ORIGINAL ARTICLE

Asbestos Lung Burden Does Not Predict Survival in Malignant Pleural Mesothelioma: A Necropsy-Based Study of 185 Cases

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ABSTRACT

Introduction: Malignant pleural mesothelioma is an asbestos-related disease with poor survival. The prognostic role of histologic subtype is well established. Some studies (without a biological hypothesis) suggested that higher asbestos lung burden is associated with reduced survival.

Methods: We selected subjects from two series of necropsies: residents in Brescia province (North-West Italy) and workers (or persons living with them) employed in the Monfalcone shipyards (North-East Italy). Asbestos fibers and asbestos bodies in lung samples were counted using a scanning electron and an optical microscope, respectively. Separately in the two series, we analyzed median survival time and fitted multivariable Cox regression models (adjusted for sex, period and age at diagnosis, and histopatholocical diagnosis) to calculate hazard ratios (HRs) and 95% confidence intervals (CIs) for three levels of asbestos fiber counts (reference: <1 million fibers/g of dry lung tissue).

Results: We analyzed 185 necropsies, 83 in Brescia and 102 in Monfalcone. Despite a much higher lung burden in Monfalcone patients, median survival was slightly shorter in Brescia (8.3 mo) than in Monfalcone (10.2 mo). In Brescia, medium (1.0–9.9) and high (10+) fiber burden HRs were 0.91 (95% CI: 0.54–1.53) and 1.23 (95% CI: 0.41–3.70), respectively. In Monfalcone, the corresponding HRs were 1.18 (95% CI: 0.59–2.35) and 1.63 (95% CI: 0.77–3.45), respectively.

Conclusions: No relationship between asbestos lung burden and survival was found. Histologic subtype was the strongest prognostic determinant.

Keywords: Pleural mesothelioma; Survival; Asbestos lung burden; Asbestos fibers; Asbestos; Mortality

Introduction

Malignant pleural mesothelioma (MPM), like other malignant mesotheliomas (MMs), is a cancer causally associated with asbestos exposure. Despite current available therapy, the prognosis is poor, with estimated median survival time of approximately 10 months in population-based settings.[1] Selected patients with early stage disease receiving combined treatments or the multimodality approach had significantly longer median survival.[2–4] Several studies revealed that predictors of better survival were younger age at diagnosis, sex, epithelioid histotype, and pathologic stage.[5–10] Moreover, a recent article revealed that mesotheliomas in carriers of *BAP1* germline mutations are significantly less aggressive.[11]

To the best of our knowledge, six studies evaluated the association of survival in patients with MM or MPM with lung asbestos burden, measured as concentration of asbestos fibers (AFs) or asbestos bodies (ABs).[12–17] Four of them found an association.[12,13,15,17] Several studies analyzed the association of MPM survival with history of asbestos exposure (a rather crude index of lung asbestos

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burden)[1,18–27]; of these, only four old studies found an association.[19–21,24]

We are aware of no plausible biological hypothesis to support a role of lung asbestos burden in affecting survival after mesothelioma occurrence. To shed light on this controversy, we exploited existing information from two series of necropsies performed on patients with MPM in the context of criminal trials in Northern Italy. The specific aim of our study was to evaluate the relationship between asbestos lung AF burden and survival separately within these two groups, which together represent the second largest necropsy series with information on survival and lung asbestos burden. AB counts are presented for descriptive purposes, but they were not used in survival because they were unavailable for a substantial proportion of subjects.

Materials and Methods

Study Subjects

Subjects were selected from two series of necropsies performed from 1994 to 2017. The first group of 83 autopsies was performed in the context of the activity of the Malignant Mesothelioma Registry of Brescia Province (Lombardy region, North-West Italy), as described in detail elsewhere.[28] All individuals in Brescia had a histologically confirmed MPM diagnosis.

The second group of necropsies was performed in the context of criminal trials regarding a large shipyard in Monfalcone (Friùli Venezia-Giulia region, North-East Italy) on patients affected by MPM (definite or suspected) and previously exposed to asbestos (shipyard workers or their family members).[29] In particular, this work was originally carried out in the framework of an expert testimony to the public prosecution office by one of us (PGB). From this series, we selected necropsy reports for 102 subjects with definite (histologically confirmed) MPM.

