Elemental and immunohistochemical analysis of the lungs and hilar lymph node in a patient with asbestos exposure, a pilot study

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Abstract

Objectives Studies have shown that inhaled mine dust, such as asbestos, can be translocated to various organs including the lymph nodes. Recently, we have established a protocol that enables us to identify inhaled elements using paraffin embedded lung specimens by in-air micro-particle-induced X-ray emission (micro-PIXE). However, little research has examined the concentration of these inhaled fibers in various organs or the mechanisms of their translocation. In this study, we compared the concentration of inhaled fibers in the lung parenchyma to the concentration in the hilar lymph node as well as to determine the elemental spatial distribution of the inhaled fibers in a patient with occupational asbestos exposure.

Methods Lung tissues and hilar lymph node in a patient with asbestos exposure were used in this study. Elemental analysis was performed by in-air micro-PIXE. Immunohistochemical analysis was performed using anti CD163, smooth muscle actin, vimentin and β-catenin antibody. Results The analysis revealed that the amount of inhaled silicon was approximately 6 times higher in the lymph node than in the lungs. The spatial analysis showed that silicon, iron and aluminium were colocalized in the hilar lymph node. The immunohistochemical analysis showed localized agreement of the inhaled fibers with macrophages, smooth muscle actin, and vimentin in the hilar lymph node.

Conclusions This study showed that in-air micro-PIXE could be useful for analyzing the elemental distribution and quantification of inhaled fibers in the human body.

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Furthermore, immunohistochemistry in combination with in-air micro-PIXE analyses may help to determine the mechanism of mine dust distribution in vivo.

**Keywords** Elemental analysis · Lymph node · Smooth muscle actin · Asbestos · PIXE

**Abbreviations**
- micro-PIXE: Micro particle-induced X-ray emission
- EMT: Epithelial to mesenchymal cell transition
- SMA: Smooth muscle actin
- TTF-1: Thyroid transcription factor-1
- CK14: Cytokeratin 14
- Mg: Magnesium
- Si: Silicon
- Al: Aluminium
- Fe: Iron
- S: Sulphur
- SEM: Scanning electron microscopy
- TEM: Transmission electron microscopy
- EDX: Energy dispersive X-ray spectroscopy
- ppmv: Parts per million by volume

**Introduction**

The distribution of inhaled asbestos in the human body is frequently analyzed by electron microscopy of biological tissue [1, 2]. This method has shown that silicate particles and asbestos tend to be more concentrated in the hilar lymph nodes than the lung parenchyma [2, 3]. This may be because the lymphatic ducts relocate fibers from the lung parenchyma to the hilar lymph nodes as well as the pleura and more distant lymph nodes [4]. However, the mechanism for transporting these inhaled fibers has not been established.

In recent years, microparticle-induced X-ray emission (micro-PIXE) has been applied to measure the elemental composition and concentration of elements in biological samples [5]. We have established a protocol that enables us to identify inhaled atomic elements using paraffin embedded lung specimens while simultaneously leaving the samples unchanged [6]. The protocol involves specific approaches to determine the concentration of atomic elements and their distribution in tissue.

Several hypotheses regarding asbestos-induced carcinogenesis have been proposed. For example, epithelial to mesenchymal cell transition (EMT) has been shown to be an important process involved in the progression of carcinogenesis and fibrosis caused by inhaled fibers such as asbestos [7, 8]. Furthermore, an in vitro study showed that A549 lung cancer cells exposed to asbestos exhibited smooth muscle actin (SMA) expression [9] and the lungs of rats exposed asbestos showed an increase of SMA expression by immunohistochemistry [10].

In this study, we used in-air micro-PIXE to quantify inhaled fibers in the lung parenchyma and the hilar lymph nodes of a patient with occupational asbestos exposure. This is the first study to use in-air micro-PIXE to compare the ratio of inhaled fibers in the lungs to the ratio of inhaled fibers in the lymph node. In addition, to gain a better understanding of the relocation mechanisms of inhaled fibers, we used immunohistochemistry for macrophages in the lung parenchyma and the hilar lymph node. Furthermore, an immunohistochemical analysis of SMA, vimentin and β-catenin was performed to examine the association between carcinogenesis and EMT in a patient with double primary lung cancers.

