Review

Elemental analysis of occupational and environmental lung diseases by electron probe microanalyzer with wavelength dispersive spectrometer

Toshinori Takadaa,*, Hiroshi Moriyamaa, Eiichi Suzukib

aDivision of Respiratory Medicine, Graduate School of Medical and Dental Sciences, Niigata University, Niigata, Japan
bDepartment of General Medicine, Niigata University Medical and Dental Hospital, Niigata, Japan

Abstract

Occupational and environmental lung diseases are a group of pulmonary disorders caused by inhalation of harmful particles, mists, vapors or gases. Mineralogical analysis is not generally required in the diagnosis of most cases of these diseases. Apart from minerals that are encountered rarely or only in specific occupations, small quantities of mineral dusts are present in the healthy lung. As such when mineralogical analysis is required, quantitative or semi-quantitative methods must be employed. An electron probe microanalyzer with wavelength dispersive spectrometer (EPMA–WDS) enables analysis of human lung tissue for deposits of elements by both qualitative and semi-quantitative methods. Since 1993, we have analyzed 162 cases of suspected occupational and environmental lung diseases using an EPMA–WDS. Our institute has been accepting online requests for elemental analysis of lung tissue samples by EPMA–WDS since January 2011. Hard metal lung disease is an occupational interstitial lung disease that primarily affects workers exposed to the dust of tungsten carbide. The characteristic pathological findings of the disease are giant cell interstitial pneumonia (GIP) with centrilobular fibrosis, surrounded by mild alveolitis with giant cells within the alveolar space. EPMA–WDS analysis of biopsied lung tissue from patients with GIP has demonstrated that tungsten and/or cobalt is distributed in the giant cells and centrilobular fibrosing lesion in GIP. Pneumoconiosis, caused by amorphous silica, and acute interstitial pneumonia, associated with the giant tsunami, were also elementally analyzed by EPMA–WDS. The results suggest that commonly found elements, such as silicon, aluminum, and iron, may cause occupational and environmental lung diseases.

© 2013 The Japanese Respiratory Society. Published by Elsevier B.V. All rights reserved.

Abbreviations: EPMA, electron probe microanalyzer; WDS, wavelength dispersive spectrometer; TBBs, transbronchial biopsies; EDS, energy dispersive spectrometers; HE, hematoxylin and eosin; GIP, giant cell interstitial pneumonia; HRCT, high-resolution CT; BALF, bronchoalveolar lavage fluid; UIP, usual interstitial pneumonia

*Correspondence to: Division of Respiratory Medicine, Graduate School of Medical and Dental Sciences, Niigata University, 1-757 Asahimachi-dori, Chuo-ku, Niigata 951-8510, Japan. Tel.: +81 252272200; fax: +81 252270775.
E-mail addresses: ttakada@med.niigata-u.ac.jp (T. Takada), hiroshim@med.niigata-u.ac.jp (H. Moriyama), eiichi@med.niigata-u.ac.jp (E. Suzuki).

2212-5345/$ – see front matter © 2013 The Japanese Respiratory Society. Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.resinv.2013.05.002
Contents

1. Introduction ............................................................................................................... 6
2. Analytical methods for mineral particle analysis of lung tissue . ......................... 6
   2.1. Pathologic examination ................................................................................... 6
   2.2. Elemental analysis of human lung tissue .......................................................... 7
3. Application of EPMA–WDS analysis to human lung tissue ............................... 7
   3.1. EPMA–WDS procedure for analysis for human lung tissue ......................... 7
   3.2. Summary of elemental analysis of occupational and environmental lung diseases ........................................................................................................ 8
   3.3. Online inquiries for EPMA–WDS elemental analysis of lung tissue .......... 8
4. Hard metal lung disease ......................................................................................... 9
   4.1. Exposure to hard metal .................................................................................. 9
   4.2. Clinical presentation ...................................................................................... 10
   4.3. Pathology and elemental analysis .................................................................. 10
   4.4. Diagnostic evaluation .................................................................................... 10
5. Other occupational and environmental lung diseases ........................................ 12
   5.1. Pneumoconiosis due to amorphous silica ...................................................... 12
   5.2. Acute interstitial pneumonia associated with the 2011 earthquake disaster .... 12
6. Conclusions ........................................................................................................... 12
Conflict of interest ..................................................................................................... 13
Acknowledgments ....................................................................................................... 13
References .................................................................................................................... 13

