

## **Supplemental material**

### **Title**

The asbestos fibre burden in human lungs - New insights into the chrysotile debate.

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Additional Table 1 with detailed asbestos fibre burden counting results.

Additional Table 2 summary of asbestos fibre burden counting results.

### Patient 1

78-year-old male, ship's carpenter, dock/ transport worker, 12 fibre years  
No plaques on X-ray evaluation; resection of left upper lobe for adenocarcinoma.

Diagnosis at surgery: adenocarcinoma with grade I asbestosis, focal accumulation of asbestos bodies at tumour margin, RB-ILD (respiratory bronchiolitis interstitial lung disease) including DIP (desquamative interstitial pneumonitis).

Lung dust analysis per gram of wet lung tissue:

Sample 1: 500 asbestos bodies, 300 structures with non-identifiable core fibre

Sample 2: 1900 asbestos bodies, 1100 structures with non-identifiable core fibre.

Autopsy findings (time interval of tissue sampling: 14 years): metastatic small-cell carcinoma in the right lung, no recurrence of the adenocarcinoma, bilateral grade I asbestosis, hyaline calcifying plaques on costal pleura and diaphragm.

Lung dust analysis per gram of wet lung tissue:

Left residual lung

Sample 1: 224 asbestos bodies, 194 asbestos fibres, 82 structures with non-identifiable core fibre

Sample 2: 303 asbestos bodies, 404 asbestos fibres, 303 structures with non-identifiable core fibre

Right residual lung

Sample 1: 714 asbestos bodies, 102 asbestos fibres, 204 structures with non-identifiable core fibre

Sample 2: 307 asbestos bodies, 79 asbestos fibres, 59 structures with non-identifiable core fibre

Fibre type (FE-SEM): 90 % amphiboles, 10 % chrysotile.

Fibre type (TEM): 85 % amphiboles, 15 % chrysotile.

### Patient 2

65-year-old male working in a pressing plant

Plaques on X-ray evaluation, COPD (chronic obstructive pulmonary disease), wedge excision on the right for round foci, suspicion of TB (tuberculosis).

Diagnosis at surgery: TB, COPD, grade I asbestosis, asbestos body count probably too low because of TB.

Lung dust analysis per gram of wet lung tissue:

1280 asbestos bodies

Autopsy findings (time interval between tissue samplings: 21 years): pleural plaques, grade I asbestosis, high-grade COPD, high-grade chronic and active, in part hypertrophic bronchitis with mucopurulent exacerbation, variable bronchiolectases, extensive disseminated bronchopneumonic infiltrates, high-grade, in part subpleurally bullous lung emphysema.

Lung dust analysis per gram of wet lung tissue:

Left lung

Sample 1: 6733 asbestos bodies, 396 asbestos fibres, 5347 structures with non-identifiable core fibre

Sample 2: 13861 asbestos bodies, 198 asbestos fibres, 7921 structures with non-identifiable core fibre

Right residual lung:

Sample 1: 27921 asbestos bodies, 3366 asbestos fibres, 41980 structures with non-identifiable core fibre

Sample 2: 15644 asbestos bodies, 4356 structures with non-identifiable core fibre.

Fibre type (FE-SEM): 65 % amphiboles, 35 % chrysotile.

Fibre type (TEM): 70 % amphiboles, 30 % chrysotile.

### Patient 3

76-year-old male, seaman

Resection of right lower lobe for adenocarcinoma

Diagnosis at surgery: peripheral bronchioloalveolar carcinoma with central fibroelastotic scarring, locoregional infiltration of blood vessels, residual lung tissue with circumscribed atypical adenomatous hyperplasia, moderate RB-ILD, low-grade centroacinar and subpleural emphysema, low-grade pleural lipomatosis, minimal anthracosis.