For both series, with histologically confirmed diagnosis in life, information on demographics, age at MPM diagnosis and death, histologic subtype, and pleural plaques was collected. AF counts and percentage of amphibole fibers were available for all cases, whereas AB counts were available for only 22 (26.5%) individuals in Brescia and 90 (88.2%) in Monfalcone.

Information on presence of asbestosis at histologic examination was also available in the shipyard group. According to the criteria of Asbestosis Committee of the College of American Pathologists and Pulmonary Pathology Society, asbestosis diagnosis was done in the presence of two or more ABs per cm^2 of a 5-μm-thick fresh lung section, in combination with interstitial fibrosis of the appropriate pattern.[30]

In both series, necropsy had been ordered by the public prosecutor in the context of criminal trials. For this reason, informed consent was not required; institutional review board approval was obtained.

Analysis of ABs and AFs

Following the guidelines proposed by the European Respiratory Society, lung tissue samples of 1 cm^3 were collected from different parts of the lung and stored in a formaldehyde solution.[31] Samples were then prepared by lyophilization, plasma asher digestion, and filtration. The analyses were carried out using a scanning electron microscope equipped with x-ray fluorescence microanalyzer at 12,000 magnifications (ZEISS EVO 40; Zeiss and Oxford XMAX microanalyzer; Oxford) as described in detail elsewhere.[32,33]

Fiber concentration was expressed as total number of fibers (amphibole: amosite and crocidolite, and chrysotile; noncommercial amphibole: tremolite and actinolite) with length of more than 1 μm/g of dry lung tissue with 95% confidence interval (CI). This method does not allow differentiation of amphibole fibers if they have a diameter of less than 0.08 μm; for this reason, crocidolite and amosite were counted together and reported as amphiboles.

In the same laboratory, 50 mg freeze-dried lung sample was treated according the method ISTISAN 17/12[34] to count AB with optical microscope at 500 magnifications. The concentration was expressed as number of AB/g of dry lung tissue with 95% CI. The laboratory is accredited according to the International Organization for Standardization 17025 by ACCREDIA on both methods (laboratory n. 1324 sede E). AB and AF concentrations were expressed in thousands and millions per gram of dry lung tissue, respectively. Both AF and AB analyses were performed between 2000 and 2017 blindly of subjects' disease and occupational history.

Statistical Analysis

We compared subjects from Brescia MPM Registry and Monfalcone shipyard using chi-square (for categorical variables) or Wilcoxon-Mann-Whitney (for quantitative variables) tests. AF lung burden in these two series was compared with that previously measured in 13 subjects from the Brescia province never exposed to asbestos (11 men, two women, aged 54–67 y) who died from cardiovascular disease.[35]

We informally compared median survival times between the series of Brescia (lower exposure) and Monfalcone (higher exposure). Nevertheless, because the two groups largely differed for several potential confounding variables (including the period of diagnosis), formal

survival analyses were performed separately within the two series of necropsies. Survival time was computed starting from the date of histologically confirmed MPM diagnosis. We fitted univariate and multivariable (adjusted for gender, period of diagnosis, age at diagnosis, and MPM morphology) Cox proportional-hazards regression models to calculate hazard ratios (HRs) and 95% CIs for three levels of AF counts. We tested the proportional-hazards assumption on the basis of Schoenfeld residuals. Given that AB counts were missing for a number of subjects, we did not use them in Cox analyses. Statistical analyses were performed with Stata 17 (StataCorp, 2021).

Results

Subjects from Brescia MPM Registry included more women, were younger, and had less pleural plaques than subjects from Monfalcone shipyard (Table 1). Distribution of histologic MPM types was similar in the two groups. Approximately one-fourth of subjects from Monfalcone had histologically confirmed asbestosis. Subjects from Brescia MPM Registry had much lower AB and AF counts. In both series, the large majority of AF were amphiboles, but in lung tissue samples from Brescia, patients were found to have less amphibole fibers, either in absolute or relative terms. Nevertheless, a negligible difference (1.9 mo) in median survival time was observed between the two groups of subjects (8.3 mo in Brescia, 10.2 mo in Monfalcone). Both series had higher lung concentrations of AFs compared with unexposed controls (Fig. 1). AF and AB counts were highly correlated (Pearson's correlation coefficient on log-transformed counts: $r = 0.83$, $p < 0.001$).