1. Introduction

Occupational and environmental lung diseases are a group of pulmonary disorders caused by inhalation of harmful particles, mists, vapors or gases. Occupational lung diseases, caused by exposure to harmful substances in the work place, are classified into two categories: diseases that are not occupation-specific, such as occupational asthma, and diseases related to a specific occupation, such as asbestosis, coal worker’s pneumoconiosis, berylliosis, and farmer’s lung. Lung diseases related to specific occupations usually develop slowly, are associated with exposure to toxic dust (such as asbestos and silica) over a period of 10–20 years, and can lead to interstitial lung disease and severe lung fibrosis. Pneumoconiosis, a type of interstitial lung disease, is caused by inhaling inorganic dusts, particularly coal dust [1]. Diagnosis of pneumoconiosis is relatively simple and is based on chest radiographs, pulmonary function tests, and the occupational history of the patient. Mineralogical analysis is not generally required in the diagnosis of most cases of occupational and environmental lung diseases. However, when mineralogical analysis is required, quantitative and semi-quantitative methods must be employed, as the lungs of even healthy individuals contain small quantities of mineral dust deposits, with the exception of those minerals and elements that are rarely or only encountered in specific occupations. For example, tungsten and cobalt are only observed within the lungs of subjects who have been exposed to hard metals [2,3]. Since the techniques of mineralogical analysis are time-consuming and expensive, they have been mostly used as research procedures only.

An electron probe microanalyzer (EPMA) is an analytical tool used to non-destructively determine the chemical composition of small volumes of solid materials. It works in a similar manner to a scanning electron microscope. When the sample is bombarded with an electron beam, x-rays generated from elements within the sample are detected by an electron microprobe. The wavelengths of the emitted x-rays are characteristic to the elements that are being analyzed. EPMA with a wavelength dispersive spectrometer (EPMA–WDS) has been extensively used in the field of material sciences. We have utilized EPMA–WDS to analyze human lung tissue in both qualitative and semi-quantitative ways to create element distribution maps [4]. In this review, we discuss the clinical application of EPMA–WDS as a promising technique for mineralogical analysis of lung samples from patients with occupational and environmental lung diseases.

2. Analytical methods for mineral particle analysis of lung tissue

2.1. Pathologic examination

Histologic examination is the basic pathologic tool for classifying occupational and environmental lung diseases. Dust resides in the lungs of all individuals, particularly smokers and those living in polluted environments. It is typically observed around the small airways, blood vessels, and in the subpleural and interlobular connective tissue. However, in individuals with occupational and environmental dust exposure, fairly large numbers of particles are observed in these locations, as well as in the alveolar spaces, and the alveolar interstitium. Mineralogical analysis is not generally required in the diagnosis of most cases of occupational and environmental lung diseases, as simple light microscopic observation and polarization is sufficient.

A surgical lung biopsy is a procedure to remove samples of lung tissue for examination under a microscope and mineralogical analysis. Although transbronchial biopsies (TBBs) may also be used for pathologic examination and elemental analysis, they have limited value to the pathologists particularly when dealing with suspected cases of occupational and environmental lung diseases. TBBs usually contain the peripheral connective tissues, which are a common repository
for inhaled dust [5,6]. Thus, if TBBs are used for elemental analysis, detection of elements may be falsely negative owing to the small sample size and the uneven distribution of the deposited dust.

2.2. Elemental analysis of human lung tissue

Various techniques of elemental analysis of human lung tissue have been described (Table 1). Liquid analysis includes atomic absorption spectrometry, plasma optical emission mass spectrometry, and ionic-coupled plasma emission spectrometry [7,8]. These techniques can detect elements in dissolved tissue solution, but cannot correlate the anatomical relationship between elements and the lung architecture, because the sample is destroyed by digestion or ashing. In contrast, solid analysis uses thick or thin sections of specimens without tissue destruction.