**Materials and methods**

**Patients and sample preparation**

A 76 year-old patient did not have any calcification of thoracic lymph node and history of tuberculosis. His work involved cutting asbestos, and he was exposed to asbestos as his work involved electrical insulation and welding for next 14 years. The patient was a former smoker with a history of smoking 55 pack-years. Double primary lung cancers were found by regular physical checkup for pleural thickening. Histologically, the tumor in the upper right lobe consisted of large epithelial cells with vesicular nuclei, which formed solid cell nests, trabecles, and small cell nests without any tubules, keratinization or rosettes. They did not express Thyroid transcription factor-1 (TTF-1), napsin, p63, cytokeratin 14 (CK14), synaptophysin, CD56, carcinoembryonic antigen (CEA), lymphatic endothelial marker (D2-40), calretinin or Wilms’s tumor-1 (WT-1). The tumor was diagnosed as a large cell carcinoma. The tumor in the lower left lobe was composed of solid cell nests and cribriform structures differentiate into squamous cells. The luminal surface of the cribriform structures stained positively with Alcian blue-PAS staining, with intracytoplasmic mucin, even though they did not express TTF-1 or napsin. Consequently, the tumor was diagnosed as adenosquamous carcinoma. These pathological features suggest that both of the tumors were primary lung cancers.

Lung specimens without occupational dust exposure were analyzed in this study as a control lung. The patient was a 74 year-old woman who had undergone partial resection of the lower right lung by video-assisted thoracic surgery for early stage (STAGE IA) adenocarcinoma. She was a housekeeper with no history of smoking or occupational dust exposure. A lesion specimen from...
the healthy lung specimen was used for PIXE analysis. The surgically obtained paraffin embedded tissue specimens were observed using a polarized-light microscope (ECLIPSE LV100 microscope, Nikon, Tokyo, Japan). As shown in Fig. 1, a polycarbonate membrane was placed on a glass slide and 15 µm of a paraffin embedded tissue specimen was placed and attached to the polycarbonate membrane for preparing samples of PIXE analysis. Then, the tissue specimen along with the polycarbonate membrane was cut to a diameter of approximately 10 mm. The sample was roughly trimmed to a small size (<10 mm) and placed on a circular acrylic-holder with a pinhole at the center and fixed with epoxy adhesive. In PIXE, a proton ion beam passes through the pinhole to the tissue. Deparaffinization or other specific treatments were not required prior to PIXE analysis. This study was conducted in accordance with the Declaration of Helsinki and was approved by the Gunma University Hospital Institutional Review Board.

**In-air micro PIXE analysis**

In-air micro-PIXE was developed to obtain distribution maps of characteristic X-rays from elements as described previously [5, 6]. In in-air PIXE analysis, an external proton beam through thin polycarbonate film is irradiated onto the sample in an atmosphere. In our study, the elemental map of S which was distributed almost uniformly throughout the tissue was used to identify the shape of the cells and to demonstrate surfactant. The Mg, Si, Al and Fe to S ratios were used to compare the inhaled atomic elements [11].