Among subjects from Brescia MPM Registry, the adjusted HRs were high for those aged 75 or more years at diagnosis and 3 to 4 times elevated HRs for people with biphasic and sarcomatoid MPM, but there were no important differences according to AF counts (HR = 1.23

Table 1. Characteristics of 185 Subjects With MPM Included in a Necropsy Study, Italy, 1994 to 2017

	Brescia MPM Registry	Monfalcone Shipyard	
Variables	n (%)	n (%)	*p* Value[a]
All subjects	83 (100)	102 (100)	
Sex			
Male	65 (78.3)	91 (89.2)	0.04
Female	18 (21.7)	11 (10.8)	
Period of diagnosis			
1994-2007	56 (67.5)	11 (10.8)	<0.001
2008-2012	11 (13.2)	53 (52.0)	
2012-2017	16 (19.3)	38 (37.2)	
Age at diagnosis (y), mean (range)	67.5 (49-91)	73.6 (58-93)	<0.001
<65	34 (41.0)	17 (16.7)	0.002
65-69	16 (19.3)	19 (18.6)	
70-74	11 (13.2)	20 (19.6)	
75-79	11 (13.2)	16 (15.7)	
80+	11 (13.2)	30 (29.4)	
Histologic subtype			
Epithelioid	46 (55.4)	46 (45.1)	0.17
Biphasic	29 (34.9)	37 (36.3)	
Sarcomatoid	8 (9.6)	19 (18.6)	
Histologic asbestosis			
No	NA	74 (72.5)	<0.001
Yes	NA	28 (27.5)	
Pleural plaques			
No	52 (62.6)	13 (12.8)	<0.001
Yes	31 (37.4)	89 (87.2)	
Asbestos bodies,[b] median (range)	7.5 (0.2-120)	90 (0.2-8.900)	<0.001
Asbestos fibers,[c] median (range)	1.0 (0.1-400)	6.9 (0.2-256)	<0.001
Amphibole fibers,[c] median (range)	0.7 (0.0-400)	6.7 (0.2-256)	<0.001
Amphiboles (%), median (range)	90 (10-100)	100 (29-100)	
Survival (mo), median (range)	8.3 (0.1-88.2)	10.2 (0.3-79.4)	0.19

[a]From chi-square (categorical variables) or Wilcoxon-Mann-Whitney (quantitative variables) tests.
[b]Thousands per gram of dry lung tissue. Values available for 22 subjects form the Brescia MPM Registry and 90 from Monfalcone shipyard.
[c]Millions per gram of dry lung tissue.
MPM, malignant pleural mesothelioma; NA, not available.

