0.87	0.56	1.28
MN peritoneum	136	28.5	4.77**	4.00	5.64	35	5.2	6.75**	4.70	9.39
MN respiratory organs	3207	2155.3	1.49**	1.44	1.54	217	62.6	3.47**	3.02	3.96
MN larynx	141	162.9	0.87	0.73	1.02	2	1.6	1.24	0.15	4.48
MN lung	2415	1918.6	1.26**	1.21	1.31	78	54.6	1.43**	1.13	1.78
MN pleura	611	46.0	13.28**	12.24	14.37	134	4.7	28.44**	23.83	33.69
MN uterus						34	35.7	0.95	0.66	1.33
MN ovary						43	31.1	1.38*	1.00	1.87
MN prostate	352	361.4	0.97	0.87	1.08					
MN bladder	291	249.2	1.17*	1.04	1.31	19	9.5	1.99**	1.20	3.11
MN unspecified site	220	158.3	1.39**	1.21	1.59	19	18.1	1.05	0.63	1.64
Psychiatric diseases	143	161.0	0.89	0.75	1.05	51	34.6	1.47*	1.10	1.94
Neurological diseases	275	361.2	0.76**	0.67	0.86	45	63.3	0.71*	0.52	0.95
Cardiovascular diseases	5452	6209.0	0.88**	0.85	0.90	909	912.2	1.00	0.93	1.06
Respiratory diseases	1413	1113.4	1.27**	1.20	1.34	154	108.7	1.42**	1.20	1.66
Digestive diseases	932	1034.5	0.90**	0.84	0.96	118	104.3	1.13	0.94	1.36
Genitourinary diseases	184	219.0	0.84*	0.72	0.97	31	27.8	1.12	0.76	1.58
Asbestosis	366	1.22	300.72**	270.70	333.17	51	0.13	389.61**	290.09	512.27
Pneumoconioses	455	50.4	90.3**	82.2	99.0	53	0.3	193.6**	145.0	253.21
Accidents and violence	851	1004	0.85**	0.79	0.91	76	78.6	0.97	0.76	1.21
Poorly specified causes	230	120.9	1.90**	1.66	2.16	75	32.93	2.28**	1.79	2.86

* $p<0.05$; ** $p<0.01$

Table 5.4. Number of observed deaths, standardized mortality ratio (SMR) and 95% confidence interval (95%CI) by time since first exposure (latency).

MEN	**<10**		**10-19**		**20-29**		**30-39**		**40-49**		**50+**	
	n	SMR (95% CI)	n	SMR (95% CI)	n	SMR (95% CI)	n	SMR (95% CI)	n	SMR (95% CI)	n	SMR (95% CI)
All causes	446	0.76** (0.69-0.83)	1440	0.89** (0.84-0.93)	3499	1.04*(1.00-1.07)	5131	1.06** (1.03-1.09)	4619	1.10** (1.07-1.13)	3235	1.10** (1.07-1.14)
Malignant neoplasm	100	0.62** (0.50-0.75)	512	0.91* (0.83-0.99)	1483	1.15** (1.09-1.21)	2261	1.19** (1.15-1.24)	1918	1.24** (1.19-1.30)	1087	1.29** (1.22-1.37)
MN peritoneum	1	0.81 (0.02-4.50)	2	0.61 (0.07-2.19)	15	2.25** (1.26-3.70)	34	3.91** (2.71-5.46)	55	9.74** (7.34-12.68)	29	9.89** (6.62-14.20)
MN respiratory organs	36	0.68* (0.48-0.94)	223	1.08 (0.95-1.24)	661	1.40** (1.30-1.51)	1068	1.59** (1.50-1.69)	810	1.58** (1.47-1.69)	409	1.68** (1.52-1.85)
MN larynx	1	0.18 (0.0-1.02)	13	0.65 (0.35- 1.11)	42	1.03 (0.74-1.39)	34	0.69* (0.48-0.96)	38	1.17 (0.83 – 1.61)	13	0.88 (0.47- 1.51)
MN lung	32	0.70* (0.48-0.99)	185	1.03 (0.89-1.20)	518	1.25** (1.14-1.36)	809	1.35** (1.26-1.45)	575	1.24** (1.15-1.35)	296	1.35** (1.20-1.51)
MN pleura	-		19	6.19** (3.73-9.67)	87	10.57** (8.47-13.04)	212	14.99** (13.04-17.15)	195	14.93** (12.90-17.17)	98	14.36** (11.66-17.50)
Respiratory diseases	14	0.77 (0.42-1.30)	57	0.88 (0.67-1.14)	201	1.20* (1.04-1.37)	379	1.33**(1.20-1.47)	410	1.34** (1.21-1.48)	352	1.30**(1.16-1.44)
Asbestosis	-		10	179.7** (86.2-330.6)	42	281.1**(202.6-380.0)	99	291.6** (237.0-355.0)	112	301.6** (248.3-362.9)	103	352.5** (287.7-427.5)
Cardiovascular diseases	103	0.75** (0.61-0.91)	358	0.73** (0.66 – 0.81)	938	0.83** (0.78-0.88)	1473	0.87** (0.83-0.92)	1423	0.92** (0.87-0.97)	1157	0.95 (0.90-1.01)
Digestive diseases	34	0.63** (0.44-0.88)	96	0.64** (0.52-0.78)	240	0.96 (0.84-1.09)	255	0.92 (0.81-1.04)	182	0.95 (0.82-1.10)	125	1.14 (0.95-1.36)
Accidents and violence	114	0.78** (0.65-0.94)	158	0.82* (0.70-0.96)	185	0.81** (0.70-0.94)	183	0.87 (0.75-1.00)	118	0.85 (0.70-1.01)	93	1.07 (0.86-1.30)

WOMEN	**<10**		**10-19**		**20-29**		**30-39**		**40-49**		**50+**	
	n	SMR (95% CI)	n	SMR (95% CI)	n	SMR (95% CI)	n	SMR (95% CI)	n	SMR (95% CI)	n	SMR (95% CI)
All causes	21	1.04 (0.65-1.59)	66	0.86 (0.67-1.10)	216	1.02 (0.89-1.16)	479	1.13* (1.03-1.23)	710	1.18** (1.10-1.27)	1011	1.26** (1.18-1.34)
Malignant neoplasm	8	0.95 (0.41-1.87)	32	0.94 (0.65-1.33)	103	1.19 (0.97-1.44)	178	1.22* (1.05-1.41)	239	1.40** (1.23-1.59)	258	1.55** (1.37-1.76)
MN peritoneum	-		-		2	2.51 (0.30-9.08)	3	2.16 (0.45-6.31)	7	4.80** (1.93-9.90)	23	20.01** (12.68-30.02)
MN respiratory organs	2	3.52 (0.43-12.70)	4	1.48 (0.40-3.78)	24	2.93** (1.88-4.36)	56	3.76** (2.84-4.88)	73	3.88** (3.04-4.87)	58	3.34** (2.53-4.32)
MN larynx	-		-		-		1	2.41 (0.06-13.47)	-		1	2.71 (0.07-15.11)
MN lung	2	4.28 (0.52-15.47)	2	0.87 (0.11-3.14)	13	1.83 (0.97-3.12)	16	1.23 (0.70-2.00)	25	1.52 (0.98-2.24)	20	1.31 (0.80-2.03)
MN pleura	-		2	11.51* (1.39-41.60)	10	19.01** (9.11-34.95)	37	34.39** (24.21-47.41)	48	32.26** (23.79-42.77)	37	26.23** (18.46-36.15)
Respiratory diseases	-		-		3	0.38 (0.08-1.12)	22	1.18 (0.74-1.78)	38	1.23 (0.87-1.69)	91	1.88** (1.51-2.31)
Asbestosis	-		-		-		5	222.75** (72.30-519.80)	11	258.00** (128.79-461.63)	35	689.69**(480.40-959.20)
Cardiovascular diseases	6	1.55 (0.57-3.38)	17	0.85 (0.50-1.36)	54	0.77 (0.58-1.00)	161	0.95 (0.81-1.11)	259	0.99 (0.87-1.12)	412	1.06 (0.96-1.17)
Digestive diseases	1	0.70 (0.02-3.90)	2	0.36 (0.04-1.29)	13	0.97 (0.52 – 1.66)	29	1.28 (0.86 – 1.84)	39	1.38 (0.98 – 1.89)	34	1.03 (0.71 – 1.44)
Accidents and violence	6	2.03 (0.75 -4.44)	7	1.19 (0.48 - 2.46)	8	0.80 (0.34-1.57)	13	0.85 (0.45 – 1.45)	17	0.89 (0.52 – 1.42)	25	0.99 (0.64 – 1.46)

(*) $p<0.05$; (**) $p<0.01$; (-) no cases

Table 5.5. Number of observed deaths, standardized mortality ratio (SMR) and 95% confidence interval (95%CI) by duration of employment