**Immunohistochemistry**

All sections were incubated at 60 °C for 30 min and deparaffinized in xylene, rehydrated, and incubated with fresh 0.3 % hydrogen peroxide in 100 % methanol for 30 min at room temperature to block endogenous peroxidase activity. After rehydration by means of a graded series of ethanol, antigen retrieval was performed with the ImmunoSaver antigen retriever system (Electron Microscopic Sciences, Hattsfield, PA, USA) at 98–100 °C for 30 min, and sections were subsequently passively cooled to room temperature. After the sections had been rinsed in 0.1 M phosphate-buffered saline (pH 7.4), nonspecific binding sites were blocked by incubation with protein block serum-free reagent (Dako, Carpinteria, CA, USA) for 30 min. The sections were then incubated with anti CD163 antibodies in a 1:100 dilution (Novocastra, Leica Biosystems Inc., Richmond, USA) as described previously [12], anti SMA (Dako, Carpinteria, CA, USA) [13], anti-vimentin (Cell Signaling Technology, Danvers, MA, USA) and anti β-catenin (Carboxy-terminal, Cell Signaling Technology) antibodies at a dilution of 1:400 overnight at 4 °C and incubated with secondary antibodies at room temperature for 30 min, respectively. The reactions were visualized with a Histofine Simple Stain MAX PO (Multi) kit (Nichirei Biosciences, Tokyo, Japan) according to the
manufacture’s instructions. The chromogen 3,3′-diaminobenzidine tetrahydrochloride was applied as a 0.02 % solution in 50 mM ammonium acetate–citric acid buffer (pH 6.0) containing 0.005 % hydrogen peroxide. The sections were lightly counterstained with hematoxylin and mounted.

Statistical analysis

Statistical analyses were performed using the GraphPad Prism software program version 6 (GraphPad Software, San Diego, CA, USA). The amount of each element in the lung parenchyma and the lymph node were calculated and analyzed by Bonferroni’s multiple comparison test after one-way ANOVA.

Results

A 76 year-old patient who had double primary lung cancers with pleural thickness and calcification (Fig. 2a–c) was enrolled in this study. Figure 2d shows the representative X-ray spectrum of the right and left lung as well as the right hilar lymph node obtained from in-air micro-PIXE. The elemental peaks in the right hilar lymph node seemed to be higher than those in the lung parenchyma. A 2-dimensional elemental map of each element showed that Al, Fe, Mg and Si were co-localized in the right hilar lymph node but not in the lung parenchyma (Fig. 3a). To compare the relative ratio of each element in the lung parenchyma and the right hilar lymph node, we analyzed the elements relative to S using an in-air micro-PIXE analysis. The amount of Si in the lymph node was significantly higher than the amount in the right or left lung parenchyma (Fig. 3b). The amount of Mg and Fe in the right hilar lymph node was significantly higher than the amount in the left lung parenchyma. The amount of Al in the lymph node was found to be 3 times higher than that in the right or left lung parenchyma. However, the differences were not significant. These results indicated that the relocated inhaled fibers were drained by the lymph ducts and aggregated in the lymph nodes. In this study, we also examined elemental analysis in the lung specimens without occupational dust exposure as a control and very low levels

Fig. 2 Double primary lung cancers with asbestosis and representative images of the elemental analysis by micro-PIXE. A 76-years-old man was exposed to asbestos over the 15 years that he worked at a shipyard. He had pleural plaques with calcification (white arrowheads) (a) and diagnosed with asbestosis without fibrosis. Three years after diagnosis, a right upper lobectomy with right hilar lymph dissection for primary large-cell lung cancer (b) was conducted. The following year, another primary adenosquamous lung cancer in the opposite left lobe (c) was found. Subsequently, a simple left lower segmental resection was performed without lymph node dissection. Representative images of the elemental analysis of both the lungs and the hilar lymph node using in-air micro-PIXE (d)
of Fe and Si were detected by PIXE analysis (Fig. 3b). The elements of Mg and Al in the control lung tissue sample were under detection level by PIXE, indicating that these elements might not be distributed in the control lung tissue sample more than at least pg [14].

Since a polarized microscope showed that small fibers were visible in the hilar lymph node, we examined the role of the macrophages for transporting inhaled fibers. Figure 4a shows the immunohistochemical analysis of the anti-CD163 antibodies, a potent surface marker of macrophages. The analysis showed a strong staining pattern in the right hilar lymph node, but a weak pattern in the lung parenchyma. Figure 4a shows the co-localization between inhaled polarized fibers and macrophages. The high-power field image showed aggregation of macrophages around inhaled fibers (Fig. 4b).