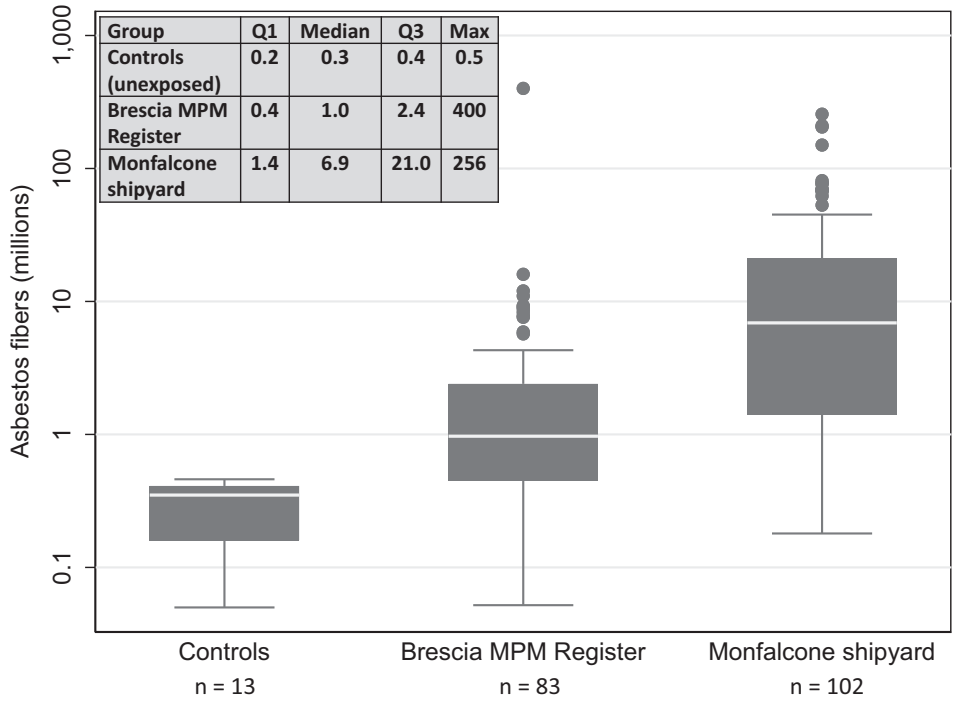

Figure 1. Asbestos fiber lung burden (millions per gram of dry lung tissue) in 185 subjects with MPM included in a necropsy study, Italy, 1994 to 2017. Values for 13 unexposed controls from the Brescia province evaluated in a previous study are illustrated for comparison.[35] Max, maximum; MPM, malignant pleural mesothelioma; Q1 and Q3, first and third quartiles (25th and 75th percentiles).

only in the highest AF category with a wide CI, $p = 0.71$) (Table 2).

In Monfalcone shipyard workers, the adjusted HRs were much higher for those diagnosed in recent periods, elevated in subjects with biphasic MPM (HR = 1.89) and strongly elevated in those with sarcomatoid histological subtype (HR = 4.34). Again, no important differences according to AF counts were found (HR = 1.63 only for those in the highest AF category, with a wide CI, $p = 0.20$). In neither group, we found evidence of violation of proportional hazards. Multivariable Cox models in which we analyzed categories of amphiboles yielded similar HRs (results not shown). In Monfalcone subjects, we found no association between survival and presence of histologically confirmed asbestosis (adjusted HR = 1.10, 95% CI: 0.64–1.88, $p = 0.73$).

Discussion

In this study, in two necropsy series, we found no association between asbestos lung burden and survival, either in univariate or multivariable Cox analysis. Furthermore, survival was similar between the two groups of patients, despite much higher lung concentrations of AFs, amphibole fibers, and ABs in the Monfalcone shipyard group compared with the Brescia MPM Registry group. Finally, within the Monfalcone shipyard series, no association was found between survival and histologic asbestosis (a marker of high asbestos exposure).

Previous studies that evaluated the role of asbestos lung burden or asbestos exposure as a prognostic factor in patients with MPM found controversial results. Of the seven studies (including the present one) based on lung AF and AB concentrations, four studies found a positive association (Table 3, upper part).[12,13,15,17]

The first was a Finnish study on 41 patients with mesothelioma, 40 with MPM and one with peritoneal mesothelioma. The main finding was a better survival ($p = 0.031$, Mantel-Cox test) in eight patients with low lung asbestos burden (<1 million fibers/g), compared with 19 with high burden (≥1 million fibers/g). Moreover, survival was worst ($p = 0.021$) in 15 patients with greater than or equal to 50% crocidolite/amosite fibers, best in four patients with greater than or equal to 50 antophyllite fibers and intermediate in eight patients with greater than or equal to 50% other fibers.[12]

In a second study performed in Germany on 73 MPM, survival was shorter in patients with high lung fiber concentration (≥70 AF/g) compared with those with less than 70 AF/g (35 versus 50 wk, respectively, $p = 0.04$).[13] This finding was confirmed in a multivariable (unspecified) analysis ($p = 0.02$).

A third study in the United States on a group of 80 surgical patients reported an association between survival and AB count (low, moderate, high).[15]

Table 2. HRs and 95% CIs According to Selected Variables in 185 Subjects With MPM Included in a Necropsy Study, Italy, 1994 to 2017

	Brescia MPM Registry			Monfalcone Shipyard		
Variables	n deaths	Crude HR	Adjusted HR (95% CI)[a]	n deaths	Crude HR	Adjusted HR (95% CI)[a]
Sex						
Male	65	Reference	Reference	91	Reference	Reference
Female	18	0.61	0.57 (0.29-1.13)	11	1.30	1.58 (0.67-3.70)
Period of diagnosis						
1994-2007	56	Reference	Reference	11	Reference	Reference
2008-2012	11	0.50	0.57 (0.24-1.35)	53	2.71	3.25 (1.52-6.93)
2012-2017	16	1.40	1.43 (0.76-2.69)	38	3.67	6.37 (2.67-15.2)
Age at diagnosis (y)						
<65	34	Reference	Reference	17	Reference	Reference
65-69	16	1.90	1.79 (0.91-3.52)	19	0.60	0.36 (0.18-0.72)
70-74	11	1.54	1.05 (0.42-2.62)	20	0.95	0.30 (0.13-0.69)
75-79	11	1.78	2.16 (1.02-4.57)	16	1.11	0.83 (0.40-1.72)
80+	11	2.43	2.90 (1.35-6.20)	30	1.38	0.68 (0.34-1.37)
Histologic subtype						
Epithelioid	46	Reference	Reference	46	Reference	Reference
Biphasic	29	4.11	3.47 (1.84-6.53)	37	1.62	1.89 (1.14-3.14)
Sarcomatoid	8	3.73	3.75 (1.37-10.3)	19	4.34	5.07 (2.63-9.78)
Asbestos fibers[b]						
<1	43	Reference	Reference	14	Reference	Reference
1.0-9.9	36	0.97	0.91 (0.54-1.53)	46	0.91	1.18 (0.59-2.35)
10+	4	0.62	1.23 (0.41-3.70)	42	0.88	1.63 (0.77-3.45)