MEN	**0-9**		**10-19**		**20-29**		**30+**	
	n	SMR (95% CI)	n	SMR (95% CI)	n	SMR (95% CI)	n	SMR (95% CI)
All causes	6866	1.08** (1.06-1.11)	4420	1.06** (1.02-1.09)	4735	1.03* (1.00-1.06)	2349	0.96* (0.92-0.99)
Malignant neoplasm	2611	1.14**(1.09-1.18)	1775	1.22** (1.16-1.27)	1956	1.18** (1.13-1.24)	1019	1.14** (1.08-1.22)
MN peritoneum	34	3.11** (2.16-4.35)	28	4.27** (2.84-6.17)	48	6.63** (4.89-8.79)	26	6.87** (4.48-10.06)
MN respiratory organs	1123	1.43** (1.35-1.52)	806	1.62** (1.51-1.74)	857	1.52** (1.42-1.62)	421	1.36** (1.23-1.50)
MN larynx	56	0.97 (0.73 – 1.25)	29	0.74 (0.49- 1.06)	48	1.11 (0.82 – 1.47)	8	0.36 ** (0.16 – 0.71)
MN lung	851	1.22** (1.14-1.30)	615	1.40** (1.29-1.51)	630	1.25** (1.16-1.36)	319	1.15* (1.03-1.28)
MN pleura	201	10.95** (9.49-12.58)	147	14.32** (12.10-16.83)	174	14.98** (12.84-17.38)	89	15.36** (12.34-18.90)
Respiratory diseases	464	1.25** (1.14-1.37)	366	1.34** (1.21-1.49)	391	1.30** (1.17-1.44)	192	1.13 (0.97-1.30)
Asbestosis	45	96.71** (70.54-129.40)	109	404.11** (331.81-487.48)	136	435.73** (365.57-515.43)	76	447.33** (352.44-559.89)
Cardiovascular diseases	1950	0.92 **(0.88-0.96)	1299	0.86 ** (0.81 – 0.91)	1482	0.89 ** (0.95 – 0.94)	721	0.79 ** (0.74 – 0.85)
Digestive diseases	364	0.96 (0.87 – 1.07)	224	0.87 * (0.76 – 0.99)	235	0.88 (0.78 – 1.01)	109	0.82 * (0.68 – 1.00)
Accidents and violence	427	0.93 (0.84 – 1.02)	186	0.78 ** (0.67 – 0.90)	162	0.77 ** (0.65 – 0.89)	76	0.81 (0.64 – 1.02)

WOMEN	**0-9**		**10-19**		**20-29**		**30+**	
	n	SMR (95% CI)	n	SMR (95% CI)	n	SMR (95% CI)	n	SMR (95% CI)
All causes	1073	1.16** (1.09-1.23)	639	1.15** (1.06-1.24)	580	1.21** (1.12-1.32)	211	1.17* (1.01-1.33)
Malignant neoplasm	323	1.13* (1.01-1.26)	222	1.47** (1.28-1.67)	195	1.46** (1.26-1.68)	78	1.90** (1.50-2.38)
MN peritoneum	4	1.69 (0.46-4.32)	16	12.29** (7.02-19.96)	9	7.84** (3.58-14.87)	6	16.60** (6.09-36.12)
MN respiratory organs	86	2.88** (2.30-3.56)	54	3.58** (2.69-4.67)	52	3.77** (2.81-4.94)	25	6.54** (4.23-9.66)
MN larynx	1	1.32 (0.03 – 7.37)	-		1	2.89 (0.07 – 16.09)	-	
MN lung	42	1.60** (1.15-2.16)	17	1.30 (0.76-2.08)	11	0.92 (0.46-1.65)	8	2.43* (1.05-4.79)
MN pleura	41	20.07** (14.40-27.22)	37	31.24** (21.99-43.06)	39	33.37** (23.73-45.61)	17	54.09** (31.51-86.60)
Respiratory diseases	55	1.22 (0.92-1.59)	38	1.32 (0.93-1.81)	41	1.66** (1.19-2.25)	20	1.97** (1.21-3.05)
Asbestosis	9	192.96** (88.23-366.31)	16	438.20** (250.46-711.61)	15	402.98** (225.53-664.64)	11	1045.30** (521.79-1870.33)
Cardiovascular diseases	399	1.08 (0.98 – 1.19)	236	0.96 (0.84 – 1.09)	198	0.95 (0.82 – 1.09)	76	0.86 (0.67 – 1.07)
Digestive diseases	58	1.27 (0.96 – 1.64)	25	0.92 (59.5 – 1.36)	29	1.25 (0.84- 1.79)	6	0.73 (0.26 – 1.59)
Accidents and violence	29	0.82 (0.55 – 1.17)	28	1.38 (0.92 – 2.00)	11	0.67 (0.33 – 1.19)	8	1.27 (0.55 – 2.50)

(*) $p<0.05$; (**) $p<0.01$; (-) no cases

Table 5.6. Number of observed and expected deaths, standardized mortality ratio (SMR) and 95% confidence interval (95%CI) by time since first exposure (latency) and duration of employment.

Latency	Duration of employment											
	0-9			10-19			20-29			30+		
	Obs	Exp	SMR (95%CI)	Obs	Exp	SMR (95%CI)	Obs	Exp	SMR (95%CI)	Obs	Exp	SMR (95%CI)
Pleural neoplasm												
0-9	0	0.73	-									
10-19	9	1.71	5.27** (2.41-10.01)	12	1.53	7.82** (4.04-13.66)						
20-29	39	3.92	9.95** (7.07-13.60)	34	2.54	13.39** (9.28-18.72)	24	2.30	10.45** (6.70-15.55)			
30-39	89	5.75	15.48** (12.43-19.05)	68	3.80	17.89** (13.89-22.67)	71	4.33	16.40** (12.81-20.69)	21	1.34	15.72** (9.73-24.03)
40-49	74	5.68	13.03** (10.23-16.35)	52	2.48	21.00** (15.68-27.53)	81	4.21	19.25** (15.29-23.93)	36	2.19	16.47** (11.53-22.80)
50+	31	2.60	11.93** (8.10-16.93)	18	1.10	16.36** (9.69-25.85)	37	1.95	18.96** (13.35-26.13)	49	2.59	18.95** (14.02-25.06)
Lung neoplasm												
0-9	34	45.85	0.74 (0.51-1.04)									
10-19	116	92.60	1.25* (1.03-1.50)	71	88.36	0.80 (0.63-1.01)						
20-29	215	166.06	1.30** (1.13-1.48)	179	125.36	1.43** (1.23-1.65)	137	130.73	1.05 (0.88-1.24)			
30-39	249	198.57	1.25** (1.10-1.42)	223	138.28	1.61** (1.41-1.84)	269	191.4	1.41** (1.24-1.58)	84	82.70	1.02 (0.81-1.26)
40-49	202	156.80	1.29** (1.12-1.48)	110	72.94	1.51** (1.24-1.82)	170	139.76	1.22* (1.04-1.41)	118	108.68	1.09 (0.90-1.30)
50+	77	64.38	1.20 (0.94-1.49)	49	28.38	1.73** (1.28-2.28)	65	52.73	1.23 (0.95-1.57)	125	89.56	1.40** (1.16-1.66)

* $p<0.05$; ** $p<0.01$

Table 5.7. Number of observed, standardized mortality ratio (SMR) and 95% confidence interval (95%CI) by year of first employment

MEN	**<= 1949**		**1950-1959**		**1960-1969**		**1970-1979**		**1980-1989**		**1990-1992**	
	n	SMR (95% CI)	n	SMR (95% CI)	n	SMR (95% CI)	n	SMR (95% CI)	n	SMR (95% CI)	n	SMR (95% CI)
All causes	5341	1.08** (1.05–1.11)	4157	1.06** (1.02-1.09)	5747	1.05**(1.03-1.08)	2608	0.97 (0.93 – 1.01)	504	1.02 (0.94 – 1.12)	13	0.67 (0.36-1.15)
Malignant neoplasm	1857	1.23** (1.17-1.29)	1661	1.20 ** (1.14-1.26)	2475	1.16** (1.12-1.21)	1171	1.08** (1.02-1.14)	195	1.09 (0.94 – 1.25)	2	0.27* (0.03-0.96)
MN peritoneum	33	4.98** (3.43-7.00)	53	8.95**(6.70-11.70)	40	4.08** (2.91-5.55)	10	1.93 (0.93-3.55)	0	-	-	
MN respiratory organs	720	1.48** (1.38-1.59)	788	1.62** (1.51-1.74)	1117	1.49** (1.40-1.58)	503	1.35** (1.24 – 1.47)	79	1.36* (1.08 – 1.69)	-	
MN larynx	36	0.86 (0.60-1.19)	31	0.82 (0.56 – 1.16)	50	0.93 (0.69-1.23)	20	0.78 (0.48-1.20)	4	1.10 (0.30 – 2.82)	-	
MN lung	600	1.40** (1.29-1.52)	574	1.32** (1.22-1.44)	792	1.18**(1.10-1.27)	390	1.17** (1.06-1.29)	59	1.14 (0.86 – 1.46)	-	
MN pleura	82	9.85** (7.83-12.23)	172	17.91**(15.33-20.79)	257	14.84**(13.08-16.77)	86	9.48** (7.58 – 11.71)	14	8.60** (4.70 – 14.43)	-	
Respiratory diseases	480	1.17**(1.07-1.28)	371	1.36**(1.23-1.51)	416	1.38** (1.25-1.52)	122	1.08 (0.89 – 1.28)	24	1.44 (0.93 – 2.15)	-	
Asbestosis	124	434.3**(361-518)	129	389.8**(325.4-463.1)	101	237.3**(193.3-288.4)	11	75.4** (37.6 – 134.9)	1	35.64 (0.89 – 198.56)	-	
Cardiovascular diseases	1908	0.92** (0.88-0.96)	1242	0.86**(0.81-0.90)	1550	0.87**(0.83-0.91)	629	0.81** (0.75-0.88)	121	1.00 (0.83 – 1.20)	2	0.48 (0.06 – 1.72)
Digestive diseases	282	1.03 (0.92-1.16)	206	0.85* (0.74-0.97)	291	0.88* (0.79-0.99)	126	0.78** (0.65 – 0.93)	27	0.99 (0.65 – 1.44)	-	
Accidents and violence	170	0.91 (0.78-1.06)	136	0.78** (0.66-0.92)	286	0.88 * (0.78-0.98)	198	0.81** (0.70 – 0.93)	57	0.81 (0.62 – 1.05)	4	1.28 (0.35 – 3.28)

