Industrial Health 2023, **61**, 134–139

Original Article

Observation of the same asbestos body by both phase contrast microscope and analytical transmission electron microscope

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Received November 30, 2021 and accepted April 23, 2022
Published online in J-STAGE February 17, 2023
DOI https://doi.org/10.2486/indhealth.2021-0269

Abstract: The amount of asbestos body (AB) in the human lungs is used as an index to assess asbestos lung cancer (ALC). This study reports a new method to observe the same AB previously observed by analytical transmission electron microscope (ATEM) by phase contrast microscope (PCM) or the contrary order. Four kinds of specimens were prepared from the lung tissue of an asbestos related worker: ordinary PCM specimen (A); PCM specimen (B) of which the cover glass was stripped off and ashed at a low temperature; transmission electron microscope (TEM) specimen (C); and PCM specimen (D) covered a TEM specimen (C) with immersion liquid and cover glass. These specimens were all observed by PCM, and the specimen (C) by analytical TEM (ATEM). The results showed that the TEM specimen (C) is transparent in visible light and we can also see the particles by PCM. The image by PCM of the TEM specimen (C) showed very similar features to that of PCM specimens (A) and (B). Accordingly, we could observe various same particles by both ATEM and PCM. In conclusion, the method observing the same AB by both PCM and ATEM will contribute to standardize the recognition of AB for PCM analysts.

Key words: Asbestos, Asbestos body (AB), Phase contrast optical microscope (PCM), Analytical transmission electron microscope (ATEM), Asbestos lung cancer (ALC), Risk of asbestos exposure, The worker's accident compensation system, The asbestos-related health damage relief system

Introduction

Asbestos body (AB) is sometimes recognized in the thin section of lung tissue as a peculiar particle by optical microscopy. AB consists of asbestos fiber (AF) coated with iron rich protein, such as ferritin or hemosiderin. AB is regarded as a result of a defense reaction of a living body. It is known that the existence of ABs in the lungs is a form of proof of a person's exposure to asbestos. Exposure is found mainly in former asbestos workers, but sometimes in the general population.

The number of patients with lung cancer in Japan was about 123,000 people in 2018[1)]. The leading cause of lung cancer is smoking, but there are various other causes. The percentage of lung cancer caused by asbestos exposure has

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not been clearly known. The existence of pleural plaque indicates the person had been exposed to asbestos and can be observed by chest X-ray or CT image. ABs and AFs can be seen in the lung tissue specimen by optical and/or electron microscopy. However, these findings do not always indicate the lung cancer due to asbestos exposure. Now, it is widely accepted that a cumulative exposure of 25 fiber-years increases the risk of lung cancer 2-fold. A 2-fold risk of lung cancer is approximately equal to 5,000 to 15,000 ABs per gram of dry lung tissue[2].

One of the authors (K.N.) examined the lung cancer cases of about 250 patients who were hospitalized in Cancer Institute Hospital in 1970s to 1999 for their amounts of ABs in the lungs, and found the patients of 4.3 percent had over 5,000AB/g (dry lung)[3, 4]. During these years, the number of lung cancer patients increased from 17,000 to 68,000 per year in Japan.

An employed worker who is estimated as 2-fold risk of lung cancer can receive compensation as asbestos lung cancer (ALC) through the Worker's Accident Compensation System (Industrial Accident Compensation Insurance) administered by the Ministry of Health, Labour and Welfare[5].

A person with ALC who has not been covered by this system because of the self-employed worker, neighbor living near asbestos factory, and a family member of the asbestos exposed worker can receive aid through the Asbestos-Related Damage Relief System (Act on Asbestos Health Damage Relief) administered by the Ministry of Environment of Japan, and operated by Environmental Restoration and Conservation Agency of Japan[6, 7].

Now, the amounts of ABs or AFs in a person's lungs have sometimes been employed to judge whether or not a case is ALC. Clinical technologists of Rosai Hospitals have been given training in the measurement of ABs in the lung tissues since 2006. The training has been offered by two separate organizations: Japan Organization of Occupational Health and Safety (JOHAS) and Environmental Restoration and Conservation Agency of Japan (ERCA).