[a]Each variable adjusted for the others.
[b]Millions per gram of dry lung tissue.
CI, confidence interval; HR, hazard ratio.

Nevertheless, we note that in that study a very unusual choice of the reference category (the moderate instead of the low one) was made when performing a multivariable Cox analysis. As a matter of fact, no trend in reduced survival with increasing AF lung burden was found: survival was worse in the low exposure category than in the moderate one. Considering the width of CIs, the adjusted HR in the low burden (<100 AB/g) category (HR = 3.0, CI: 0.95–5.0, 37 patients) was not much different from the HR in the high (>1099 AB/g lung) category (HR = 4.8, CI: 1.5–15.0, 22 patients).

The fourth recent study in Finland is the largest conducted so far, being based on 590 subjects (527 men and 43 women) identified within the national mesothelioma registry. After excluding 50 subjects with MPM diagnosed at autopsy, an association between reduced survival and lung AF burden was found in multivariable Cox models. More specifically, the authors found a reduced HR (i.e., higher survival in those with high lung burden) using the initial lung asbestos burden (referred to as "initial HR") and a high HR (i.e., lower survival in those with high lung burden) using lung asbestos burden during the follow-up period (referred to as "HR change"). It is unclear to us how the authors performed a time-dependent analysis given that asbestos lung burden was measured at a single time point (death).[17]

Conversely, two studies found no association between lung asbestos burden and survival. A large study (404 patients, 387 [95.8%] with MPM, 16 with peritoneal, and one with pericardial mesothelioma) with available information on asbestos lung burden was performed within the German mesothelioma register (1987–1999).[14] Survival in 345 MM cases with high lung burden (>22 AB/cm^3) was 13.2 months, almost identical to that in 59 cases with less than 22 AB (13.3 mo) (p = 0.59, log-rank test). Recently, a study in Italy evaluated survival in 59 MM and found no correlation between survival and lung AF burden (Spearman's rho = 0.09, p = 0.45).[16] Analyses of different AF types (chrysotile, crocidolite, amosite, anthophyllite, and tremolite) yielded correlation coefficients ranging from −0.15 to +0.18.

In a nonsystematic search, we found 11 studies that evaluated the relationship of MM or MPM survival with history of asbestos exposure (Table 3, lower part).[1,18–27] Only four of them performed between 1983 and 1989 found a positive association.[19–21,24]

A study in United Kingdom on 106 patients with MPM found better survival in patients unexposed to asbestos.[19] The authors had information on AB counts for a subset of 88 patients, but these data were not used in survival analyses. A large study in the United States found ecological associations between shorter survival

Table 3. Studies That Evaluated the Association Between MM and/or MPM Survival and Lung Asbestos Burden or Asbestos Exposure History