WOMEN	**<= 1949**		**1950-1959**		**1960-1969**		**1970-1979**		**1980-1989**		**1990-1992**	
	n	SMR (95% CI)	n	SMR (95% CI)	n	SMR (95% CI)	n	SMR (95% CI)	n	SMR (95% CI)	n	SMR (95% CI)
All causes	1172	1.18** (1.12–1.25)	705	1.15** (1.07-1.24)	465	1.14** (1.04-1.25)	124	1.25* (1.04 – 1.49)	36	1.32 (0.93 – 1.83)	1	3.62 (0.09 – 20.15)
Malignant neoplasm	305	1.39** (1.23–1.55)	253	1.34** (1.18-1.51)	187	1.30**(1.12-1.50)	53	1.17 (0.88 – 1.53)	19	1.39 (0.84 – 2.18)	1	7.70 (0.19 – 42.88)
MN peritoneum	20	10.22**(6.25-15.79)	11	6.94** (3.47-12.42)	3	2.50 (0.52 – 7.31)	1	2.90 (0.07 – 16.15)	-		-	
MN respiratory organs	59	3.18** (2.42-4.11)	77	3.88** (3.06-4.85)	64	3.88** (2.99 – 4.95)	11	1.89 (0.94 – 3.38)	6	3.25* (1.19-7.08)	-	
MN larynx	-	-	1	2.04 (0.05 – 11.35)	1	2.60 (0.07 – 14.46)	-	-	-		-	
MN lung	19	1.20 (0.72- 1.87)	31	1.79 ** (1.21 – 2.54)	23	1.59* (1.01 – 2.39)	1	0.19 (0.01 – 1.07)	4	2.37 (0.65 – 6.07)	-	
MN pleura	40	26.99**(19.3-36.8)	44	28.90**(21.00-38.79)	40	31.24**(22.32-42.54)	8	22.98** (9.92 – 45.28)	2	25.81 ** (3.12-93.22)	-	
Respiratory diseases	89	1.63**(1.31-2.01)	45	1.49* (1.08- 1.99)	17	0.87 (0.51 – 1.40)	2	0.55 (0.07 – 1.97)	1	1.27 (0.03 – 7.06)	-	
Asbestosis	33	782.3**(539-1099)	14	322.1**(176.1-540.4)	3	81.8** (16.9 – 239.0)	1	137.1* (3.43 – 764.05)	-		-	
Cardiovascular diseases	467	0.95 (0.86-1.04)	240	0.99 (0.87 – 1.12)	160	1.11 (0.95 – 1.30)	36	1.42 (0.997 – 1.97)	6	1.16 (0.43 – 2.52)	-	
Digestive diseases	52	1.18 (0.88-1.54)	29	0.89 (0.60 – 1.28)	31	1.47 (0.99-2.08)	5	0.99 (0.32 – 2.31)	1	0.80 (0.02 – 4.43)	-	
Accidents and violence	33	0.96 (0.66-1.34)	15	0.71 (0.40 – 1.17)	15	0.97 (0.54-1.60)	10	1.88 (0.90 – 3.46)	3	1.47 (0.30 – 4.30)	-	

* p<0.05; ** p<0.01

5.2 The "Balangero" mine cohort

Follow-up was completed for the 98% of the subjects (953/974). During the 1965-2013 period, the cohort contributed 27,175 person-years (py). Table 5.8 shows the main characteristics of the subjects. At the end of follow-up, the 38.8% of the subjects were alive, the 58.8% deceased, 0.2% moved abroad and 2.2% were lost to follow up. Although most of the workers (66% of the subjects) were first employed before 1970, 34% started employment after 1970.

Table 5.8. Cohort study of workers in the Balangero chrysotile mine. Descriptive information of the cohort.

	N	%
Status at follow-up		
Alive	378	38.8
Deceased[a]	573	58.8
Emigrated[b]	2	0.2
Lost to follow-up	21	2.2
Year of first exposure		
[1921,1946)	298	30.6
[1946,1960)	178	18.3
[1960,1970)	170	17.4
[1970,1989]	328	33.7
Age of first exposure		
[13,30)	673	69.1
[30,66]	301	30.9
Duration of exposure		
[0.5,10)	300	30.8
[10,20)	329	33.8
[20,30)	237	24.3
[30,47]	108	11.1
Period since first exposure		
[0.5,20)	93	9.5
[20,30)	140	14.4
[30,40)	284	29.2
[40,78]	457	46.9
Period since cessation of exposure		
0	63	6.5
(0,1)	18	1.8
[1,10)	118	12.1
[10,66)	775	79.6
Total	**974**	**100**

a. 74 deaths before 1965; 42 causes of death unknown
b. 2 emigrated before 1965

Table 5.9 presents the analysis of mortality for the main causes of death. A statistically significant increase in overall mortality (SMR=1.28; p<0.01) was observed. An increase of mortality close to statistical significance was observed for all cancers. Among the different cancer sites, a statistically significant increase for pleural cancer (SMR: 4.30; p<0.01) was observed. An increase of mortality was found for lip, oral cavity and pharynx cancer (9 obs vs 4.2 exp), lung cancer (40 obs vs 35.1 exp) and peritoneal cancer (2 obs vs 0.6 exp) but was not statistically significant. Among non-neoplastic diseases, a significant increase in mortality for asbestosis (SMR=375.1; p<0.01) was observed. Thirty-three deaths for cirrhosis and other chronic liver diseases (SMR=2.28; p<0.01) were detected.

Table 5.9. Cause-specific mortality. Reference: Piedmont population mortality rates.

CAUSES OF DEATH	OBS	EXP	SMR	95% C.I.
All causes	499	389.6	1.28**	1.17-1.40
Malignant neoplasm	135	123.2	1.10	0.92-1.30
MN lip, oral cavity and pharynx	9	4.2	2.1	0.98-4.06
M.n. digestive organs and peritoneum	42	42.5	0.99	0.71-1.34
M.n. stomach	13	10.49	1.24	0.66-2.12
M.n. intestine and rectum	14	13.0	1.07	0.59-1.80
M.n. colon	11	8.5	1.30	0.65-2.32
M.n. rectum	3	4.3	0.69	0.14-2.03
M.n. peritoneum and retroperitoneum	2	0.6	3.25	0.39-11.75
M.n. respiratory organs	53	40.8	1.30	0.97-1.70
M.n. larynx	3	3.7	0.81	0.17-2.38
M.n lung	40	35.1	1.14	0.81-1.55
M.n. pleura	6	1.4	4.30**	1.58-9.37
M.n. bladder	4	5.7	0.70	0.19-1.80
M.n. nervous system	3	2.6	1.16	0.24-3.39
M.n. unspec	4	3.0	1.32	0.36-3.37
Psychiatric disorders	1	3.4	0.29	0.01-1.64
Cardiovascular diseases	164	150.4	1.09	0.93-1.27
Ischemic cardiopathy	48	54.2	0.89	0.65-1.17
Respiratory diseases	67	27.3	2.45**	1.90-3.12
Asbestosis	36	0.1	375.06**	262.68-519.23
Pneumoconiosis	38	0.9	43.82**	31.01-60.15
Digestive diseases	39	25.9	1.51*	1.07-2.06
Cirrhosis and other chronic liver diseases	33	14.5	2.28**	1.57-3.20
External causes	25	22.4	1.12	0.72-1.65
Poorly defined	25	3.6	6.87**	4.45-10.14

*p<0.05**p<0.01

The analyses by duration of exposure (table 5.10) showed an increase in mortality for peritoneal malignancies, statistically significant in the class 20-30 years of duration but not in the subsequent class of duration. Mortality for pleural cancer showed a statistically significant increase after 30 years of exposure. The mortality for asbestosis increased in all classes of duration, with a statistically significant trend ($p<0.001$).

Table 5.11 presents SMR analyses by latency for selected causes of death. Deaths from malignant cancer of the pleura were observed after at least 30 years of latency, the class in which there is a statistically significant increase of mortality (SMR=7.26; $p<0.05$) and in the class of higher latency (over 40 years) the SMR stopped growing. Both cases of death due to malignant neoplasm of the peritoneum were observed in the latency class "30–40 years" and the SMR was statistically significant (SMR=11.1; $p<0.05$). The SMR for lung cancer was irregularly shaped and did not present an increase with the increase of latency.

Table 5.12 presents the analysis for exposure and latency. The reduced number of subjects imposed classes of latency and duration of exposure rather large. For pleural cancer, the cases were observed after 30 years of latency and the SMR increases with increasing duration of exposure (SMR=4.42 for a duration<30 years and SMR=8.68 for duration over 30 years). With regard to lung cancer, an irregular pattern was observed, which does not allow interpretations. Three cases of deaths from asbestosis were observed before 30 years of latency, with SMR statistically significant. In the latency class 30 and over, there was a statistically significant increase in the SMR for asbestosis from the category 0-29 years of exposure to the category over 30 years.

As regards mortality by time since last exposure (table 5.13), for the malignant neoplasm of the pleura, the observed SMR increased statistically significantly in the first year after the last exposure and then SMRs showed a reduction (1-9 years: SMR: 5.48; 10 years and over: SMR: 3.48).