This paper reports a new method to observe the same AB by both phase contrast microscope (PCM) and by analytical transmission electron microscope (ATEM) ** intending to improve the accuracy in the recognition of AB by PCM.

** ATEM is a transmission electron microscope equipped with an energy dispersive X-ray (EDX) analyzer.

Material and Method

Lung tissue is sometimes excised from lung cancer patient in the purpose of diagnosis and/or cure. Lobectomy by thoracoscopy is becoming popular treatment of early lung cancer. Non-tumorous lung tissue is occasionally used for the determination of ABs and/or AFs to assess whether the patient's cumulative exposure to asbestos is equivalent to a two-fold risk of lung cancer.

The case used in this study was requested by a medical inspector to Yokohama Rosai Hospital to measure the amount of AB in the lungs. The written information of the case by the medical inspector was as follows: Man of 70's; Piping worker; Asbestosis; Sample information: Pathological dissection, Lower lob of right lung, Formalin fixation. The amount of AB measured by our laboratory was measured over 80,000AB/1 gram dry lung.

A piece of the lung tissue autopsied on the person received from the medical inspector was dissolved in an alkaline solution, and the residue containing the ABs and asbestos fibers (AFs) was washed with water and kept as 50 ml in a glass bottle following the procedure by Kohyama and Suzuki (1991)[8]. The manual of the sample preparation and counting method of AB has been published by JOHAS and ERCA[9].

Taking an aliquot from the suspension sample of the lung tissue of the case, we prepared the following four specimens in sequence after each observation: an ordinary PCM specimen with immersion liquid of Entellan^{R} new and cover glass; a PCM specimen (B) of which the cover glass was stripped off by immersion in xylene for one to three days and the membrane filter was ashed at a low temperature; ordinary TEM specimens (C) which were made from a PCM specimen (B); and a PCM specimen (D) which was a TEM specimen (C) covered with immersion liquid and cover glass. The TEM specimens (C) were made from a PCM specimen (B) following the procedures of the carbon extraction method by Kohyama, *et al.* (1991 & 1996)[8, 10], which is fully introduced in the textbook 'Analytical Chemistry of Aerosols' by Dr. Spurny[11].

We observed the images of ABs on each specimen by PCM and ATEM in detail. At the beginning we observed a TEM specimen (C) by PCM for the shapes of ABs and suspected ABs, and then we observed the TEM specimen (C) by ATEM for the same ABs and suspected ABs analyzing the core fibers by EDX analysis.

The case used in this study does not require the ethical examination and informed consent in accordance with the 10th article of the detailed enforcement of the ethical code of the Ethics Committee of the Yokohama Rosai Hospital[12]. This was confirmed by the chairman of the committee. The authors declare no conflict of interest.