Lung dust analysis per gram of wet lung tissue:

S6: 314 asbestos bodies, 17 asbestos fibres, 124 structures with non-identifiable core fibre

S10: 431 asbestos bodies, 20 asbestos fibres, 88 structures with non-identifiable core fibre.

Autopsy findings (time interval between tissue samplings: 8 years): tumour recurrence with extensive intrapulmonary metastatic spread, pleural metastases, pleural hyaline plaques, circumscribed grade I asbestosis

Lung dust analysis per gram of wet lung tissue:

Left lung

Segment 2: 800 asbestos bodies, 500 structures with non-identifiable core fibre

Segment 10: 200 asbestos bodies, 200 asbestos fibres, 200 structures with non-identifiable core fibre

Right residual lung

Sample 1: 300 asbestos bodies

Sample 2: 297 asbestos bodies

Fibre type (FE-SEM): 20 % amphiboles, 80 % chrysotile.

Fibre type (TEM): 30 % amphiboles, 70 % chrysotile.

### Patient 4

62-year-old male, welder and assembly fitter, 73 fibre years

Plaques on X-ray evaluation, wedge excision of right upper lobe for round focus, numerous postoperative complications including bronchopulmonary fistula, creation of a tracheostoma, several, probably embologenic, cerebral insults, myocardial infarction

Diagnosis at surgery: inflammatory- partly granulomatous changes, no malignancy, grade I asbestosis

Lung dust analysis per gram of wet lung tissue:

1167 asbestos bodies and 917 structures with non-identifiable core fibre

Autopsy findings (time interval between tissue samplings: 4 years): severe complex lung disease including bilateral, base-accentuated pleural collapse and plate atelectasis, diffuse pleural fibrosis fully encompassing the lung, with partial lung encasement under consecutive plate and infolding atelectases, subpleural fibrosis and grade I asbestosis even presenting as focal grade III asbestosis in the left subpleural base; from here originates severe purulent abscessed pneumonia of the left lung with colliquation and part carnification, focal manifestations in the presence of bilobar pneumonia

Lung dust analysis per gram of wet lung tissue:

Right lung

Segment 2: 5347 asbestos bodies, 198 asbestos fibres, 1188 structures with non-identifiable core fibre

Segment 10: 4554 asbestos bodies, 792 structures with non-identifiable core fibre

Left lung affected by pneumonia

Segment 2: 1010 asbestos bodies, 1212 structures with non-identifiable core fibre

Segment 10: 3333 asbestos bodies, 588 structures with non-identifiable core fibre

Fibre type (FE-SEM): 5 % amphiboles, 95 % chrysotile.

Fibre type (TEM): 10 % amphiboles, 90 % chrysotile.

### Patient 5

65-year-old male, papermaker

Plaques on X-ray evaluation, resection of right upper lobe for adenocarcinoma

Diagnosis at surgery: moderately differentiated adenocarcinoma with acinar, papillary, and solid parts showing locoregional infiltration of lymph and blood vessels, peritumoral chronic and active retention pneumonia, moderate chronic and active bronchitis and peribronchitis with luminal, alveolar macrophage alveolitis and RB-ILD, low-grade anthracosis, grade I asbestosis and typical hyalinised pleural plaques

Lung dust analysis per gram of wet lung tissue:

Sample 1: 2435 asbestos bodies, 2609 asbestos fibres, 696 structures with non-identifiable core fibre

Sample 2: 1400 asbestos bodies, 1700 asbestos fibres, 300 structures with non-identifiable core fibre

Autopsy findings (time interval between tissue samplings: 8 years): Recurrence of adenocarcinoma in left upper lobe with metastases in lymph nodes and pericardium, hyaline pleural plaques, grade I asbestosis

Lung dust analysis per gram of wet lung tissue:

Left lung

Segment 2: 593 asbestos bodies, 254 asbestos fibres

Segment 10: 1681 asbestos bodies, 336 asbestos fibres

Right lung

Segment 4/5: 3190 asbestos bodies, 259 asbestos fibres

Segment 10: 2018 asbestos bodies, 183 asbestos fibres

### Patient 6

70-year-old male, joiner at shipyard

Fibrosis on X-ray evaluation, hyaline pleural plaques, wedge resection for round foci, autopsy after multiple focal pneumonias and cardiac failure although a year ago pulmonary function parameters had been at the high end of the normal range.