Table 5.14 presents the analysis of mortality by year of first exposure. Overall mortality increased until 1960 (SMR 123.6 and 153.8; $p<0.01$). Three of the six cases of death from cancer of the pleura started working before 1946 (SMR=6.8; $p<0.05$). The mortality rate was higher than expected for asbestosis, in a statistically significant way, until the period of 1960-1969.

Table 5.15 shows the analysis of mortality by age at first exposure. The SMR for pleural cancer was higher among subjects employed after the age of 30 than before 30. The SMR for asbestosis was higher among those employed before age 30.

As regards the analysis of incidence of PMM, six cases were observed (table 5.16) and SIR for PMM was 6.3 (CI95% 2.3-13.7). An increase by duration of exposure was observed (SIR=3.8 for a duration <10 years; SIR=6.3 for 10-19 years; SIR=8.2 for 20+ years). Five cases out of 6 had more than 40 years of latency (SIR=10.4: CI95% 3.4-24.3).

Table 5.10. Cause-specific mortality by duration of exposure. Reference: Piedmont population mortality rates.

CAUSE OF DEATH	<10			10-20			20-30			30+		
	OBS	**SMR**	**CI95%**	**OBS**	**SMR**	**CI95%**	**OBS**	**SMR**	**CI95%**	**OBS**	**SMR**	**CI95%**
All causes	93	1.10	0.88-1.34	144	1.40**	1.18-1.65	166	1.29**	1.10-1.50	96	1.31*	1.06-1.60
Malignant neoplasm	24	0.89	0.57-1.33	42	1.23	0.89-1.67	43	1.10	0.80-1.48	26	1.12	0.73-1.64
M.n. digestive organs and peritoneum	5	0.55	0.18-1.29	12	1.01	0.52-1.77	15	1.10	0.61-1.81	10	1.26	0.60-2.32
M.n. intestine and rectum	2	0.72	0.09-2.59	4	1.11	0.30-2.83	3	0.72	0.15-2.10	5	2.06	0.67-4.80
M.n. peritoneum and retroperitoneum	-			-			2	10.50*	1.27-37.92	-		
M.n. respiratory organs	12	1.36	0.70-2.38	18	1.58	0.93-2.49	14	1.10	0.60-1.84	9	1.15	0.53-2.19
M.n lung	9	1.19	0.54-2.25	16	1.63	0.93-2.65	9	0.82	0.38-1.56	6	0.89	0.33-1.93
M.n. pleura	1	3.02	0.07-16.82	1	2.38	0.06-13.26	2	4.85	0.59-17.52	2	8.68*	1.05-31.36
Cardiovascular diseases	26	0.86	0.56-1.27	46	1.19	0.87-1.59	62	1.18	0.91-1.51	30	1.02	0.69-1.46
Respiratory diseases	8	1.52	0.65-2.99	13	1.99*	1.06-3.40	19	1.94*	1.17-3.03	27	4.75**	3.13-6.91
Asbestosis	1	53.24*	1.33-296.63	4	184.95**	50.40-473.55	16	486.56**	278.10-790.14	15	661.09**	369.99-1090.36
Pneumoconiosis	2	13.27*	1.61-47.96	5	24.44**	7.93-57.03	16	49.54**	28.31-80.45	15	79.42**	44.45-130.99
External causes	8	1.07	0.46-2.11	6	0.96	0.35-2.09	8	1.38	0.59-2.71	3	1.04	0.21-3.05

*p<0.05**p<0.01

Table 5.11. Cause-specific mortality by time since first exposure (latency). Reference: Piedmont population mortality rates.

CAUSE OF DEATH	<20			20-30			30-40			40+		
	OBS	SMR	CI 95%	OBS	SMR	CI 95%	OBS	SMR	CI 95%	OBS	SMR	CI 95%
All causes	34	1.23	0.85-1.72	62	1.17	0.90-1.50	136	1.41**	1.18-1.66	267	1.26**	1.11-1.42
Malignant neoplasm	9	1.24	0.57-2.36	17	0.93	0.54-1.50	42	1.22	0.88-1.65	67	1.06	0.82-1.34
M.n. digestive organs and peritoneum	1	0.44	0.01-2.48	7	1.12	0.45-2.31	14	1.15	0.63-1.93	20	0.92	0.56-1.42
M.n. intestine and rectum	-			3	1.71	0.35-5.00	2	0.56	0.07-2.01	9	1.28	0.58-2.43
M.n. peritoneum and retroperitoneum	-			-			2	11.14*	1.35-40.26	-		
M.n. respiratory organs	4	1.74	0.47-4.45	4	0.61	0.17-1.57	18	1.47	0.87-2.33	27	1.37	0.90-1.99
M.n. lung	4	2.10	0.57-5.39	2	0.36	0.04-1.31	12	1.15	0.60-2.01	22	1.28	0.80-1.93
M.n. pleura	-			-			3	7.26*	1.50-21.21	3	4.16	0.86-12.16
Cardiovascular diseases	12	1.64	0.85-2.87	22	1.24	0.78-1.88	36	1.01	0.70-1.39	94	1.05	0.85-1.28
Respiratory diseases	1	1.12	0.03-6.25	3	1.24	0.25-3.61	15	2.68**	1.50-4.41	48	2.61**	1.93-3.46
Asbestosis	1	329.54**	8.24-1836.22	2	171.69**	20.77-620.22	6	299.80**	110.02-652.51	27	440.54**	290.32-640.98
Pneumoconiosis	1	28.23	0.71-157.30	2	15.51*	1.88-56.03	7	28.80**	11.58-59.35	28	60.91**	40.47-88.03
External causes	4	0.67	0.18-1.72	6	1.34	0.49-2.92	9	1.86	0.85-3.53	6	0.84	0.31-1.83

*p<0.05**p<0.01

Table 5.12. Cause-specific mortality by time since first exposure and duration of exposure. Reference: Piedmont population mortality rates.

	Duration of exposure					
	0-29			30+		
Latency	Ob	Exp	SMR	Obs	Exp	SMR
Pleural neoplasm						
0-19	0	0.06	-			
20-29	0	0.20	-			
30+	4	0.90	4.42* (1.21-11.33)	2	0.23	8.68* (1.05-31.36)
Lung neoplasm						
0-19	4	1.90	2.10 (0.57-5.39)			
20-29	2	5.50	0.36 (0.04-1.31)			
30+	28	20.91	1.34 (0.89-1.93)	6	6.75	0.89 (0.33-1.93)
Peritoneum neoplasm						
0-19	0	0.06	--			
20-29	0	0.11	--			
30+	2	0.33	6.06 (0.73-21.88)	0	0.11	--
Asbestosis						
0-19	1	0.00	329.54** (8.24-1836.22)			
20-29	2	0.01	171.69** (20.77-620.22)			
30+	18	0.06	307.11** (182.01-485.37)	15	0.02	661.09** (369.99-1090.36)

*$p<0.05$ **$p<0.01$

Table 5.13. Cause-specific mortality by time since last exposure. Reference: Piedmont population mortality rates.

CAUSE OF DEATH	<1			1-9			10+		
	OBS	**SMR**	**CI 95%**	**OBS**	**SMR**	**CI 95%**	**OBS**	**SMR**	**CI 95%**
All causes	8	1.98	0.86-3.91	85	1.47**	1.17-1.81	386	1.28**	1.16-1.42
Malignant neoplasm	4	3.06	0.83-7.84	25	1.26	0.81-1.86	102	1.08	0.88-1.31
M.n. digestive organs and peritoneum	1	2.31	0.06-12.89	9	1.32	0.60-2.50	32	0.97	0.66-1.37
M.n. intestine and rectum	-			3	1.60	0.33-4.68	11	1.06	0.53-1.89
M.n. peritoneum and retroperitoneum	-			2	16.27*	1.97-58.79	-		
M.n. respiratory organs	2	4.13	0.50-14.93	9	1.23	0.56-2.34	40	1.31	0.94-1.79
M.n. lung	-			6	0.98	0.36-2.12	32	1.21	0.83-1.70
M.n. pleura	1	89.38*	2.23-498.02	1	5.48	0.14-30.53	4	3.48	0.95-8.92
Cardiovascular diseases	-			30	1.49*	1.00-2.13	126	1.04	0.86-1.23
Respiratory diseases	1	6.07	0.15-33.84	10	3.44**	1.65-6.32	56	2.40**	1.81-3.12
Asbestosis	1	1018.85**	25.47-5677.02	5	356.76**	115.80-832.53	30	394.73**	266.32-563.50
Pneumoconiosis	1	83.00*	2.07-462.47	5	28.82**	9.36-67.26	32	51.50**	35.23-72.70
External causes	-			9	1.97	0.90-3.74	14	1.12	0.61-1.88

Subjects still working at the end of follow up were excluded

*$p<0.05$ **$p<0.01$

Table 5.14. Cause-specific mortality by year of first exposure. Reference: Piedmont population mortality rates.