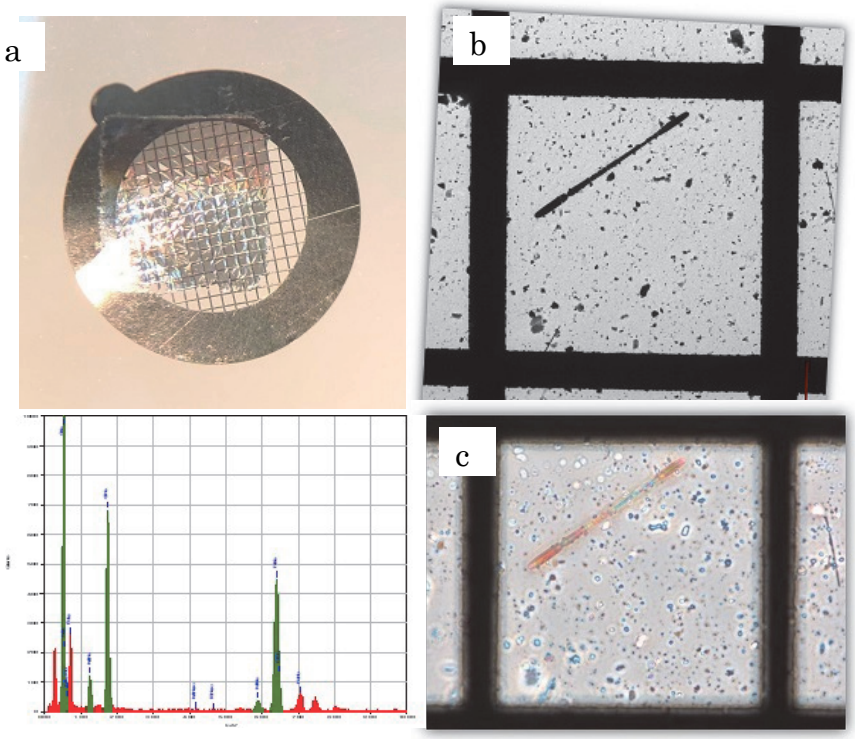

Fig. 1. TEM specimen (C) observed by a loupe (a), by TEM (b) and by PCM (c). The EDX spectrum shows the core fiber of the AB is amosite. The TEM grid is 110 µm square.

Results and Discussion

We found the TEM specimen (C) is transparent in visible light and that the particles on the TEM specimen can be seen by PCM (Fig. 1c). As a result, we could observe the same particle by both PCM and ATEM and confirm the particle as AB by the EDX analysis of the core fiber.

The particles on a PCM specimen (B), the cover glass was stripped off and the membrane filter was ashed at a low temperature, showed a slightly higher contrast than those of an ordinary PCM specimen (A) (Figs. 2a, b). The PCM image of an ordinary TEM specimen (C) showed a very similar contrast to those of a PCM specimen (B) (Figs. 2b, d). The image of the PCM specimen (D), which was a TEM specimen (C) covered with immersion liquid and cover glass, showed very similar features to that of an ordinary PCM specimen (A) (Figs. 2a, e).

Through these observations, we confirmed that the PCM images of an ordinary TEM specimen (C) showed similar details to those of a PCM specimen (B). Therefore, we can correctly identify the particle seen by PCM on a TEM specimen (C) through the observation of the same particle by ATEM.

Examples of pair images observed by the present method can be seen below:

Fig. 3a shows an image of one AB by PCM, but Fig. 3b seen by ATEM shows the AB actually consists of two ABs. However, it would be impossible to count the AB as two by PCM observation. This would be a limitation of PCM.

Fig. 4 shows a fibrous particle seen by both PCM and ATEM. The PCM image shows somewhat like AB (Fig. 4a), but it is not AB according to the rule of measurement manual of AB[9]. The image observed by ATEM (Fig. 4b) shows a fiber (amosite) simply coexisting with a particle, which is clearly not AB. This provides important information to the person conducting measurements with regard to their judgment of AB.

The observation of a core fiber stretched from AB is an important marker for the judgment of AB by PCM. Fig. 5 shows the images of the same AB observed by both PCM and ATEM. The stretched core fiber is crocidolite as shown by the EDX spectrum and the fiber diameter is approximately 0.1 µm (Fig. 5b). The fiber could not be seen on the PCM image in the area indicated by arrow on Fig. 5a. We could understand the diameter of this fiber was well below the visibility of PCM. It has been stated that the lower limit of visibility of PCM is around 0.25 µm[13]. As shown here,