Although asbestos-induced lung cancer is different from that caused by tobacco smoke [15], the mechanism of carcinogenesis with asbestos exposure remains unclear. The patient may have developed these lung cancers because of two factors, occupational asbestos exposure and cigarette smoking since smoking may have increased the risk of lung cancer induced by asbestos exposure [16]. Recently, EMT is thought to be implicated in carcinogenesis and fibrosis caused by asbestos exposure in vitro [9]. Since the asbestos exposure in this case occurred over 40 years prior to this investigation, we also examined the presence of SMA which is a marker of EMT to determine the effects of long-term, latent exposure to inhaled fibers. Immunohistochemistry using anti-SMA antibodies demonstrated the presence of SMA with the inhaled fibers in the lung parenchyma and right hilar lymph node.
Since high-power field imaging showed SMA expression around the inhaled fibers in the right hilar lymph node (Fig. 5b), we examined the expression of vimentin and β-catenin as EMT markers. Vimentin and β-catenin were also expressed around inhaled fibers in the hilar lymph node similar with SMA expression.

**Discussion**

This study supports research by Dodson et al. indicating that asbestos fibers are found more often in the thoracic lymph nodes than the lung tissues after occupational [1] as well as non-occupational exposures [17]. The analyses conducted by Dodson et al. used electron microscopy to measure the asbestos fibers in tissues and showed that the relative ratio of asbestos fibers in the thoracic lymph nodes was about 10 times higher than that in the lung tissues. Our results are consistent with previous reports and suggest that in-air micro-PIXE is useful for examining the distribution of inhaled fibers. It was difficult to observe asbestos bodies in the hematoxylin and eosin stained sections of the lungs and hilar lymph node. Dodson et al. reported that the length of the asbestos fibers accumulating in the lymph nodes is less than 5 µm; thus, asbestos bodies are not seen in most cases [18, 19]. Consistent with this, while most of the fibers observed in the hilar lymph node using polarized light microscopy were less than 5 µm, some fibers were almost 5 µm in length (Fig. 5). Fibers in lungs may be drained into the lymphatic nodes, and the decrease in the concentration of fibers in lungs and the shortening of fibers in lymph nodes might occur over a period [17].

Scanning electron microscopy (SEM) or transmission electron microscopy (TEM) with energy dispersive X-ray spectroscopy (EDX) requires an electron beam, which is lighter than a proton microbeam, and these methods result in greater background noise and lesser sensitivity than PIXE analysis [20, 21]. Destructive analytical methods such as lung digestion are thought to overlook and not to detect fibers <0.2 µm diameter [22]. Previous studies compared elemental analyses of brain tissue by PIXE and SEM. In the comparison, SEM with EDX analysis could not detect the peak of Fe, Cu, and Zn levels, while PIXE could detect these elemental peaks, indicating that PIXE has more elemental analysis sensitivity for human tissues [23]. Therefore, PIXE analysis is useful for the analysis of asbestosis as well as SEM or TEM with EDX analysis. The in-air micro-PIXE technique is also beneficial because the

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**Fig. 4** Localization of inhaled fibers and macrophages in the lungs and lymph node. Co-localization of inhaled fibers with macrophages (a). The lower column shows high-power field images from the polarized microscope and the anti-CD163 staining of the hilar lymph node (b). White arrowheads indicate polarized elements. Black arrowheads indicate co-localized anti-CD163 antibodies.
Fig. 5 Localization of inhaled fibers and EMT markers in the lungs and lymph node. Co-localization of inhaled fibers with SMA (a). High-power field images from the polarized microscope and anti-SMA antibody staining of the hilar lymph node (b). The staining of EMT markers, vimentin and β-catenin, in the hilar lymph node (c). White arrowheads indicate polarized elements. Black arrowheads indicate SMA, vimentin, and β-catenin expression around inhaled fibers, respectively.
sample can be analyzed for the spatial–temporal elemental distribution and it does not damage the tissue sample. Elemental analysis showed that light elements such as Mg tend to be visualized diffusely throughout the tissue in a two-dimensional mapping. Although the intensity of the green image of the upper column was stronger than that of the green image in the lower column in Fig. 3a, Mg was visualized diffusely in the lymph node. It is believed that the appearance of background color for light elements is due to the Bremsstrahlung effect produced when ion-beams pass through matter rapidly.