CAUSE OF DEATH	<1946			1946-1959			1960-1969			1970+		
	OBS	SMR	CI 95%	OBS	SMR	CI 95%	OBS	SMR	CI 95%	OBS	SMR	CI 95%
All causes	235	1.24**	1.08-1.40	140	1.54**	1.29-1.81	73	1.23	0.96-1.54	51	1.04	0.77-1.37
Malignant neoplasm	53	1.04	0.78-1.36	39	1.32	0.94-1.81	23	1.02	0.64-1.52	20	1.00	0.61-1.54
M.n. digestive organs and peritoneum	18	0.97	0.57-1.53	12	1.21	0.63-2.12	8	1.08	0.47-2.13	4	0.61	0.17-1.56
M.n. intestine and rectum	8	1.40	0.60-2.76	2	0.67	0.08-2.43	2	0.87	0.11-3.16	2	0.98	0.12-3.54
M.n. peritoneum and retroperitoneum	1	4.22	0.10-23.51	1	6.70	0.17-37.33	0	--		0	--	
M.n. respiratory organs	21	1.35	0.84-2.07	16	1.58	0.90-2.57	9	1.11	0.51-2.11	7	0.99	0.40-2.04
M.n. lung	14	1.07	0.58-1.79	12	1.38	0.71-2.41	8	1.13	0.49-2.22	6	0.97	0.36-2.11
M.n. pleura	3	6.80*	1.40-19.87	1	3.44	0.09-19.17	1	3.15	0.08-17.53	1	2.90	0.07-16.17
Cardiovascular diseases	86	1.02	0.81-1.26	45	1.29	0.94-1.73	23	1.21	0.77-1.82	10	0.82	0.39-1.50
Respiratory diseases	40	2.51**	1.79-3.42	21	3.16**	1.95-4.83	5	1.59	0.52-3.71	1	0.63	0.02-3.50
Asbestosis	21	425.96**	263.67-651.13	12	407.69**	210.67-712.17	3	237.86**	49.08-695.11	0	--	
Pneumoconiosis	23	43.19**	27.38-64.80	12	49.89**	25.78-87.15	3	40.81**	8.42-119.27	0	--	
External causes	4	0.53	0.15-1.37	5	1.16	0.38-2.71	6	1.35	0.50-2.94	10	1.62	0.78-2.98

*$p<0.05$ **$p<0.01$

Table 5.15. Cause-specific mortality by age of first exposure. Reference: Piedmont population mortality rates.

CAUSE OF DEATH	<30			30+		
	OBS	**SMR**	**CI 95%**	**OBS**	**SMR**	**CI 95%**
All causes	326	1.35**	1.21-1.51	173	1.16	1.00-1.35
Malignant neoplasm	88	1.11	0.89-1.37	47	1.07	0.78-1.42
M.n. digestive organs and peritoneum	26	0.98	0.64-1.43	16	1.01	0.58-1.64
M.n. intestine and rectum	8	0.98	0.42-1.93	6	1.23	0.45-2.68
M.n. peritoneum and retroperitoneum	1	2.49	0.06-13.90	1	4.67	0.12-26.05
M.n. respiratory organs	33	1.24	0.85-1.74	20	1.42	0.87-2.20
M.n. lung	25	1.08	0.70-1.60	15	1.25	0.70-2.06
M.n. pleura	3	3.31	0.68-9.68	3	6.14*	1.27-17.95
Cardiovascular diseases	97	1.10	0.89-1.34	67	1.08	0.84-1.37
Respiratory diseases	49	3.02**	2.24-4.00	18	1.62	0.96-2.56
Asbestosis	26	400.91**	261.89-587.42	10	321.20**	154.02-590.69
Pneumoconiosis	27	50.56**	33.32-73.56	11	33.03**	16.49-59.09
External causes	20	1.29	0.79-1.99	5	0.72	0.23-1.69

*$p<0.05$ **$p<0.01$

Table 5.16. Incidence and/or mortality for mesothelioma

	Age	Year of diagnosis of mesothelioma	Year of death	Cause of death
1.	78	2006	2006	pleural cancer
2.	87	2012	2012	pleural cancer
3.	73	2008	2008	pleural cancer
4.	64	1985	1985	pleural cancer
5.	62	-	1975	pleural cancer
6.	74	1992	1993	pleural cancer
7.	77	1994	1995	lung cancer
8.	86	2005	2012	cardiomyopathy
9.	56	-	1982	peritoneal cancer
10.	64	-	1973	peritoneal cancer

5.3 Case control study in the area of Casale Monferrato

The study included 223 eligible cases (Mean age: 68.4; DS:11.3; Males: 62%) and 552 controls (Mean age: 65.4; DS: 12.1; Males: 61%). Two hundred cases (89.7%) and 348 (63.0%) controls accepted the invitation and were interviewed. Distribution by sex of participating cases and controls was similar. Cases were older than controls because of the oversampling of controls matched to cases under 60 and of the different age distribution of non-participating controls. Interviews took slightly longer to cases than to controls. Interviews were face to face in 54% of the cases (because of death or poor health condition, 46% of interviews were with a close relative) and for nearly all controls. Such differences were statistically significant and these variables were adjusted for in the analyses.

Table 5.17. Descriptive information of the interviewed subjects.

	Cases	**Controls**
	N (%)	N (%)
Male	127 (63.5)	220 (63.2)
Female	73 (36.5)	128 (36.8)
Total	**200 (100.0)**	**348 (100.0)**
Age (mean±SD)	68.3 (11.4)	63.3 (11.6)
Duration of interview (minutes; mean±SD)	74.2 (18.3)	70.7 (17.1)
Type of interview		
To subject	108 (54.0)	323 (92.8)
To relatives	92 (46.0)	25 (7.2)
Vital status		
Deceased	48 (24.0)	1 (0.3)
Alive	152 (76.0)	347 (99.7)
Residence at the interview		
Casale Monferrato	134 (67.0)	105 (30.2)
Other town of the LHA	66 (33.0)	243 (69.8)

Two thousand five hundred and eighty nine occupational, residential or domestic asbestos exposure circumstances were reported or identified by expert assessment in the 548 questionnaires (705 occupational, 1515 residential and 369 domestic exposures).

Table 5.18 presents results by classes of cumulative dose index. A trend in the risk of pleural malignant mesothelioma was observed with increasing occupational and non occupational cumulative exposure. ORs increased with cumulative exposure index ($p<0.0001$) from 4.4 (CI 95% 1.7 to 11.3) (<1 f/mL-years) to 62.1 (CI 95% 22.2 to 173.2) (≥10 f/mL-years) when both occupational and non-occupational exposures were considered. Among subjects never occupationally exposed, corresponding ORs were 3.8 (CI 95% 1.3 to 11.1) and 23.3 (CI 95% 2.9 to 186.9) (reference: background level of asbestos exposure). Only 20 cases and 9 controls had been employed in the AC industry, a proportion that is not surprising given that the Casale Monferrato plant had closed operations in 1986 and two-thirds of the cohort members have died.

The exposure-response relationship appeared steeper when not only definite, but also probable and possible exposures were taken into account (table 5.19).

Risk estimates by type of exposure shows the exposure-response trend for pleural malignant mesothelioma irrespective of the route of exposure (occupational, environmental or domestic) (table 5.20).

Table 5.18. Risk of mesothelioma in relation to cumulative dose index

All subjects

	Cases	Controls	OR (95% CI)
Fibres/ml-years	N (%)	N (%)	
background level (<0.1)	8 (4.0)	106 (30.5)	1 (ref)
>=0.1 - <1	26 (13.0)	108 (31.0)	4.4 (1.7-11.3)
>=1 - <10	113 (56.5)	115 (33.0)	17.5 (7.3-41.8)
>=10 (Mean 201; Range 10-4128)	53 (26.5)	19 (5.5)	62.1 (22.2-173.2)
Total	200 (100.0)	348 (100.0)	

Non-occupationally exposed only

	Cases	Controls	OR (95% CI)
Fibres/ml-years	N (%)	N (%)	
background level (<0.1)	7 (8.3)	85 (42.3)	1 (ref)
>=0.1 - <1	15 (17.9)	58 (28.9)	3.8 (1.3-11.1)
>=1 - <10	57 (67.9)	56 (27.9)	14.8 (5.7-38.6)
>=10 (Mean 16; Range 10-24.2)	5 (6.0)	2 (1.0)	23.3 (2.9-186.9)
Total	84 (100.0)	201 (100.0)	

*The models are adjusted for age, sex, type of interview

Table 5.19. Risk of mesothelioma in relation to probability of exposure

Definite only

	Cases	Controls	OR (95% CI)
Fibre/ml-years	N (%)	N (%)	
background level (<0.1)	16 (8.0)	130 (37.4)	1 (ref)
>=0.1 - <1	35 (17.5)	100 (28.7)	3.9 (1.8-8.2)
>=1 - <10	102 (51.0)	100 (28.7)	11.2 (5.6-22.6)
>=10 (Mean of fibres in this class: 219; Range of fibres in this class: 10-4128)	47 (23.5)	18 (5.2)	34.9 (14.4-84.4)
Total	200 (100.0)	348 (100.0)	

Definite and probable

	Cases	Controls	OR (95% CI)
Fibre/ml-years	N (%)	N (%)	
background level (<0.1)	13 (6.5)	126 (36.2)	1 (ref)
>=0.1 - <1	32 (16.0)	100 (28.7)	4.1 (1.8-9.1)
>=1 - <10	107 (53.5)	104 (29.9)	12.6 (6.1-26.2)
>=10 (Mean of fibres in this class: 216; Range of fibres in this class:10-4128)	48 (24.0)	18 (5.2)	39.4 (15.8-98.0)
Total	200 (100.0)	348 (100.0)	

Definite, probable and possible

	Cases	Controls	OR (95% CI)
Fibre/ml-years	N (%)	N (%)	
background level (<0.1)	8 (4.0)	106 (30.5)	1 (ref)
>=0.1 - <1	26 (13.0)	108 (31.0)	4.4 (1.7-11.3)
>=1 - <10	113 (56.5)	115 (33.0)	17.5 (7.3-41.8)
>=10 (Mean of fibres in this class: 201; Range of fibres in this class:10-4128)	53 (26.5)	19 (5.5)	62.1 (22.2-173.2)
Total	200 (100.0)	348 (100.0)	