Study (First Author, Country, y)	No. of Cases	Case Source	Period	AB or AF Lung Tissue Counts	Association
Lung asbestos burden					
Tammilehto, Finland, 1992	27 MM (26 MPM)	Hospital series	1982-1989	AF g/dry t. SEM-EDS/TEM	Yes
Kayser, Germany, 1999	73 MPM	Hospital series	1995-1996	AF g/wet t. LM	Yes
Neumann, Germany, 2001	404 MM (387 MPM)	National MM registry	1987-1999	AB/cm^3 LM or g/wet t. LM	No
Christensen, USA, 2008	80 MPM	Surgical series	2000-2006	AB g/wet t. LM	Yes
Visonà, Italy, 2021	59 MM	Forensic series	2000-2018	AF $>$ 5 μg/dry t. SEM-EDS	No
Laaksonen, Finland, 2022	590 MPM (540 analyzed)	National MM registry	2000-2012	AF g/dry t. TEM	Yes
This study, Italy, 2022	185 MPM	Forensic series	1994-2017	AF $>$1 μg/dry t. SEM-EDS	No
Asbestos exposure history					
Musk, Australia, 1982	81 MPM	Hospital series	1957-1980	NA	No
Law, UK, 1983	106 MPM	Hospital series	1957-1980	NA	Yes
Spirtas, USA, 1988	1475 MM	SEER	1973-1984	NA	Yes
Ruffie, Canada, 1989	118 MPM	Hospital series	1965-1984	NA	Yes
Edwards, UK, 2000	107 MM	Hospital series	1990-2001	NA	No
Gorini, Italy, 2005	381 MPM	Regional MM registry	1988-2000	NA	No
Flores, USA, 2007	945 MPM	Hospital series	1990-2005	NA	Yes
Ak, Turkey, 2009	235 MPM	Hospital series	1991-2008	NA	No
Montanaro, Italy, 2009	4100 MPM	National MM registry	1990-2001	NA	No
Nojiri, Japan, 2010	314 MPM	Hospital series	1996-2006	NA	No
Berardi, Italy, 2016	62 MPM	Hospital series	2003-2013	NA	No

AB, asbestos bodies; AF, asbestos fibers; LM, light microscopy; MM, malignant mesothelioma; MPM, malignant pleural mesothelioma; NA, not applicable; SEER, Surveillance, Epidemiology and End Results Program of the National Cancer Institute; SEM-EDS, scanning electron microscope-energy-dispersive x-ray spectroscopy; t., lung tissue; TEM, transmission electron microscope; UK, United Kingdom; USA, United States of America.

and presence of shipbuilding as a major industry.[20] The authors concluded that there was "weak support for the hypothesis that asbestos-exposed cases of mesothelioma have worse survival experience than other cases." Finally, a study in Canada on 188 MPM with complete data found that asbestos exposure was an important predictor of survival at multivariate analysis.[21] Recently, a study in the United States reported a positive association between survival and history of asbestos exposure at univariate analysis, but not in multivariable analysis.[24] In general, these studies are much less informative, because history of asbestos exposure is a crude proxy for asbestos lung burden.

Our study had several strengths. First, this is one of the largest necropsy studies with documented AF lung burden. The lung content of AFs is a widely accepted index of lifetime cumulative asbestos exposure.[36–39] Second, it included two series with very different asbestos lung burdens. Third, accurate lung burden assessment was performed in a reference regional laboratory. Our study also had some limitations. First, AB concentrations were missing in many patients. Nevertheless, AF counts were available for all patients with MPM. Second, we did not have information on patient's treatment or BAP1 germline mutations, which could differ between the two series. We performed, however, separated analyses, and we found no association between lung asbestos burden and survival in neither of the two necropsy series.

We were not able to find in the literature convincing hypotheses on the role of lung asbestos burden on survival. One could hypothesize that a higher burden is a marker of a higher degree of lung fibrosis, which might affect lung function. Although we find a negligible relationship between histologic asbestosis and survival in shipyard patients, one should consider that histologically documented fibrosis rarely implies relevant lung function loss; in fact, only a few subjects had radiologically confirmed asbestosis. Because germline testing was not performed in any of the studies, the discrepancies among different studies might in theory be explained by different proportions of subjects with BAP1 mutations in the different series. Nevertheless, a relationship between BAP1 and lung asbestos burden would be necessary to confound the association between lung asbestos burden and survival.

In conclusion, the results of our study, based on a large series of autopsies and high-quality laboratory data, do not confirm the inverse relationship between asbestos lung burden or exposure and survival observed in previous studies. In contrary, our results are in agreement with other studies revealing lack of association between lung asbestos burden or exposure and survival. We confirmed that the strongest predictor is

histologic type. Results of this study may be relevant for clinicians and in medico-legal settings.

CRediT Authorship Contribution Statement

Pietro Gino Barbieri: Conceptualization, Investigation, Data curation.

Dario Consonni: Methodology, Software, Formal analysis, Writing—review and editing.

Anna Somigliana: Lung sample analysis.

Ethics Approval

The study was approved by the Ethical Committee of the Friuli Venezia-Giulia region (n. 2019/13818, May 21, 2019).

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