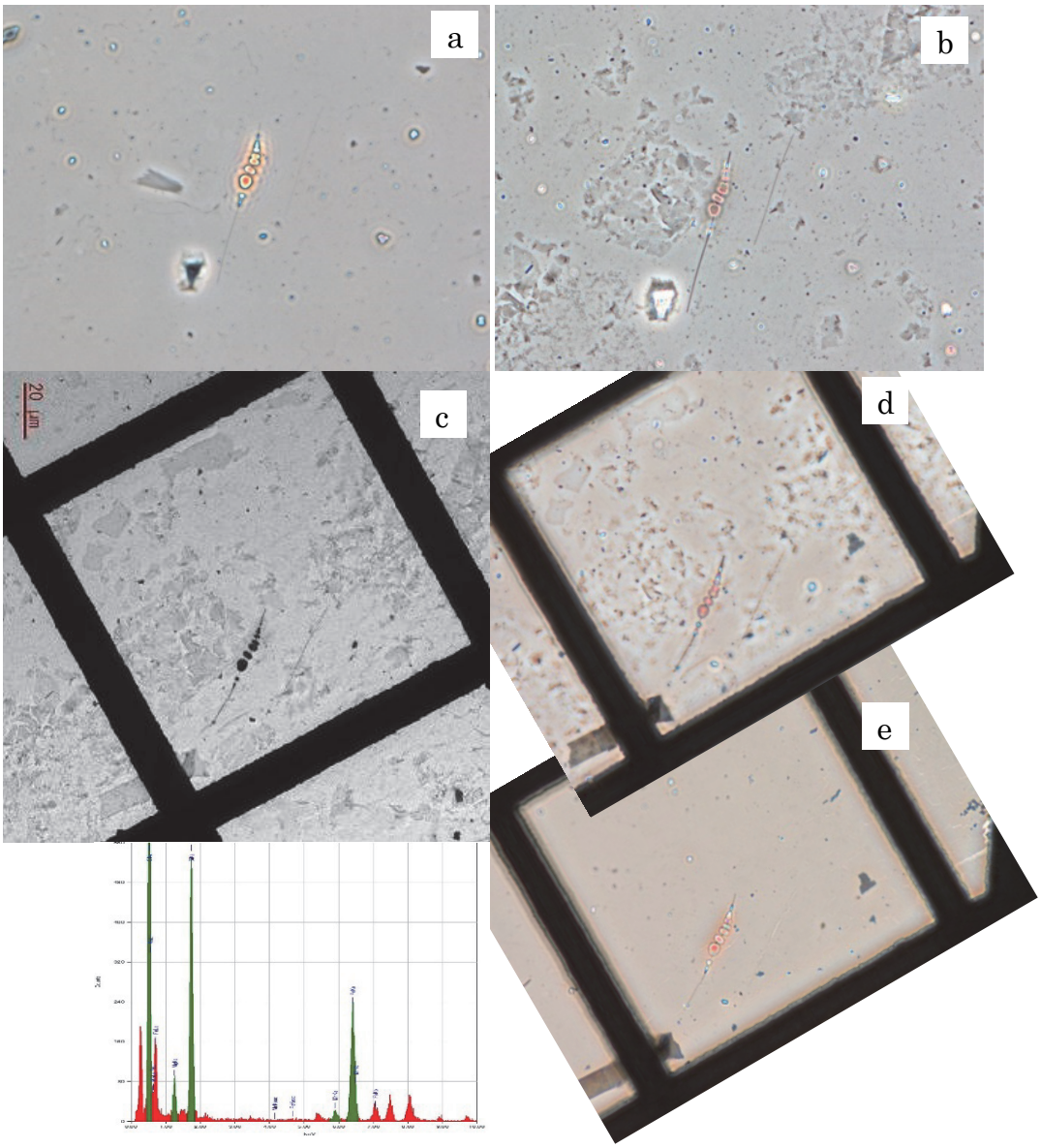

Fig. 2. AB observed by PCM and ATEM on each specimen.
a: PCM image of ordinary PCM specimen (A) with cover glass, b: PCM image of PCM specimen (B) of which the cover glass stripped off and the membrane filter was ashed at a low temperature, c: TEM image of TEM specimen (C), d: PCM image of the TEM specimen (C), e: PCM specimen (D) covered a TEM specimen (C) with cover glass.
The EDX spectrum shows the core fiber is amosite.

this method would be effective to measure the lower limit of visibility of PCM directly and precisely.

Although PCM observation can not specify the core fiber of AB, this method can specify the core fiber by the EDX analysis of ATEM because the same AB observed by PCM can be analyzed by ATEM for the kind of the core fiber. Therefore, this method is also expected to clearly establish whether there is a certain relationship between the shapes of ABs and the kind of fiber.