Table 5.20. Risk of mesothelioma by cumulative exposure and route of exposure

Occupational exposures

	Cases	Controls	OR (95% CI)
Fibre/ml-years	N (%)	N (%)	
background level (<0.1) (a)	8 (4.0)	106 (30.5)	1 (ref)
background level (<0.1) (b)	87 (43.5)	129 (37.1)	12.0 (5.0-28.7)
>=0.1 - <1	23 (11.5)	61 (17.5)	7.6 (2.8-20.5)
>=1 - <10	43 (21.5)	37 (10.6)	19.3 (7.2-51.7)
>=10 (Mean: 260 f/mly; range:10-4126 f/mly)	39 (19.5)	15 (4.3)	63.5 (21.8-185.0)
Total	200 (100.0)	348 (100.0)	

Environmental exposures

	Cases	Controls	OR (95% CI)
Fibre/ml-years	N (%)	N (%)	
background level (<0.1) (c)	8 (4.0)	106 (30.5)	1 (ref)
background level (<0.1) (d)	27 (13.5)	54 (15.5)	8.6 (3.3-22.6)
>=0.1 - <1	69 (34.5)	119 (34.2)	10.0 (4.2-24.1)
>=1 - <10	89 (44.5)	68 (19.5)	23.5 (9.6-57.2)
>=10 (Mean 15 f/mly; range: 10-24 f/mly)	7 (3.5)	1 (0.3)	51.5 (4.4-605.6)
Total	200 (100.0)	348 (100.0)	

Familial/Domestic

	Cases	Controls	OR (95% CI)
Fibre/ml-years	N (%)	N (%)	
background level (<0.1) (e)	8 (4.0)	106 (30.5)	1 (ref)
background level (<0.1) (f)	136 (68.0)	200 (57.4)	11.6 (5.0-27.3)
>=0.1 - <1	24 (12.0)	22 (6.3)	20.3 (7.2-57.4)
>=1 - <10	27 (13.5)	18 (5.2)	25.6 (8.9-73.5)
>=10 (Mean 16 f/mly; range: 11-28 f/mly)	5 (2.5)	2 (0.6)	43.9 (6.3-303.6)
Total	200 (100.0)	348 (100.0)	

*The models are adjusted for age, sex, type of interview

(a,c,e) no occupational, environmental or familial/domestic exposures above background
(b) no occupational, but one or more environmental or familial/domestic exposures above background
(d) no environmental, but one or more occupational or familial/domestic exposures above background
(f) no familial/domestic, but one or more occupational or environmental exposures above background

A significantly increased OR was observed when father or mother or spouse were occupationally exposed to asbestos. An increase, close to statistical significance, was present also for other family members. Family members of 33 cases (16%) and 26 controls (7%) were reported to have brought work clothes home for cleaning.

Table 5.21. Risk of mesothelioma in relation to occupational asbestos exposure of family members (having vs not having an exposed family member)

Exposed family members	**Cases**	**Controls**	**OR^{adj} (95%CI)**	**Cases**	**Controls**	**OR^{c} (95%CI)**
	N (%)	N (%)		N (%)	N (%)	
Father and/or mother	19 (9.5)	21 (6.0)	2.6 (1.3-5.5)	17 (9.4)	19 (5.6)	3.1 (1.4-6.5)
Spouse	14 (7.0)	8 (2.3)	2.6 (0.9-7.5)	12 (6.7)	8 (2.4)	2.2 (0.7-6.8)
Other	9 (4.5)	7 (2.0)	2.2 (0.7-7.1)	7 (3.9)	6 (1.8)	2.5 (0.7-8.8)
Any family members	38 (19.0)	35 (10.1)	2.2 (1.2-4.0)	33 (18.3)	32 (9.4)	2.4 (1.3-4.4)
No family members/cohabitants occupationally exposed to asbestos	162 (81.0)	313 (90.0)	1 (ref)	147 (81.7)	307 (90.6)	1 (ref)

*The models are adjusted for age, sex, type of interview
†OR^{c} : OR in the stratum of subjects with no occupation in the AC industry
‡OR^{adj} : OR adjusted by occupational exposure to asbestos in the AC industry

Table 5.22 shows the results on risk associated with domestic exposure due to the use of asbestos containing tools or the presence of AC materials at home or around it. Having a garden or courtyard paved with asbestos cement tailings, an asbestos cement roof or buildings near home were also associated with a significant increase in the OR.

Table 5.22. Risk of mesothelioma in relation to environmental and domestic exposure to asbestos with reference to the entire life history (exposed vs not exposed).

Type of Exposure	Cases	Controls	OR^{adj} (95%CI)	Cases	Controls	OR^{c} (95%CI)
Asbestos-cement (AC) roof	60 (30.0)	75 (21.6)	2.4 (1.4-4.2)	55 (30.5)	72 (21.2)	2.5 (1.4-4.5)
Use of utensils of asbestos material	41 (20.5)	86 (24.7)	1.3 (0.7-2.3)	39 (21.7)	86 (25.4)	1.2 (0.7-2.2)
Garden or courtyard pavement with AC tailings	18 (9.0)	13 (3.7)	3.4 (1.4-8.4)	16 (8.9)	12 (3.5)	3.6 (1.4-9.2)
AC buildings in the garden or courtyard	102 (51.0)	147 (42.2)	1.9 (1.2-3.2)	91 (50.5)	142 (41.9)	2.1 (1.2-3.4)
Any of the above categories	152 (76.0)	221 (63.5)	1.9 (1.2-3.0)	137 (76.1)	214 (63.1)	2.0 (1.2-3.2)
None of the above categories	48 (24.0)	127 (36.5)	1 (ref)	43 (23.9)	125 (36.9)	1 (ref)

*The models are adjusted for age, sex, type of interview
†OR^{c} : OR in the stratum of subjects with no occupation in the AC industry
‡OR^{adj} : OR adjusted by occupational exposure to asbestos in the AC industry

5.4 Genetic risk studies

The interactions between genetics and asbestos exposure, and their effects in modulating pleural malignant mesothelioma risk in Italian population were studied among 759 subjects, 392 PMM cases and 367 controls, after standard GWAS quality control procedure. Patients and controls were genotyped for more than 370.000 SNPs and the GWAS results showed that the combined effects of several genetic polymorphisms could contribute together with asbestos exposure in the development of PMM.

The statistical analyses of interaction between SNPs and asbestos considered the fifteen Single Nucleotide Polymorphisms (SNPs) with highest significance levels as reported in Matullo et al. [2013].

Table 5.23 reports the subjects included in the analysis by sex, centre, age and asbestos exposure and table 5.24 reports RERI, SI, V and the statistical significance of the interaction term in the multiplicative logistic regression model for each SNP.

Six of these SNPs (rs1508805, rs2501618, rs4701085, rs4290865, rs10519201, rs763271) showed deviation from the additive model according to RERI and SI index, four of which (rs1508805, rs2501618, rs4701085, rs10519201) also deviate from the multiplicative model. After accounting for multiple comparisons using Bonferroni correction of $p = 0.003$ (0.05/15) the interaction remained statistically significant on the multiplicative scale, for rs1508805 and rs4701085.

The gene-asbestos interaction analysis suggests that gene-asbestos interaction may play an additional role on MM susceptibility, given that asbestos exposure appears as the main risk factor [Tunesi et al., 2015].

Table 5.23. Summary statistics of subjects included in the interaction analysis. Percentages are reported for cases and control groups.

		CENTRE						Overall sample	
		Casale Monferrato		Genova		Torino			
		controls	cases	controls	cases	controls	cases	controls	cases
		N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Gender	Females	75 (31.65)	75 (32.61)	19 (25.33)	6 (8.22)	17 (30.91)	27 (30.34)	111 (30.25)	108 (27.55)
	Males	162 (68.35)	155 (67.39)	56 (74.67)	67 (91.78)	38 69.09)	62 (69.66)	256 (69.75)	284 (72.45)
	Total	237 (50.75)	230 (49.25)	75 (50.68)	73 (49.32)	55 (38.19)	89 (61.81)	367 (48.35)	392 (51.65)
Asbestos exposure	No	54 (22.78)	4 (1.74)	41 (54.67)	10 (13.70)	18 (32.73)	3 (3.37)	113 (30.79)	17 (4.34)
	Yes	183 (77.22)	190 (82.61)	34 (45.33)	63 (86.30)	37 (67.27)	86 (96.67)	254 (69.21)	339 (86.48)
	Not - available		36 (15.65)						36 (9.18)
	Age (mean ± SD)	63.36 ±11.06	67.61 ±11.14	58.59 ±15.03	69.64 ±9.64	68.31 ±8.80	68.74 ±8.84	63.11 ±12.01	68.25 ±10.39

Table 5.24. Results for gene-environment interaction analysis for each candidate SNP and asbestos exposure, adjusted for age, gender, PCA cluster, and centre.