Furthermore, the preparation of paraffin-embedded samples for an in-air micro-PIXE analysis is simple and easy. While PIXE analysis has the benefit of sensitivity for elemental analysis, the equipment involves a powerful proton ion beam, which is very expensive. If the cost of the powerful ion beam equipment can be reduced, PIXE can become a more familiar and useful elemental analysis method for investigating environmental health. This may be achieved by a progress in the study of PIXE by more researchers to elucidate the environmental disorders.

The sensitivity of PIXE analysis using standard reference material in the macroporous cation-exchange resin (Macro-Prep 25S) was previously determined [14]. When 270 parts per million by volume (ppmv) of Al-containing or 135 ppmv of Fe-containing resin was analyzed, PIXE detected $100 \pm 16$ count or $33 \pm 11$ count/pg with a nanoclone beam irradiation, respectively, suggesting that PIXE analysis can detect Al or Fe to have one pg element in the tissue. Although we have not determined the sensitivity of Si and Mg using Macro-Prep 25S by PIXE, the sensitivity of other elements such as S, Ca, Mn, and Co was found to be similar to the sensitivity of Al or Fe. Therefore, detection limit of Si and Mg might be also similar to that of Al or Fe.

The presence of aluminium in lung parenchyma and hilar lymph node was detected by PIXE analysis in this study. It has been reported that exposure to aluminium from welding fumes induces pulmonary aluminosis [24, 25], and the inhalation of aluminium into lung parenchyma was revealed by PIXE analysis in our previous study [26]. Therefore, the presence of aluminium in this case may be due to exposure to welding fumes since asbestos does not contain aluminium. Since this case had a history of occupational asbestos exposure and bilateral pleural calcification, the results of elemental analysis by PIXE detecting Mg, Si and Fe may indicate the inhalation of asbestos into the lungs.

The lymphatic system is an important route for asbestos relocation to the extrathoracic and intrathoracic organs, where the asbestos content of the hilar lymph nodes can exceed that found in the lungs by 3–40 times [27, 28]. Shorter fibers can be phagocytosed by alveolar macrophages and removed by ciliated epithelium in the proximal airways [29]. Asbestos accumulation in guinea pig macrophages could still be seen 2 years after a 6-week exposure to amosite [30]. Similarly, our results indicated that macrophages were accumulated and co-localized with inhaled fibers in the hilar lymph nodes suggesting that macrophage migration does act as a mechanism to transport small inhaled fibers from the lung parenchyma to the hilar lymph nodes. Interestingly, the inhaled fibers were still co-localized with the macrophages as much as 40 years post-asbestos exposure.

An immunohistochemical analysis in rats with asbestos-induced pulmonary fibrosis showed that macrophages first accumulated at the specific locations where fibers are initially deposited and subsequently, result in SMA expression in the adjacent developing lesions [10]. Consistent with these findings, the immunohistochemical analysis in our study also demonstrated that macrophages and SMA were accumulated in the right hilar lymph node where small inhaled fibers were translocated in the human body. The results indicate that inhaled fibers may be translocated from the lung parenchyma to the thoracic lymph nodes. The inhaled fibers may be also involved with the expression of EMT markers such as SMA, vimentin, and β-catenin indicating EMT processes in local tissues. Previous studies reported an increased risk of non-small-cell lung cancer in patients with asbestosis than in those exposed to asbestos but do not having asbestosis [31]. This case had lung cancers without evident pulmonary fibrosis, indicating that history of heavy smoking rather than occupational asbestos exposure causes carcinogenesis. Maeda et al. reported that continuous exposure of T-cells to asbestos exhibited enhanced binding of vimentin to asbestos [32]. Consistently, our results showed that hilar lymph node accumulated the elements of Mg, Fe, Si, and Al along with highly expressed vimentin and SMA around fibers visualized by polarized microscope. Vimentin and SMA are well known EMT markers. The development of EMT is a key factor in the pathogenesis of cancer and pulmonary fibrosis [7]. Therefore, the EMT, in this case, may accelerate carcinogenesis by increasing vimentin and SMA expression in addition to cigarette smoking. However, further studies are needed to examine the relationship between EMT processes and carcinogenesis after asbestos exposure.