EPMA was developed in 1947 to carry out nondestructive elemental analysis at resolutions approaching those of the transmission electron microscope. The technique has found wide application in mineralogy, metallurgy, and solid-state science, as well as in the fields of clinical and life sciences. When combined with energy dispersive spectrometers (EDS), EPMA can simultaneously analyze all elements and map chemical elements in lung tissue with a very high resolution [2,3,9]. EPMA–WDS has been widely used to provide element maps with a spatial resolution in the order of 1 μm, and WDS is almost 10 times more sensitive than EDS for all elements [10]. However, most studies that have analyzed organic samples by EPMA used EDS, as the high temperatures generated by the stronger beam used in WDS to detect trace amounts of elements resulted in burning the tissue sample. In order to overcome these shortcomings, Watanabe et al. developed an improved EPMA–WDS technique for element analysis of 2-μm tissue sections [11].

3. Application of EPMA–WDS analysis to human lung tissue

3.1. EPMA–WDS procedure for analysis for human lung tissue

Lung tissues are inflated, fixed with formalin, and then embedded in pure paraffin. 3 μm sections are floated on water drops on carbon blocks. By quickly removing the water with filter paper and completely drying, the tissue section is strongly attached to the carbon block. Paraffin is thoroughly removed by xylene and the sample is coated with carbon evaporation films. Using a smoothly polished carbon block to support a thin section prevents excessive rises in temperature. As a result, this allows the sample to be irradiated with an electron beam of 0.6 mA at 20 kV, which is 10 times stronger than is usually used. This allows sufficient x-ray signals to be emitted from trace amounts of atoms in the specimen without causing serious thermal damage. Background x-rays emitted from the carbon support have the lowest wavelength since carbon is the lightest element.

Serial sections of the paraffin embedded sample can be used for EPMA–WDS analysis and simultaneous pathological examination. For pathological analysis, we use the conventional hematoxylin and eosin (HE) stain as well as detecting collagen

Table 1 – Techniques of elemental analysis of human tissues.

<table>
<thead>
<tr>
<th>Method</th>
<th>Applications and limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid analysis</td>
<td>Sample generally destroyed, localization of particles not usually possible</td>
</tr>
<tr>
<td>Atomic absorption spectrometry</td>
<td>Very sensitive for trace elements, but not all elements entifable</td>
</tr>
<tr>
<td>Plasma optical emission mass spectrometry</td>
<td>Tissue section can be used, localization of specific particles theoretically possible</td>
</tr>
<tr>
<td>Ionic-coupled plasma emission spectrometry</td>
<td>Equipment available in many labs, very high resolution and simultaneous analysis of all elements</td>
</tr>
<tr>
<td>Solid analysis</td>
<td></td>
</tr>
<tr>
<td>EPMA–EDS</td>
<td></td>
</tr>
<tr>
<td>EPMA–WDS</td>
<td>Ten times more sensitive than EDS, can yield a map of element distribution</td>
</tr>
</tbody>
</table>

Abbreviation: EPMA, electron probe microanalyzer, EDS, energy dispersive spectrometer and WDS, wavelength dispersive spectrometer.
and other connective tissue by the elastica van Gieson method. This enables us to observe the distribution of elements within the tissue structure by comparing the images of the histopathology of the tissue with the EPMA–WDS images (Fig. 1).

3.2. Summary of elemental analysis of occupational and environmental lung diseases

We have analyzed 162 suspected cases of occupational and environmental lung diseases since 1993 using EPMA–WDS (Table 2). Two thirds of the cases were from institutes of other prefectures (provinces of Japan) than Niigata, and two cases were from the United States of America. Diagnoses of the cases analyzed by EPMA–WDS are summarized in Table 3. When tungsten and/or cobalt were detected, a diagnosis of hard metal lung disease was made, as these elements are only found in the lung tissues of patients who have worked in the hard metal industry. It should be noted that in more than half of the patients, we were unable to make a definite diagnosis, even using EPMA–WDS. In addition to silicon, aluminum, and iron, which are commonly found in the lung tissue, we also detected minor elements, including barium, copper, antimony, vanadium, gold, zirconium, indium, cerium, lanthanum, lead, arsenic, and neodymium. Inhalation of some of these minor elements is associated with occupational and environmental lung diseases [12].

3.3. Online inquiries for EPMA–WDS elemental analysis of lung tissue

We accepted inquiries for elemental analysis of lung tissue by EPMA–WDS until 2010 by personal communication. In January 2011, we opened an online inquiry website, in Japanese and English, on the homepage of the Internal Medicine II, Niigata University Medical School. Researchers and clinicians, who are interested in elemental analysis of lung tissue by EPMA–WDS, can access the following website for more information: (http://www.med.niigata-u.ac.jp/in2/) (Fig. 2). After submitting an online form, a follow-up e-mail reply is sent within 5 business days. In the future, we plan to provide a webpage containing information on hard metal lung disease and related diseases.