Diagnosis at surgery: multiple intrapulmonary lymph nodes with focal pulpal hyperplasia and increased dust storing, residual paranodal lung tissue showing subpleural transformation, in part essentially preserved alveolar septal structure, but with distinct connective tissue widening of the septa in the reticular architecture, in parts associated with initial honeycombing, peribronchiolar dense lymphocyte and lymphofollicular infiltrates, in the alveoli plenty of activated macrophages with focal iron deposits, pulmonary arteries with fibromuscular wall thickening and initial plexiform vascular lesions, in some instances asbestos bodies in the parabronchiolar connective tissue septa, hyaline pleural plaques

Comment: Correlating with unexplained round foci: intrapulmonary lymph nodes; interstitial lung disease shows an overlap pattern commensurate with NSIP (non-specific interstitial pneumonitis) of the fibrotic subtype with hints of an UIP (usual interstitial pneumonia)/honeycombing, with the combination of the lymphocyte/lymphofollicular component and the vascular lesions suggesting an autoimmune disease. Since findings – including the presence of asbestos bodies – indicate much more than fibrosis of respiratory bronchioles, they do no longer correspond to a grade I asbestosis, and neither the NSIP nor the lymphocyte/inflammatory component are characteristic of an asbestosis of a higher grade. Thus a multi-etiological clinical picture with an asbestos-dependent and an asbestos-independent component is to be postulated, and it is safe to say that asbestos must be seen as essentially co-responsible and possibly directing the course of deterioration.

Lung dust analysis per gram of wet lung tissue:

Sample 1: 605 asbestos bodies

Sample 2: 49 asbestos bodies per gram of lung tissue

Autopsy findings (time interval between tissue samplings: 6 years): immunological component declining in favour of marked progressive fibrosis; now reticular connective tissue widening of alveolar septa adjacent to terminal honeycomb air spaces with high-grade epithelial transformation, bronchiolisation and cuboid metaplasias, also evidence of asbestos bodies in the septa associated by granulocyte/inflammatory infiltrates, subpleural fibrosis and pleural fibrosis.

Comment: Again, no classical isolated minimal asbestosis present. Considering the course of the pathology, it can be postulated that the potential autoimmune disease was "searing" and the asbestos-associated fibrogenic effect took the foreground.

Lung dust analysis per gram of wet lung tissue in advanced autolytic changes:

Right lung

Upper lobe: 1414 asbestos bodies und 101 structures with non-identifiable core fibre

Lower lobe: 1682 asbestos bodies und 561 structures with non-identifiable core fibre

Left lung

Upper lobe: 1327 asbestos bodies und 1531 structures with non-identifiable core fibre

Lower lobe: 1442 asbestos bodies und 288 structures with non-identifiable core fibre.

#### Patient 7

79-year-old male, insulation lagger in shipbuilding, changed employment upon diagnosis of asbestosis

Plaques on X-ray evaluation, wedge excision of lingula when pleural mesothelioma was suspected, autopsy in status post gastric cancer

Diagnosis at surgery: pleurisy, plaques, grade I asbestosis, no malignancy

Lung dust analysis per gram of wet lung tissue:

Left upper lobe: 4528 asbestos bodies

Autopsy findings (time interval between tissue samplings: 7 years): complex lung and pleura pathology, chronic, in part exacerbated bronchitis, emphysema with fine to medium bullae, mostly subpleural, partially advanced irregular lung fibrosis in the presence of an asbestosis up to grade II, rounded atelectases, severe pleurisy with chronic progressive thickening, diffuse pleural fibrosis (hyalinosis complicata) and lung encasement, bilateral clod-like calcification of pleural plaques, subpleural fibrosis, chronically active pleurisy, rounded atelectasis, „destroyed lung“, severe shrinking of lungs, especially on the right, no tumour recurrence.