In summary, more small inhaled fibers were found in the hilar lymph node rather than the lung parenchyma in a patient exposed to asbestos. These fibers were co-localized with macrophages even after a long latency period. Inhaled fibers were similarly co-localized with SMA expression in both the lung parenchyma and the hilar lymph node. This study extends and supports previous research that used electron microscopes to examine the amount of inhaled fibers in various organs, and shows that in-air micro-PIXE
may be useful for estimating the distribution of inhaled fibers in various organs.

**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

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**References**

1. Dodson RF, Williams MG Jr, Corn CJ, Brollo A, Bianchi C. Asbestos content of lung tissue, lymph nodes, and pleural plaques from former shipyard workers. Am Rev Respir Dis. 1990;142:843–7.

2. Dumortier P, De Vuyst P, Yernault JC. Comparative analysis of inhaled particles contained in human bronchoalveolar lavage fluids, lung parenchyma and lymph nodes. Environ Health Perspect. 1994;102(Suppl 5):257–9.

3. Cox-Ganser JM, Burchfiel CM, Fekedulegn D, Andrew ME, Ljutic D, Filipovic T, et al. The interstitial expression of alpha-smooth muscle actin in glomerulonephritis is associated with renal function. Med Sci Monit. 2012;18:CR235–40.

4. Ishida M, Teshima K, Kusunoki S, Aoki T, Takahashi K, et al. Distribution of asbestos bodies and calcium in the lung tissue of asbestos-exposed individuals. J Occup Med. 1988;30:151–4.

5. Shimizu Y, Matsuzaki S, Satoh T, Koka M, Yokoyama A, Ohkubo T, et al. In-air microparticle induced X-ray emission analysis of asbestos and metals in lung tissue. Int J Immunopath Pharmacol. 2008;21:567–76.

6. Shimizu Y, Matsuzaki S, Satoh T, Koka M, Yokoyama A, Ohkubo T, et al. In-air microparticle induced X-ray emission analysis of particles in interstitial pneumonia lung tissue obtained by transbronchial biopsy. J Clin Biochem Nutr. 2011;49:125–30.

7. Kamp DW. Asbestos-induced lung diseases: an update. Transl Res. 2009;153:143–52.

8. Chen T, Nie H, Gao X, Yang J, Pu J, Chen Z, et al. Epithelial-mesenchymal transition involved in pulmonary fibrosis induced by multi-walled carbon nanotubes via TGF-beta-Smad signaling pathway. Toxicol Lett. 2014;226:150–62.

9. Tamminen JA, Myllariemi M, Hyytiainen M, Keski-Oja J, Koli K. Asbestos exposure induces alveolar epithelial cell plasticity through MAPK/Erk signaling. J Cell Biochem. 2012;113:2234–47.

10. Perdue TD, Brody AR. Distribution of transforming growth factor-beta 1, fibronectin, and smooth muscle actin in asbestos-induced pulmonary fibrosis in rats. J Histochem Cytochem. 1994;42:1061–70.

11. Kitamura H, Ichinose S, Hosoya T, Ando T, Ikushima S, Oritsu M, et al. Inhalation of inorganic particles as a risk factor for idiopathic pulmonary fibrosis–elemental microanalysis of pulmonary lymph nodes obtained at autopsy cases. Pathol Res Pract. 2007;203:575–85.

12. Matsuzaki S, Shimizu Y, Dobashi K, Nagamine T, Satoh T, Ohkubo T, et al. Analysis on the co-localization of asbestos bodies and Fas or CD163 expression in asbestos lung tissue by in-air micro-PIXE. Int J Immunopathol Pharmacol. 2010;23:1–11.