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of cases</th>
<th>Institutes</th>
<th></th>
<th></th>
<th>USA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ours</td>
<td>Niigata prefecture</td>
<td>Other prefectures</td>
<td>USA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before 1999</td>
<td>9</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td>6</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>2001</td>
<td>7</td>
<td>0</td>
<td>4</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>2002</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>2003</td>
<td>9</td>
<td>1</td>
<td>2</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>2004</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>2005</td>
<td>17</td>
<td>2</td>
<td>2</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>2006</td>
<td>12</td>
<td>5</td>
<td>2</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>2007</td>
<td>17</td>
<td>3</td>
<td>3</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>2008</td>
<td>13</td>
<td>1</td>
<td>4</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>2009</td>
<td>19</td>
<td>1</td>
<td>1</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>2010</td>
<td>18</td>
<td>0</td>
<td>0</td>
<td>17</td>
<td>1</td>
</tr>
<tr>
<td>2011</td>
<td>15</td>
<td>0</td>
<td>4</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>2012</td>
<td>10</td>
<td>0</td>
<td>3</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>162</td>
<td>19</td>
<td>33</td>
<td>108</td>
<td>2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Occupational lung diseases</td>
<td></td>
</tr>
<tr>
<td>Hard metal lung diseases</td>
<td>37</td>
</tr>
<tr>
<td>Aluminum lung</td>
<td>12</td>
</tr>
<tr>
<td>Pneumoconiosis or related diseases</td>
<td>7</td>
</tr>
<tr>
<td>Silicosis</td>
<td>4</td>
</tr>
<tr>
<td>Welder’s lung</td>
<td>2</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td></td>
</tr>
<tr>
<td>Interstitial pneumonia with occupational history</td>
<td>45</td>
</tr>
<tr>
<td>Sarcoidosis or related diseases</td>
<td>5</td>
</tr>
<tr>
<td>Pulmonary alveolar proteinosis or related diseases</td>
<td>4</td>
</tr>
<tr>
<td>Hypersensitivity pneumonitis suspected</td>
<td>4</td>
</tr>
<tr>
<td>Others</td>
<td>42</td>
</tr>
<tr>
<td>Total</td>
<td>162</td>
</tr>
</tbody>
</table>
4. Hard metal lung disease

Hard metal lung disease is an occupational interstitial lung disease that primarily affects workers exposed to dust from the hard metal tungsten carbide. The pathology of hard metal lung disease includes a pattern of giant cell interstitial pneumonia (GIP) [9,13,14]. Liebow originally classified GIP as one of the idiopathic interstitial pneumonias [15], but it is now recognized that GIP is pathognomonic for hard metal lung disease [3].

4.1. Exposure to hard metal

Hard metal, or tungsten carbide, is a synthetic compound that is produced by combining tungsten and carbon using cobalt as a binder. The proportion of cobalt varies between 5% and 25% by weight, with a higher percentage of cobalt yielding a harder product. Tungsten carbide is almost as hard as a diamond, and thus, it is used to make machine parts that require high temperature resistance, as well as in the manufacture of tools used for drilling, cutting, machining, or grinding. The main occupational sources of exposure to hard metal include the various stages of hard metal production, the maintenance and sharpening of hard metal tools and blades, and the use of hard metal tools [16]. Diamond tools are also used to cut stones, marble, glass, and to grind or polish various materials. Although diamond tools are not composed of “hard metal” as they contain only cobalt, the manufacture of diamond tools and their use, for example, during diamond polishing by high-speed cobalt diamond disks, could lead to the same pathology of GIP as seen in hard metal lung disease [17–19].

Patients with hard metal lung disease usually have a mean exposure time to hard metal dust of more than 10 years, ranging from 2.5 to 30 years [4]. The disease may also occur after a shorter exposure time, which suggests that host susceptibility factors are also important in determining the development and the severity of the disease [20]. However, a history of exposure to hard metal dust may not be apparent in some cases. For example, office clerks working in a room next to a poorly air-conditioned hard metal factory may be exposed to hard metal dusts and develop hard metal lung disease. Some patients with GIP are unaware of such exposures, while others may have no history of exposure. A case
report from India described an office sweeper with GIP but no history of hard metal exposure [7]. In addition, the lung tissue of a 15-year-old boy with GIP, who may have been exposed to hard metal via his parents who were occupationally exposed to hard metal, was negative for tungsten and cobalt [21].