Lung dust analysis per gram of wet lung tissue:

Right lung

Segment 2: 2963 asbestos bodies, 463 asbestos fibres

Segment 10: 4483 asbestos bodies, 86 asbestos fibres

Left lung

Segment 2: 3039 Asbestos bodies

Segment 10: 5612 Asbestos bodies

### Patient 8

77-year-old male; metalworker, smith and boiler welder  
Pneumonectomy on the left for squamous cell carcinoma

Diagnosis at surgery: moderately differentiated keratinising squamous cell carcinoma of the lung with locoregional infiltration of blood vessels; in residual lung tissue: moderate RB-ILD, grade I asbestosis, focal grade II asbestosis with typical asbestos bodies in the fibrotic areas, subpleural fibrosis, low-grade anthracosis, no silicosis

Lung dust analysis per gram wet lung tissue:

Segment 2: 6433 Asbestos bodies

Segment 10: 2557 Asbestos bodies

Autopsy findings (time interval between tissue samplings: 7 years): metastatic squamous cell carcinoma infiltrating the costal pleura, heart, metastases in lymph nodes, liver, adrenal glands, thoracic vertebral bodies, plaques on the right, calcification of fibrotic diaphragm and pleura, irregular pulmonary emphysema with moderate anthracosis and anthracofibrosis, focal grade II asbestosis.

Lung dust analysis per gram wet lung tissue:

Right lung

Segment 2: 39121 Asbestos bodies,

Segment 10: 3932 Asbestos bodies, 513 Asbestos fibres

### Patient 9

82-year-old man, metalworker, heating fitter

Wedge excision on the right in suspected asbestosis, exclusion of pleural mesothelioma in the presence of pleural effusion, autopsy due to suspected abdominal mesothelioma

Diagnosis at surgery: pleural hyalinosis with pleural thickening, subpleural fibrosis with rounded and plate atelectases, high-grade chronic, luminal fibrotic alveolar macrophage alveolitis even indicating DIP (desquamative interstitial pneumonitis), severe vascular sclerosis with secondary pulmonary arterial hypertension, grade 3 after Heath and Edwards, subpleural fibrosis, central grade I asbestosis showing - focal – subpleural confluence with the fibrosis as well as rounded atelectasis, no classical pleural plaques in the excision specimen supplied.

Lung dust analysis per gram of wet lung tissue:

Sample from right lung: 9750 asbestos bodies

Autopsy findings (time interval between tissue samplings: 8 years): biphasic peritoneal mesothelioma, hyaline pleural plaques, high-grade desquamative and luminal fibrotic alveolitis with chronic and active lesions of the alveolar walls (diffuse alveolar damage - DAD), in part abnormal epithelial regeneration including bronchioloalveolar tumorlets, vascular sclerosis, ossifying pneumonitis, rounded atelectasis and grade II – III asbestosis

Lung dust analysis per gram of wet lung tissue:

Right lung

Upper lobe: 53663 asbestos bodies, few free asbestos fibres

Lower lobe: 27600 asbestos bodies, comparatively numerous free asbestos fibres

Left lung

Upper lobe: 24200 asbestos bodies, comparatively numerous free asbestos fibres

Lower lobe: 14800 asbestos bodies, comparatively numerous free asbestos fibres

### Patient 10

75-year-old male, varnisher in shipyard, tank cleaner, sandblaster operator, and "bystander"  
No plaques on X-ray evaluation, no asbestosis, lower bilobectomy on the right for squamous cell carcinoma, no asbestos bodies