		Deviation from additive model		**Deviation from multiplicative model**	
	gene	RERI (95% CI)	SI (95% CI)	V	P[a]
1	rs2236304 (*MMP14*)	10.53 (-2.30 – 23.36)	1.61 (1.11 – 2.33)	0.54	0.309
2	rs742109	-3.15 (-7.87 – 1.57)	0.64 (0.41 – 0.99)	1.24	0.780
3	rs1508805[b,c,d]	5.92 (1.72 – 10.12)	2.74 (1.60 – 4.69)	10.6	<0.001
4	rs2501618 (*CEP350*)[b,d]	10.69 (2.11 – 19.26)	2.87 (1.77 – 4.63)	4.56	0.016
5	rs4701085[b,c,d]	6.52 (1.87 – 11.18)	3.21 (1.76 – 5.88)	7.28	<0.001
6	rs4290865[d,e]	9.38 (1.15 – 17.62)	2.37 (1.52 – 3.70)	2.51	0.106
7	rs9536579[d]	-4.68 (-9.57 – 0.21)	0.51 (0.33 – 0.77)	0.96	0.951
8	rs3801094 (*ETV1*)	7.61 (-2.17 – 17.39)	1.46 (1.01 – 2.11)	0.55	0.333
9	rs7841347 (*PVT1*)	-3.80 (-9.73 – 2.13)	0.63 (0.40 – 0.99)	0.97	0.964
10	rs10519201 (*SHC4*)[b,d]	12.56 (1.78 – 23.46)	2.89 (1.75 – 4.77)	4.06	0.043
11	rs5756444[d,f]	-16.85 (-41.05 – 7.34)	0.54 (0.38 – 0.78)	0.18	0.005
12	rs7632718 (*SLC74A14*)[e]	5.87 (0.38 – 11.17)	2.78 (1.35 – 5.71)	3.24	0.070
13	rs9833191 (THRB)[d]	-10.73 (-22.41 – 0.96)	0.45 (0.29 – 0.65)	0.42	0.097
14	rs10815216[f]	-2.20 (-5.23 – 0.83)	0.57 (0.35 – 0.92)	3.32	0.048
15	rs73034881 (*SDK1/FOXK1*)	-3.91 (-7.84 – 0.02)	0.51 (0.33 – 0.81)	1.56	0.537

[a]Likelihood ratio test for multiplicative interaction term.
[b]RERI > 0, SI > 1 and *V* > 1 statistically significant.
[c]*V* index statistically significant after Bonferroni correction.
[d]After Bonferroni correction SI index statistically significant: rs1508805 95%CI 1.21–6.13; rs2501618 95%CI 1.39–5.89; rs4701085 95%CI 1.30–7.95; rs4290865 95%CI 1.22–4.62; rs9536579 95%CI 0.27–0.94; rs10519201 95%CI 1.36–6.13; rs5756444 95%CI 0.33–0.93; rs9833191 95%CI 0.24–0.79.
[e]RERI >0, SI >1 is statistically significant but *V* index is not statistically significant.
[f]*V* index statistically significant.

No BAP1 mutation carriers were observed in the 103 sporadic patients [Betti et al., 2015]. The 95% confidence interval of the prevalence of BAP1 mutations in sporadic patients was 0-3.58% using the Poisson distribution [Breslow and Day, 1980].

BAP1 mutations were studied among five MM families showing germline BAP1 mutations only in one family. For that family, the study showed another tumor-type associated with BAP1, the rare mucoepidermoide carcinoma.

Moreover, among the 40 families subsequently considered, two BAP1 and one CDKN2A germline variants were found in families with both mesothelioma and melanoma.

In a first family, the proband developed two independent cutaneous melanomas, a meningioma and an epithelioid pleural mesothelioma. The mutation in BAP1 gene was transmitted to the proband's healthy daughter.

A further patient whose father died of MM, developed cutaneous melanoma and breast cancer and carried a germline duplication in BAP1 promoter region.

In a further family, the CDKN2A germline mutation was carried by the proband affected by cutaneous melanoma and by her mother affected by both cutaneous melanoma and mesothelioma.

Two other BAP1 germline variants were identified in a patient with multiple cutaneous amelanotic melanomas. Her mother had non-Hodgkin cutaneous lymphoma and her paternal uncle had cutaneous melanoma. These variants were inherited from the mother. A healthy maternal aunt and a proband's healthy sister were also carriers. On the other hand, the proband's healthy father and the paternal uncle affected by cutaneous melanoma did not carry the variant allele [Betti et al., 2016].

Information on asbestos exposure was available for 10 out of 12 mesothelioma probands. All of them showed asbestos exposure, in agreement with literature. Possible occupational exposure was reported for four probands, para-occupational or household exposure for five patients and both possible occupational and household exposure for one proband.

One out of 28 melanoma probands showed professional or chronic UV exposure. Information regarding Fitzpatrick skin phototype (individuals with skin types I and II face the highest risk of developing skin cancer, while types V and VI are at the lowest risk) was

available for 16 out of 28 probands: one had phototype I, 10 had phototype II and 5 had phototype III.

Chapter 6

CONCLUSIONS

The project has allowed to deepen the study of the effect of asbestos exposure considering time variables, different types of exposures and the potential role of genetic factors in the development of asbestos-related diseases.

The analysis of the occurrence of pleural and peritoneal neoplasms, as well as of lung cancer and other asbestos-related diseases, by time-dependent variables was one of the major purposes that led to the construction of the pooled cohort study.

The results obtained clearly showed that pleural neoplasms risk increases only over the first four decades of time since first exposure (latency), for both men and women: after longer latency, SMR no longer increases for men and declines for women.

The study by Barone-Adesi et al. [2008] suggested that the risk for pleural cancer does not increase indefinitely but it reaches a plateau when a sufficiently long time has elapsed since the start of exposure and that this may be related to the clearance of asbestos from the start of exposure. These findings were obtained according to Berry's hypothesis [Berry et al., 1991] and observed in other studies [Berry et al., 2012]. The data from six cohort studies of exposed workers and two cohorts with residential exposure showed that the risk of pleural mesothelioma increased until 40 years since first exposure and then reached a plateau [Reid et al., 2014]. The peritoneal neoplasms risk increased over five decades of TSFE in men and all over the observation time in women. Our results for peritoneal neoplasm are similar to the results presented by Reid et al. [2014].

SMRs increased with duration of employment for asbestosis and pleural and peritoneal neoplasms in men and women, even if in men pleural cancer SMRs increased only slightly after 10-19 years duration. For lung cancer, in men the SMR increased at first in the 10-19 duration category, but decreased thereafter, whereas in women no specific trend was apparent, even if the highest estimate was in the longest duration category.

In the present study, no excess risk of laryngeal cancer was found. A meta-analysis reported the association between asbestos exposure and laryngeal cancer risk, and supported the hypothesis that exposure to asbestos was associated with an increased risk of laryngeal cancer, especially in male workers [Peng et al., 2016]. The exact mechanisms of asbestos exposure resulting in laryngeal cancer and the reason of disagreement of our results will be further investigated.

In a meta-analysis of studies of workers in which a major portion of the cohort was presumed to have been exposed to asbestos reported an increase in the pooled estimate in men (meta-SMR=1.13, 95% CI 1.02–1.26) for stomach cancer in relation to exposure to asbestos [Fortunato et al., 2015].

In a cohort of subjects occupationally exposed to asbestos, among male individuals, a significantly elevated incidence was observed for peritoneal mesothelioma, liver cancer, oesophageal cancer and for all digestive cancers combined, both including or excluding peritoneal mesothelioma. In female individuals, a significant excess of risk was found for peritoneal mesothelioma [Boulanger et al., 2015]. In our results, mortality was lower than expected in men for stomach, colon, rectum and liver cancers and diseases of the digestive tract.

A statistically significant increase of ovarian cancer mortality (43 observed vs 31.1 expected, SMR=1.38, $p<0.05$) was found in our study. An association between asbestos exposure and ovarian cancer was obtained in a meta-analysis by Camargo et al. [2011], that estimated a meta-analytical SMR of 1.77 (CI95% 1.37-2.28) over 18 studies.

The cohort showed a large increase in mortality from asbestosis: 417 deaths were observed while 1.35 were expected, corresponding to an excess of three-hundred times. In workers hired before 1970, SMR mortality was higher than in workers hired after 1970, but SMR remained well above 1 even for workers hired in 1970-1979. One death from asbestosis was observed among workers first employed in 1980-89. This observation suggests that the control of workplace exposures was still inadequate. This situation continued even in the last periods of industrial use of asbestos in Italy.

Deaths from cardiovascular diseases were fewer than expected, suggesting HWE and excluding marked differences in smoking habits with the general population.

The study of the cohort of Balangero mine is relevant because it was the most important chrysotile mine in Europe. Among this cohort, a statistically significant increase in mortality for pleural cancer was found, by us, as in the previous research [Pira et al., 2009]. The analysis by duration and latency showed an increased risk with increasing duration and the risk flattened out for latency greater than 40 years. An increase of mortality for lung cancer was observed but is not statistically significant. A significant increase (36 observed deaths vs 0.1 expected) was observed for deaths from asbestosis and mortality increased by duration of exposure.

Li et al. [2004] published a meta-analysis of 26 cohort studies of chrysotile occupational exposure and cancer mortality demonstrating an association between chrysotile exposure and lung cancer, including mesothelioma. A positive exposure-response relationship for lung cancer and pneumoconiosis more consistent for the white male workers has been detected among workers of an asbestos textile plant using chrysotile [Dement et al., 1994]. The review by Stayner et al. [1996] supported the view that occupational exposure to chrysotile is associated with an increased risk of lung cancer and mesothelioma. A clear exposure-response relationship was found for lung cancer and non-malignant respiratory diseases in a cohort of workers from a chrysotile asbestos mine in China [Wang et al., 2013]. The same trend was observed for lung cancer mortality and incidence of asbestosis in a Chinese asbestos plant [Courtice et al., 2016; Deng et al., 2012] where only chrysotile asbestos obtained from two mines in Sichuan, China, has been used in the plant [Yano et al., 2001].