13. Saratija Novakovic Z, Glavina Durdev M, Puljak L, Saraga M, Ljutic D, Filipovic T, et al. The interstitial expression of alpha-smooth muscle actin in glomerulonephritis is associated with renal function. Med Sci Monit. 2012;18:CR235–40.

14. Iwata YYN, Kitamura A, Koka M, Satoh T, Kamiya T. Calibration of several detectors on micro beam PIXE system in TIARA by standard reference material. JAEA Rev. 2014;2014–050:99.

15. Roggli VL, Sanders LL. Asbestos content of lung tissue and carcinoma of the lung: a clinicopathologic correlation and mineral fiber analysis of 234 cases. Ann Occup Hyg. 2000;44:109–17.

16. Selikoff IJ, Hammond EC, Churg J. Asbestos exposure, smoking, and neoplasm. JAMA. 1968;204:106–12.

17. Dodson RF, Huang J, Bruce JR. Asbestos content in the lymph nodes of nonoccupationally exposed individuals. Am J Ind Med. 2000;37:169–74.

18. Dodson RF, O’Sullivan MF, Huang J, Holiday DB, Hammarg SP. Asbestos in extrapulmonary sites: omentum and mesentery. Chest. 2000;117:486–93.

19. Dodson RF, Shepherd S, Levin J, Hammarg SP. Characteristics of asbestos concentration in lung as compared to asbestos concentration in various levels of lymph nodes that collect drainage from the lung. Ultrastruct Pathol. 2007;31:95–133.

20. Greshake A. The primitive matrix components of the unique carbonaceous chondrite Acfer 994: a TEM study. Geochim Cosmochim Acta. 1997;61:437–52.

21. Kuisma-Kursula P. Accuracy, precision and detection limits of SEM-WDS, SEM-EDS and PIXE in the multi-elemental analysis of medieval glass. X-Ray Spectrom. 2000;29:111–8.

22. Abraham JL, Hunt A, Burnett BR. Quantification of non-fibrous and fibrous particulates in human lungs: twenty year update on pneumoconiosis database. Ann Occup Hyg. 2002;46:397–401.

23. Johansson SAE, Campbell JL, Malmquist KG. Particle-induced X-ray emission spectrometry (PIXE). New York: Wiley; 1995. p. 14–5.

24. Miran B. Intestinal pneumonitis after acetylene welding: a case report. Int J Occup Med Environ Health. 2014;27:132–6.

25. Smolkova P, Nakladalova M. The etiology of occupational pulmonary aluminosis—the past and the present. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2014;158:535–8.

26. Chino H, Aoki T, Goto T, Koga Y, Hisada T, et al. Pulmonary aluminosis diagnosed with in-air microparticle induced X-ray emission analysis of particles. Intern Med. 2015;54:2035–40.

27. Tossavainen A, Karjalainen A, Karhunen PJ. Retention of asbestos fibers in the human body. Environ Health Perspect. 1994;102(Suppl 5):253–5.

28. Dodson RF, Williams MG Jr, Corn CJ, Brollo A, Bianchi C. A comparison of asbestos burden in lung parenchyma, lymph nodes, and plaques. Ann NY Acad Sci. 1991;643:53–60.

29. Ober dorster G. Toxicokinetics and effects of fibrous and non-fibrous particles. Inhal Toxicol. 2002;14:29–56.

30. Sjostrand M, Rylander R, Bergstrom R. Lung cell reactions in shipyard workers. Am Rev Respir Dis. 1990;142:843–7.

31. O’Reilly KMA, Mclaughlin AM, Beckett WS, Sime PJ. Asbestos-related lung disease. Am Fam Physician. 2007;75(5):683–8.

32. Maeda M, Chen Y, Kumagai-Takei N, Hayashi H, Matsuzaki H, Lee S, et al. Alteration of cytoskeletal molecules in a human T cell line caused by continuous exposure to chrysotile asbestos. Immunobiology. 2013;218:1184–91.