Diagnosis at surgery: moderately differentiated keratinized squamous cell carcinoma of the lung, peritumoral chronic and active retention pneumonia, purulent bronchiectases, residual lung tissue showing low-grade irregular emphysema, high-grade anthracosis with moderate anthracofibrosis and as a predominant sign siderosis with low-grade siderofibrosis, high-grade RB-ILD including DIP (desquamative interstitial pneumonitis), grade I asbestosis  
Lung dust analysis per gram of wet lung tissue:

Right inferior lobe: 1346 asbestos bodies, numerous opaque pin-structures and pseudoasbestos bodies

Autopsy findings (time interval between tissue samplings: 9 years): complex lung pathology, pleural fibrosis with pleural hyalinosis, subpleural fibrosis and fibrosis as well as parabronchiolar fibrosis with anthracosis and siderosis, siderofibrosis, RB-ILD including DIP, grade I-II asbestosis, no tumour recurrence

Lung dust analysis per gram of wet lung tissue:

Left lung

Segment 1/3: 900 asbestos bodies, 1000 structures with non-identifiable core fibre

Segment 8: 1269 asbestos bodies, 149 structures with non-identifiable core fibre

Right lung

Segment 3: 1200 asbestos bodies, 1000 structures with non-identifiable core fibre

### Patient 11

80-year-old male, metalworker in metallurgical plant, bike park attendant  
BAL (bronchoalveolar lavage) for suspected asbestosis

Diagnosis from BAL: numerous asbestos bodies, 90 % macrophages, few lymphocytes

Lung dust analysis per mL BAL:

Sample 1: 3.4 asbestos bodies and 1.2 structures with non-identifiable core fibre

Sample 2: 2.3 asbestos bodies and 3.4 structures with non-identifiable core fibre

Autopsy findings (time interval between samplings: 13 years): complex lung disease with pre-existing moderate obstructive, chronic and active abscessed bronchitis in the presence of moderate emphysema with fine bullae, bilateral hyaline plaques of the pleura and diaphragm, circumscribed pleural hyalinosis, rounded atelectasis with subpleural fibrosis of the "crow's feet" type, grade I asbestosis

Lung dust analysis per gram of wet lung tissue:

Right lung

Segment 2: 1584 asbestos bodies

Segment 10: 1100 asbestos bodies, 800 structures with non-identifiable core fibre

Left lung

Segment 2: 980 asbestos bodies, 490 structures with non-identifiable core fibre

Segment 10: 1863 asbestos bodies, 98 asbestos fibres, 294 structures with non-identifiable core fibre

Fibre type (FE-SEM): 20 % amphiboles, 80 % chrysotile.

Fibre type (TEM): 40 % amphiboles, 60 % chrysotile.

## Patient 12

82-year-old male, plumber, insulator in power plant, waiter

Plaques on X-ray evaluation, no asbestosis, pulmonary function tests at first revealed a restrictive ventilation disorder without an obstructive component, increasing heart failure with resulting compromised respiration, coronary heart disease with bypass surgery and biologic aortic valve replacement, autopsy for relapsing cardiac decompensation in the presence of the severe heart condition

Diagnosis from BAL: BAL with high cell content with partially activated alveolar macrophages, mucus substances with granulocyte agglutination, asbestos bodies

Lung dust analysis per mL BAL:

Sample 1: 10.3 asbestos bodies and 0.14 structures with non-identifiable core fibre

Autopsy findings (time interval between samplings: 8 years): complex lung pathology, bilateral focal round atelectases and plate atelectases as well as rounded atelectases, with lower lobe preference, recurrent pleurisy, pleural thickening, partial lung encasement, hyaline pleural plaques of up to 10 cm in size, grade I asbestosis, no heart failure cells, additional work load on right heart and aggravation of the pulmo-cardiac congestion due to asbestosis

Lung dust analysis per gram of wet lung tissue:

Right lung

Segment 2: 165347 asbestos bodies, numerous asbestos fibres, 47327 structures with non-identifiable core fibre

Segment 10: 87477 asbestos bodies, numerous asbestos fibres, 26542 structures with non-identifiable core fibre

Left lung in the presence of high-grade pneumonia

Segment 2: 36952 asbestos bodies, numerous asbestos fibres, 8381 structures with non-identifiable core fibre

Segment 10: 42593 asbestos bodies, numerous asbestos fibres, 17963 structures with non-identifiable core fibre

Fibre type: 15 % amphiboles, 85 % chrysotile.