4.2. Clinical presentation

Patients exposed to hard metal may develop occupational asthma, a syndrome resembling hypersensitivity pneumonitis, and/or interstitial lung disease, which is generally recognized as hard metal lung disease. In a typical case of hard metal lung disease, respiratory symptoms, including dry cough and shortness of breath, appear within several months to years after exposure to hard metal. These symptoms improve on holidays and exacerbate during workdays, similar in some cases to hypersensitivity pneumonitis. Sometimes, during a physical examination, fine crackles are identified during auscultation of the chest [22,23], while in advanced cases, clubbed fingers and weight loss are observed [13]. Pneumothorax is a complication sometimes observed in patients with hard metal lung disease [22,24,25]. This complication is commonly associated with patients with advanced disease who have developed lung honeycombing and multiple cysts.

Pulmonary function tests typically show restrictive ventilatory impairment, characterized by reduced total lung capacity, vital capacity, and lung diffusing capacity [21,23]. In the early stages of the disease, the restrictive changes can improve after cessation of exposure and recur on returning to the workplace. While, in the advanced stages of pulmonary fibrosis, impaired gas exchange with hypoxemia during exercises, or even at rest, is observed.

The chest radiograph typically shows a diffuse micronodular and reticular pattern predominantly in the lower lung zones. In advanced disease, the lung volume decreases and small cystic lesions i.e., honeycombing may develop. Early hard metal lung disease appears on high-resolution CT (HRCT) as diffuse centrilobular micronodular opacities and subpleural curvilinear densities with ground-glass attenuation. HRCT of this disease may also find areas of consolidation, irregular linear opacities, extensive reticular opacities and traction bronchiectasis [8,26].

Bronchoalveolar lavage fluid (BALF) from patients with hard metal lung disease is characterized by an increase in the total cell count, with increased numbers of lymphocytes and eosinophils, and a decrease in the CD4 cell:CD8 cell ratio [13,21,23,27]. The reduced CD4 cell:CD8 cell ratio suggests that the immunologic pathogenesis of this lung disease may be similar to that of hypersensitivity pneumonitis [28]. The presence of bizarre multinucleated giant cells in the BALF is characteristic of hard metal lung disease [29]. Elemental analysis of the macrophages in the BALF of patients with hard metal lung disease detected the presence of inorganic dust particles and reveal an increased amount of tungsten [30]. As such, lung biopsy is not necessary for a diagnosis of hard metal lung disease if tungsten is observed in the BALF.

4.3. Pathology and elemental analysis

The histologic pattern of GIP is characteristic of hard metal lung disease [3,31]. Features of GIP are bronchiolocentric fibrosing interstitial pneumonia with bronchiolar and peri-bronchiolar fibrosis and increased macrophages in the airspaces associated with multinucleated giant cells (Fig. 3A and B). Atypical cases of GIP resemble interstitial pneumonia or desquamative interstitial pneumonia with or without honeycomb. Multinucleated giant cells in GIP show cannibalism and contain phagocytosed cellular material, mostly derived from macrophages and neutrophils.

We applied EPMA-WDS to biopsied lung tissue from patients with hard metal lung disease and demonstrated that tungsten was distributed in the giant cells and centrilobular fibrosing lesion (Fig. 3C). Comparison of the distribution of inflammatory cells and tungsten suggested that inhaled hard metal elements caused centrilobular inflammation and fibrosis, via a mechanism involving the interaction of CD163+ macrophages with CD8+ lymphocytes [4]. Qualitative analysis of a selected area (a 10 x 10-μm² area) in a fibrosing lesion of GIP showed the presence of aluminum, silicon, titanium, chromium, iron, and tantalum, in addition to tungsten. It is reported that cobalt is only detected in approximately 10% of GIP lung tissue by EPMA-EDS [3] and in 24% of GIP lung tissue by EPMA-WDS [4]. This is most likely due to the fact that bio-soluble cobalt rapidly disappears from the lung. In usual interstitial pneumonia (UIP) pattern, tungsten was detected in the periarteriole area, and subpleural fibrosis was not associated with centrilobular fibrosis or inflammatory cell infiltration. These results suggest that those who are less susceptible to hard metal elements may directly develop UIP pattern without going through GIP.