The pair images of the same particle including ABs by both PCM and ATEM will greatly help the accurate measurement of ABs by PCM as the images of ABs confirmed by ATEM. Therefore, if the pair images are used in the professional training of personnel measuring AB offered by the JOHAS and ERCA organizations, the accuracy management will be improved. Moreover, personnel who are tasked with measuring AB will benefit from a reduction in their sense of insecurity in the judgment of AB. Thereby, it

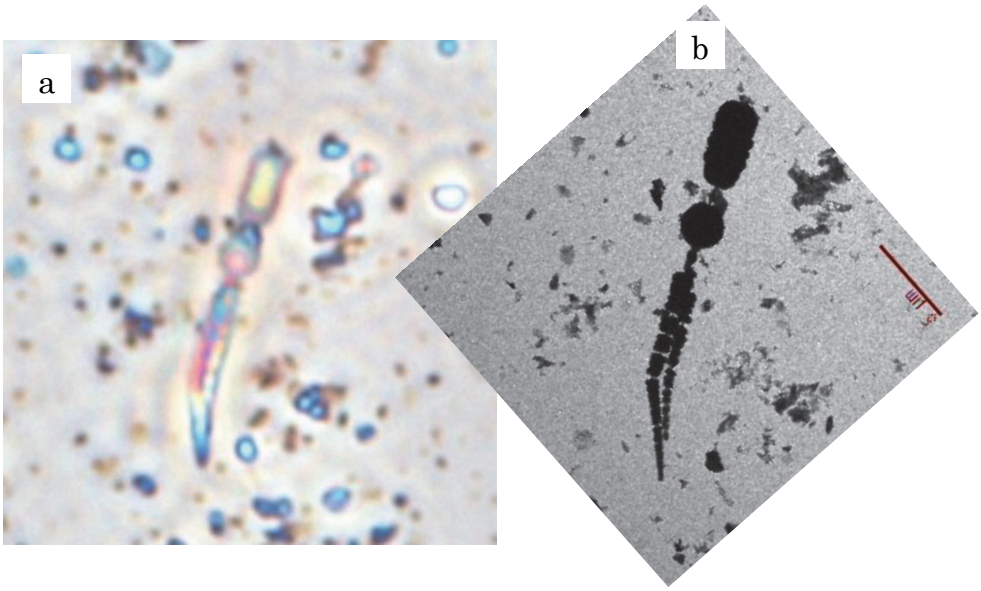

Fig. 3. A pair image of AB observed by PCM (a) and by ATEM (b).

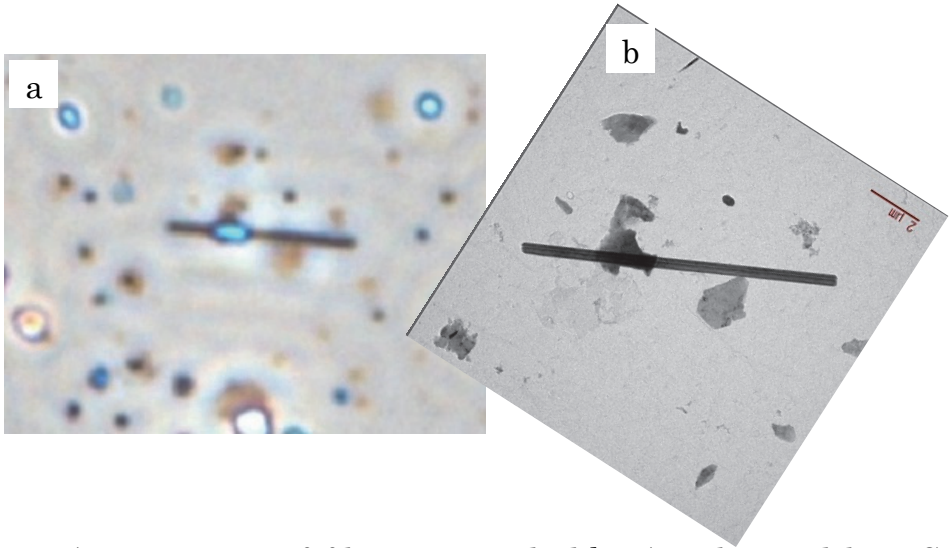

Fig. 4. A pair image of fibrous particle like AB observed by PCM (a) and by ATEM (b). The TEM image (b) shows a fiber coexisting with a particle and showing not AB.

is expected that adoption of this method will result in reduction of burden on personnel as well as shortening the measurement time of AB.