As regards the incidence of mesothelioma, Mirabelli et al. [2008] estimated a standardized incidence rate of mesothelioma (SIR) equal to 4 (95%CI 1.5–8.7) based on 6 cases in the period 1946-2006. In this study, a higher SIR was found, but considering a different period (1990-2012) using the rates of the population of the Piedmont region as a reference.

An excess of mesothelioma has been reported in South Carolina asbestos textile workers who were predominantly exposed to chrysotile asbestos imported from Quebec [Hein et al., 2007]. Hodgson and Darnton [2010] showed that per fibre risk of mesothelioma from chrysotile in textile plants is greater than it is in the mines.

The fact that the chrysotile asbestos mined in Quebec is contaminated with a small percentage (<1.0%) of amphibole (tremolite) asbestos has made the interpretation of these findings [IARC, 2012] more complex. Data from the two Chinese asbestos workers cohorts did not show that there is a difference in lung cancer mortality between textile and mining workers at a high level of exposure but textile workers appeared to have a higher risk at a low level of exposure [Wang et al., 2014]. Lung cancer is most closely associated with long, thin asbestos fibres in a study that included chrysotile asbestos textile workers in Carolina [Loomis et al., 2012].

The study showed an increase at the limit of statistical significance for malignant neoplasm of lips, oral cavity and pharynx and did not show an association with laryngeal cancer.

The data suggest a possible increased risk for stomach cancer but that did not rise to statistical significance. In the cohort of chrysotile asbestos mine in Qinghai Province of China, an exposure-response gradient was observed for stomach cancer, in which the workers at the highest exposure level had a 6.5-fold increased mortality rate than general population [Lin et al., 2014].

An increased risk of mortality for colon cancer was also found but is not statistically significant. Cancer of the colon and rectum was associated with asbestos exposure during chrysotile production, with an approximately two-fold risk in a study by Liddell et al. [1984].

A statistically significant increase of mortality for cirrhosis was found by our study and in a previous study by Pira [Pira et al., 2009]. A significant increase in mortality due to cirrhosis of the liver (SMR 2.0, 95% CI 1.1–3.6), based on 11 observed cases was also observed among workers in two Lithuanian asbestos-cement factories [Smailyte et al., 2004]. Both studies underlined the likely association with alcohol use [Pira et al., 2009; Smailyte et al., 2004].

In conclusion, this study demonstrated an excess of mortality for pleural cancer and asbestosis and an increased risk by duration of exposure after the exposure to pure chrysotile.

The case-control study of Casale Monferrato underlined the association between pleural mesothelioma and non-occupational exposures to asbestos underling an increase in risk among those living with a family member occupationally exposed to asbestos or having been exposed from domestic or environmental exposure. A study published by Maule at al. [2007] reported a risk for mesothelioma of 10.5 (CI95% 3.8-50.1) among people living near the Eternit factory of Casale Monferrato. The risk decreased when the distance of people's houses from the plant increased. For people living at 10 kms from the factory, risk is nevertheless 60% of the value reported above. A study by Hansen et al. [1998] showed that the incidence of mesothelioma increased significantly with time from first exposure for people resident at Wittenoom mine and with an increased level of exposure to crocidolite.

Environmental exposures to asbestos combined with domestic exposure were reported to be the cause of epidemics of mesothelioma among people living in villages in Turkey exposed to erionite [Baris et al. 1987] and among people residing in areas of tremolite contamination in Cyprus [McConnochie et al., 1987], Metsovo [Gogali et al., 2012] and New Caledonia [Baumann et al., 2011].

A systematic review of the quantitative relationship between PMM and asbestos exposure was carried out for the Second Italian Consensus Conference on Malignant Mesothelioma of the Pleura: the review showed that MM occurrence increases with cumulative exposure to asbestos [Magnani et al., 2013]. Our results confirm such conclusion, detecting a sharp increase in PMM risk with cumulative exposure even at low and very low-levels. Risk estimates by exposure category confirm the exposure-response trend for pleural malignant mesothelioma irrespective of the route of exposure (occupational, environmental or domestic). Results consistent with an increase of MM risk with increasing asbestos exposure were provided by other case-control studies.

Iwatsubo et al. [1998] carried out an analysis of the exposure-response relationship for PMM in five regions of France, their main interest being occupational exposures. They observed a clear trend of increase in the OR according to cumulative exposure, with an OR

of 11.3 for subjects continuously exposed to over 10 f/mly. Results of a case-control study on PMM recently published by Lacourt et al. [2014] showed a clear exposure-response relationship between quantitatively assessed occupational exposure and PMM among men.

Increasing knowledge on interaction between asbestos and genetic factors will contribute to understanding the mechanisms of action of mineral fibres, and possibly to identify families or subjects at increased risk. Expected results will be of interest to other tumors.

Familial risk for mesothelioma has not yet been fully determined. De Klerk et al. [2013] has been reported a doubled risk of mesothelioma in blood relatives of affected family members involved in asbestos milling in the Wittenoom Gorge in Western Australia [De Klerk et al., 2013].

Testa et al. [2011] reported 2 unrelated families with multiple cases of malignant mesothelioma transmitted in an autosomal dominant pattern. Affected members of both families had only household exposure to asbestos, but not occupational exposure. In 1 family, there were 5 affected individuals spanning 3 generations. There was also 1 case each of ovarian cancer, breast cancer, and renal cell carcinoma in other members of this family. The second family had 7 cases of mesothelioma. In addition, there was 1 case each of squamous cell carcinoma, basal cell carcinoma, and pancreatic cancer in other family members. One of the mesothelioma patients also had a uveal melanoma, and 1 additional family members reportedly had a uveal melanoma, but no DNA from the latter patient was available.

Because of the limited number of families reported to date, the penetrance, natural history, and frequencies of the *BAP1*-associated tumors are yet to be determined [Pilarski et al., 2016].

Growing evidence suggests that *BAP1* pathogenic variants interact with environmental asbestos exposure to increase the risk for MM [Pilarski et al., 2016].

In the first article published by Betti et al. [2015], among the five families considered, the BAP1 mutation was found only in one family suggesting that other genes could be involved in familial predisposition and association of more gene variants can increase the risk.

In a study by Ohar et al. [2016], taking into consideration 150 mesothelioma patients with a family history of cancer, 50 asbestos-exposed control individuals with a family history of cancers other than mesothelioma, and 153 asbestos-exposed individuals without familial cancer, BAP1 alterations were found in nine of 150 mesothelioma cases (6%) with a family history of cancer. No mutations were detected in the other two cohorts.

Patients with MM and a personal or family history of other types of cancer should be considered for genetic testing, aiming at identifying the families that might benefit from BAP1 mutation screening and regular clinical monitoring of family members for the purpose of early detection of cancers and clinical intervention.

The majority of MM cases with germline BAP1 mutations arise in individuals with a strong history of malignancy but the study by Testa et al. reported 7.7% (2/26) of sporadic MM cases carried BAP1 germline mutations. No BAP1 mutations were observed in the 103 sporadic cases considered in the article by Betti et al. [2015]. Studies of nearly 200 sporadic European MM cases have not identified any relevant germline BAP1 mutations [Sneddon et al., 2015].

The germline genome studies do not show an alternative role of genes in relation to asbestos exposure in the development of mesothelioma but gene variants can increase the effects of asbestos exposure. The identification of genes can increase our understanding of the etiology and pathogenesis of MM and may open new perspectives for the control and treatment of this disease [Ugolini et al., 2008].

Chapter 7

FUTURE PERSPECTIVES

In the pooled cohort study, exposure cannot be assessed on an individual basis, because of the lack of individual data on jobs and work activities of cohort members in almost all cohorts. Cohort-specific average exposures will be, however, estimated by calendar year, and will be applied to all workers in each cohort on the basis of an evaluation of information on the production cycle of the different industries such as the mineralogical varieties of asbestos used in the company over time, data monitoring of airborne fibers performed in the plant, information on the use of asbestos (direct or indirect) and so on. Using such information, a cumulative exposure index will be calculated for each subject in order to obtain an evaluation of risk on the basis of different categories of asbestos exposure.

In order to identify incident MM cases among cohort members and compare mortality with incidence, a record-linkage study with the National Mesothelioma Registry (ReNaM) files has been planned.

In the Balangero cohort, an evaluation of cumulative exposure will be performed as well. As estimates of airborne asbestos concentrations are available by work-area, but not by job, and data from the personnel records specified more often the work-area than the job, exposure assessment will be conducted at the work-area level.

Cumulative exposure of cohort members will result from the (time-dependent) sum of their yearly exposure levels and will be reported in fibre/millilitre-years (f/ml-y).

Further analyses will be performed using the data of the case control study of Casale Monferrato. In particular, the risk of pleural mesothelioma associated to the residence of the subjects, taking into account the distance from the Eternit plant, will be studied. In order to extend the “cumulative exposure” index, with the aim to accommodate the temporal variation in risk because of protracted exposure, taking into account the intensity

and timing of past exposure, distributed lag linear and non linear models will be applied. These models will be performed by means of the R software and the packages provided by Gasparrini [2010,2011,2014].

In relation to genetic studies, in order to identify variants found in the coding region which affect protein function, an exome sequencing will be performed. Further analyses are also being performed to identify specific methylation patterns taking into account a potential interaction between methylation profiles and asbestos exposure levels. Epigenetic aberrations in malignant mesothelioma may be putatively due to a direct effect of the inhaled asbestos fibers, or to the subsequent inflammation status induced by the deposition of asbestos fibers into the mesothelial cells.

Chapter 8

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