4.4. Diagnostic evaluation

There are four requirements for the diagnosis of hard metal lung disease [32]. (1) As with any occupational disease, a comprehensive and detailed work history in the hard metal industry is a key element for the diagnosis of this disease; however, it should be noted that a history of exposure to hard metal dust is sometimes not apparent. (2) Chest HRCT showing opacities consistent with hard metal lung disease, in particular centrilobular micronodular opacities, are required. (3) The observation of giant cells in the BALF and/or pathological features of GIP exhibited in lung samples; however, the absence of these cells does not exclude the possibility of the disease. (4) The detection of tungsten and/or cobalt by elemental analysis in giant cells or lung specimens, but it should be noted that cobalt is only detected in one fourth of cases with hard metal lung disease.

The presence of tungsten and/or cobalt by elemental analysis of BALF or lung specimens enables us to make a definite diagnosis of hard metal lung disease. Although the finding of GIP is almost pathognomonic of hard metal lung disease, we reported two patients whose biopsies exhibited features of GIP, but no tungsten or cobalt was detected in their lung specimens, and neither had a history of work in the hard metal industry [4]. Screening of lung tissue from patients with suspected occupational and environmental lung diseases by EPMA-WDS sometimes yields elements that have not previously been thought to cause lung injury, such as indium, vanadium, and niobium. Extrinsic elements that are barely detected with current techniques may cause
non-hard metal GIP or “idiopathic” GIP [33]. On the other hand, we reported a case with hard metal lung disease without the detection of GIP by lung histopathology [34]. A 54-year-old man employed in the hard metal industry developed bilateral fibrosis, predominantly in the upper lobes, with bilateral pleural effusions. His pathological examinations showed apical cap-like fibrosis without GIP, but by EPMA–WDS tungsten deposits were detected in the fibrotic region of the lung sample. These results suggest that a detailed work history, including employment in hard metal industry, is a key element in the diagnosis of this disease.

Fig. 3 – A representative light microscopic image and EPMA–WDS image of serial lung sections from a patient with hard metal lung disease. (A) Centrilobular fibrosis surrounded by mild alveolitis with giant cells within alveolar space is shown. (B) The rectangular area in panel A is magnified to show accumulated giant cells with cannibalistic appearance. (C) A two-dimension EPMA–WDS image of an element map corresponding to the area shown in (B), showing orange dots, indicating tungsten, accumulated in giant cells. The distribution of amino nitrogen colored green corresponds to the pathological image (B), thus a detected element is easily localized in the lung specimen. Note that tungsten is found not only in the giant cells but also in the centrilobular fibrosing lesion.
5. Other occupational and environmental lung diseases

5.1. Pneumoconiosis due to amorphous silica

Silica is most commonly found in nature as crystalline silica. Since it is a common component of sand, stone, rock, concrete, brick, block, and mortar, construction operations or glass manufacturing, foundries, and sand blasting can result in worker inhalation of small airborne crystalline silica particles, which may cause silicosis as a form of occupational lung diseases [35]. On the other hand, amorphous silica (SiO₂) is an inorganic manufactured synthetic silica material commonly used in semiconductor circuits to isolate different conducting regions. Because of its mechanical resistance, high dielectric strength, and selectivity for chemical modification, amorphous silica has also become a key material in microelectronics and chromatography. Although amorphous silica may also cause silicosis in industrial workers exposed to manufactured synthetic silica, the disease rarely occurs, most likely owing to excellent dust control.