To this end, we are further planning to collect many pair images which are observed ABs by both PCM and ATEM for typical shape, suspected shape and uncertain shape.

Conclusion

We found the TEM specimen is transparent in the visible light and can be seen by PCM. Although we cannot know the kind of core fiber of AB by PCM, we can observe the same AB by ATEM and can identify the fiber kind by the EDX analysis. Therefore, the pair images produced by this method will contribute to standardize the recognition of AB by PCM and to train the PCM analysts for AB measurement.

Acknowledgment

The authors wish to acknowledge Dr. Yasushi Shinohara, formerly executive researcher of National Institute of Oc-

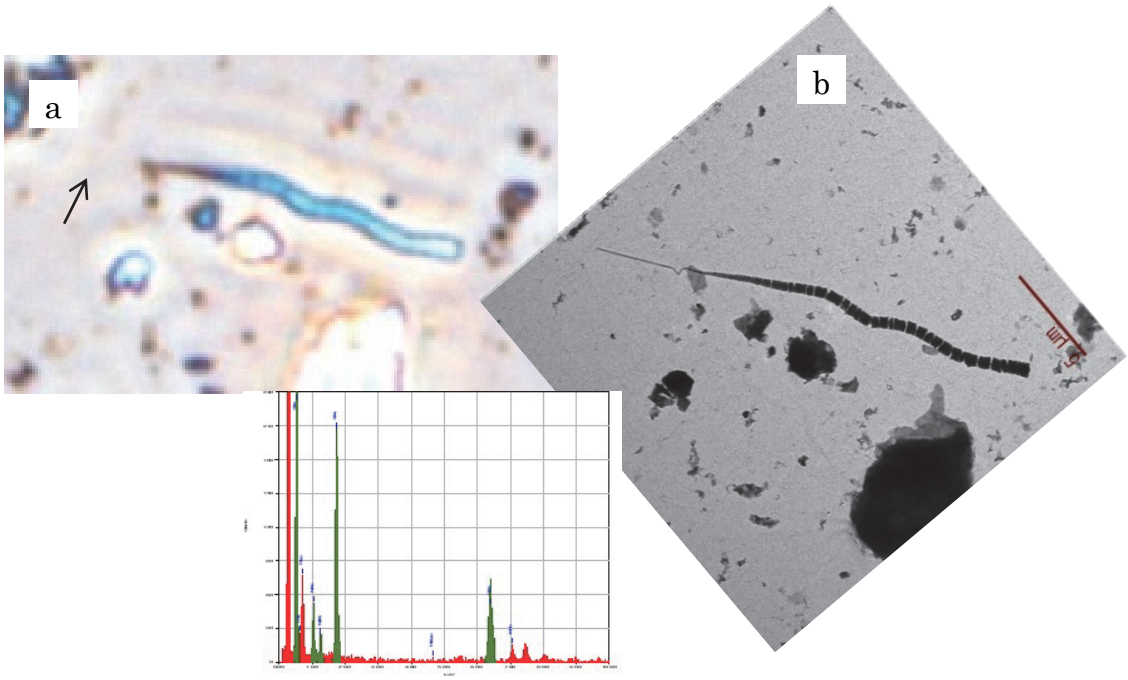

Fig. 5. A pair image of AB observed by PCM (a) and by ATEM (b).
The PCM image (a) does not show the stretched fiber (arrow area) which is seen on the image of ATEM (b). The EDX spectrum shows the core fiber is crocidolite.

cupational Health and Safety, for the kind assistance provided to the author (S.I.) in acquiring the techniques for asbestos measurement by ATEM.

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