Fibre type (FE-SEM): 15 % amphiboles, 85 % chrysotile.

Fibre type (TEM): 10 % amphiboles, 90 % chrysotile.



**Additional Table 1** Detailed fibre analyses at surgery and autopsy in comparison with corresponding time intervals.

patient	AB/ g FW; FF/ g FW surgery		AB/ g FW; FF/ g FW autopsy				Years from surgery to autopsy
	Sample 1	Sample 2	Sample 1	Sample 2	Sample 3	Sample 4	
1	500; 0	<b>1900</b> ; 0	224; 194	330; 404	<b>714</b> ; 102	307; 79	14
2	<b>1281</b> *	ND	6733; 396	13861; 198	<b>27921</b> ; 3366	15644; 0	21
3	314; 17	<b>431</b> ; 20	<b>800</b> ; 0	198; 198	297; 0	297; 0	8
4	<b>1167</b> ; 917	ND	<b>5347</b> ; 198	4554; 0	1010; 0	3333; 0	4
5	<b>2435</b> ; 2609	1400; 1700	593; 254	1681; 336	<b>3190</b> ; 259	2018; 183	8
6	<b>605</b>	49	1414	<b>1682</b>	1327	1442	6
7	<b>4528</b> ; 0	ND	2963; 463	4483; 86	3039; 0	<b>5612</b> ; 0	7
8	<b>6433</b> ; 2557	ND	<b>39121</b> ; 0	3932; 513	ND	ND	7
9	<b>9750</b>	ND	<b>53663</b> ; 100	27600; ~25000	24200; ~25000	14800; ~15000	8
10	<b>1346</b>	ND	900	<b>1269</b>	1200	ND	9
11	<b>3,4</b> **	2,2 **	1584; 0	1100; 0	980; 0	<b>1863</b> ; 83	13
12	<b>10,3</b> **	ND	<b>165347</b>	87477	36952	42593	8
<b>Median</b>	1623		2202				8
<b>Mean</b>	2854		9460				

Time intervals are also shown in figure 2 in the main manuscript, this detailed fibre analysis results are illustrated in figure 3 in the main manuscript. The highest asbestos body count value given in table 1 in the main manuscript is highlighted in bold. \* tissue with active tuberculosis, thus fibre count may be underestimated. \*\*Patients without lung tissue; values represent asbestos bodies per mL of bronchoalveolar lavage (BAL) fluid and were not considered for the calculation of mean and medians of the asbestos fibre burden. Mean and medians are calculated for the pulmonary asbestos fibre burden, this is asbestos bodies and bare fibres taken together. AB: asbestos bodies. FW: fresh weight. FF: free fibres. ND: not done.

**Additional Table 2** Summary of asbestos fibre counts by phase contrast microscopy.

	Highest asbestos body count per g of wet lung		Asbestos bodies per g of wet lung		Asbestos fibers per g of wet lung	
	Surgery	Autopsy	Surgery	Autopsy	Surgery	Autopsy
<b>Median</b>	1623	4268	1313	2018	1623	2202
<b>Mean</b>	2988	13932	2296	7505	2854	9460
<b>Minimum</b>	431	714	49	198	49	297
<b>Maximum</b>	9750	53663	9750	53663	9750	53763

Summary of all counting results from ten patients with two tissue excisions at a median interval of 8 years. Asbestos fibres are asbestos bodies and bare fibres totalized. g: gram.