Kumasaka et al. reported a male patient with pneumoconiosis induced by long-term amorphous silica exposure [36]. The patient had worked in a food-processing company for 20 years and was exposed to an absorbent composed of calcined diatomaceous earth without personal protection. His chest radiograph and HRCT showed a bilateral perihilar mass and hydropneumothorax of the right lung. The histology of the lung revealed granulomatous reaction with a large number of macrophages laden with yellow and black pigments accumulated within the alveolar spaces and incorporated into the interstitial sites. Elemental analysis of his lung specimens by EPMA–WDS demonstrated that silicon and oxygen were mapped on the same pigments phagocytosed by macrophages. In addition, semi-quantitative analysis revealed two peaks of silicon and oxygen at a ratio of silicon to oxygen suggestive of amorphous silica, SiO₂. It should be noted that the presence of silicon or oxygen in the lungs from cases of suspected occupational and environmental lung diseases alone does not confirm that inhalation of SiO₂ is the cause of disease, as both these elements are commonly found in the healthy human lung. However, their simultaneous presence on the same pigments at a molecular weight ratio consistent with SiO₂ does strongly suggest that inhaled SiO₂ is the cause of the observed granulomatous reaction. EPMA–WDS enables us to analyze human lung tissue in both a qualitative and semi-quantitative fashion, and combined with histology enables us to provide element distribution maps. As demonstrated, this technique is of great value when evaluating the presence and distribution of common elements within lung specimens from patients suspected of having occupational or environmental lung disease.

5.2. Acute interstitial pneumonia associated with the 2011 earthquake disaster

The great earthquake that shook East Japan on March 11, 2011, caused a giant tsunami which hit the coast of Eastern Japan that faces the Pacific Ocean. A type of aspiration pneumonia named “tsunami lung” occurs when people swept by tsunami waves inhale saltwater contaminated with mud and bacteria [37]. The resulting pneumonia-like infections are usually treated with antibiotics. The disastrous earthquake and tsunami has also left innumerable disaster waste, such as debris and rubble from the smashed houses, buildings and other concrete structures, as well as scrapped cars and ships. The total quantity of this waste is estimated to amount to 25 million tons. Those who work to remove and dispose of the remaining waste may inhale harmful substances from the debris and develop occupational and environmental lung diseases.

Ohkouchi et al. reported a case of acute interstitial lung disease in a patient who was involved in making wood chips from contaminated debris created by the tsunami [38]. A 61-year-old Japanese man had worked without personal protection crushing wood debris into wood chips to generate biomass fuel. He was admitted to the hospital with shortness of breath and pathological analysis of a lung specimen revealed localized organizing fibrosis, chiefly located in the subpleural lesions, and mild emphysematous changes. The localized fibrotic lesions contained various phases of diffuse alveolar damage, most of which were at the organizing phase without any apparent hyaline membrane formation. Elemental analysis of his lung specimen by EPMA–WDS demonstrated excessive silicon, aluminum, iron (ferrous oxide), and titan deposition, suggesting that the patient had inhaled a large amount of contaminated materials while making wood chips from tsunami debris.

Since silicon, aluminum, and iron are elements commonly found in the healthy human lung, the presence of these elements alone cannot contribute to a diagnosis of occupational and environmental lung diseases. However, the EPMA–WDS images demonstrated that these elements were located not only at peribronchiolar regions, but also almost throughout the lung specimen, which strongly suggested that the inhaled harmful substances containing these elements caused the interstitial lung disease. Using EPMA–WDS, you can pinpoint a particular element (or multiple elements) to a specific location within the lung as well as determine the amount of element present at that specific location. This is achieved by comparing element distribution maps with pathological findings. Thus, comparison between element maps and pathological findings may be useful in evaluating whether the presence of an element commonly found in the lung is contributing to the disease.

6. Conclusions

Inhalation of harmful particles, mists, vapors or gases may cause occupational and environmental lung diseases. Mineralogical analysis by EPMA–WDS is useful in the diagnosis of some cases of these diseases, particularly when a given element or mineral is rarely or never found within the lungs except in occupationally exposed individuals. Additionally, this technique is useful in evaluating the presence of commonly found elements in the lung, as it can analyze elements in the tissue in both a qualitative and semi-quantitative fashion, and produce element distribution maps that can be subsequently compared to the pathology of a serial tissue section. We conclude that EPMA–WDS is a promising technique of mineralogical analysis for patients with occupational and environmental lung diseases.
Conflict of interest

The authors have no conflicts of interest.

Acknowledgments

The authors would like to acknowledge Dr. Kouichi Watanabe and Mr. Masayoshi Kobayashi of EPMA Laboratory, Center of Instrumental Analysis, Niigata University, who contributed to elemental analysis of lung specimens. The authors also thank Dr. Yoshiro Endo, Nagaoka Chuo General Hospital for kindly supplying samples of hard metal lung disease.

References