Ultra-magnifying Imaging of Thoracoscopic Biopsy Specimens Using Ex vivo Endocytoscopy in Three Patients with Mesothelioma

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Abstract: We performed endocytoscopy (ECS) for the ex vivo evaluation of mesothelioma in specimens biopsied during medical thoracoscopy in three patients. We evaluated 19 biopsy specimens based on the density of nuclei and irregularity in the nuclei shape using ECS and compared them with the histopathological findings. All 10 specimens considered malignant based on ECS were diagnosed as malignant based on histopathology. The nine specimens evaluated as non-malignant based on ECS consisted of six specimens diagnosed as malignant based on histopathology and three diagnosed as non-malignant based on histopathology. ECS was feasible and had some utility for ex vivo ultra-magnifying imaging of thoracoscopic biopsy specimens from patients with mesothelioma.

Key words: endocytoscopy, thoracoscopy, nucleus

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Introduction

Endocytoscopy (ECS) is an optical magnification technique that enables endoscopic imaging at high magnification to observe and evaluate nuclei. In gastroenterology, ECS has been used in endoscopy to evaluate esophageal (1), gastric (2), and intestinal lesions (3). It has been suggested that ECS can distinguish between tumor cells and normal cells based on the shape of nuclei and the nucleocytoplasmic ratio (4). In pulmonology, there have been reports of ECS being used to evaluate intraepithelial extension of squamous cell lung cancer (5), surgical margin (6), and bronchial lesions during bronchoscopy (7). Thoracoscopy under local anesthesia (medical thoracoscopy) enables observation of the pleural cavity and a parietal pleural biopsy. It is useful for diagnosing malignant mesothelioma, lung cancer, tuberculous pleurisy, and other conditions that cannot be diagnosed with cytology or culture of pleural effusion. For malignant diseases in particular, it is important to collect an appropriate, sufficient volume of specimens using thoracoscopy in order not to delay a diagnosis.

We herein report the usefulness of ECS for the evaluation of malignancy in specimens biopsied during medical thoracoscopy.

Case Reports

All three patients who underwent thoracoscopy under local anesthesia at the Department of Internal Medicine, Division of Medical Oncology & Respiratory Medicine, Faculty of Medicine, Shimane University from October 2018 to March 2019 were included in this study. This study was conducted after obtaining approval from the ethics committee of Shimane University Hospital (study number: 3318). Informed consent was obtained from all patients.

During thoracoscopy under local anesthesia, a port was placed at the site of the pleural effusion, and a Pleuravidoscope (LTF TYPE 260; Olympus Medical Systems, Tokyo, Japan) was inserted. After aspiration of the pleural effusion fluid, the parietal pleura was observed extensively with white-light or narrow-band imaging (NBI). Elevated le-
sions or areas with blood vessels with an abrupt caliber change were biopsied using biopsy forceps. Biopsy specimens that were removed from the body were observed using an endocytoscope with a total length of 380 cm and a tip diameter of 3.2 mm (prototype, XEC-300-2; Olympus Medical Systems, Tokyo, Japan). Images were viewed at 450-fold magnification on a 14-inch monitor. The field of view was 300 μm×300 μm, the observation depth was 0-30 μm, and the horizontal resolution was 4.2 μm (Fig. 1a). The ECS images of specimens were recorded by a video recorder (Image Management Hub, IMH-20; Olympus Medical Systems).

The biopsy specimens were immersed in 10% neutral buffered formalin for 30 seconds and placed on gauze. One or two drops of 0.5% methylene blue solution were placed onto the specimen with a 1-mL syringe. The lens at the tip of the endocytoscope was attached to the specimen and slid over the surface of the specimen for observation (Fig. 1b). An ECS evaluation was performed according to the evaluation of esophageal lesions described by Kumagai et al. (8, 9). A histological examination showed that the mesothelial layer consisted of a single layer of mesothelial cells, and the parietal pleura consisted of numerous fat cells (10). Therefore, we suspected that the density of the nuclei in the intact parietal pleura on ECS would be low. The density of nuclei was defined as low when there was an area with only a few nuclei on the ECS image of the biopsy specimen and as high when the number of nuclei on the ECS image of the biopsy specimen was increased. Specimens with regularly shaped nuclei were considered non-malignant, whereas those with irregularly shaped nuclei were considered malignant, regardless of the density of nuclei.

**Patient 1**

An 89-year-old man had had left pleural effusion for 6 months before his first visit to our institution. He had a 15-year history of smoking and a history of asbestos exposure while working in the demolition industry. On a physical examination, the breath sounds in the left lung were decreased. Computed tomography (CT) of the chest showed nodular shadows in both upper lobes, pleural thickening, and left pleural effusion (Fig. 2a). Blood tests showed no significant elevations in tumor markers. The interferon-gamma release assay was positive. The left pleural effusion fluid collected by thoracentesis was exudative. There were no significant findings on cytology or bacterial culture. Medical thoracoscopy revealed multiple flat whitish lesions on the left parietal pleura (Fig. 2b). A pleural biopsy was performed. ECS of the second and third biopsy specimens showed irregularly shaped nuclei on the screen (Fig. 2c). A histopathological examination of the second and third biopsy specimens showed atypical cells with irregularly shaped nuclei. The histopathological diagnosis based on Hematoxylin and Eosin (H&E) staining and immunohistochemistry was malignant mesothelioma (Fig. 2d). The type of mesothelioma was not diagnosed. The fifth biopsy specimen had few nuclei on ECS (Fig. 2e). It was diagnosed as non-malignant based on the histopathological findings (Fig. 2f) (Table).

**Patient 2**

A 69-year-old man visited the hospital because chest X-ray showed left pleural effusion. He had no history of smoking but had worked as a plasterer for over 50 years. There were no other abnormal physical findings except for decreased breath sounds in the left lung. Chest X-ray showed left pleural effusion (Fig. 3a). Chest CT showed pleural thickening with contrast enhancement of the left dorsal pleura (Fig. 3b). The left pleural effusion was exudative. There were no significant findings on cytology or bacterial culture. Medical thoracoscopy showed reddish with multiple white plaques in the left parietal pleura (Fig. 3c). Eight bi-
**Patient 1:** Mesothelioma. (a) Chest computed tomography revealed nodular shadows in both upper lobes. Left pleural thickening was present with left pleural effusion. (b) Thoracoscopy revealed multiple flat whitish lesions on the left parietal pleura. (c) Endocytoscopy (ECS) of the third biopsy specimen showed irregularly shaped nuclei. (d) Histopathological findings of the third biopsy specimen included atypical cells with irregularly shaped nuclei [Hematoxylin and Eosin (H&E) staining, ×200]. (e) ECS of the fifth biopsy specimen showed few nuclei in the pleura. (f) A histopathological examination of the fifth biopsy specimen showed few nuclei in the pleura (H&E staining, ×200).

Other five specimens were obtained from the area with plaques, which had vessels with abrupt caliber changes at the margins of the plaques (Fig. 3d). Based on ECS, we evaluated seven specimens as malignant (Fig. 3e) and one as non-malignant. Three of eight biopsy specimens were too small to slice for a histopathological diagnosis. The other five specimens were all diagnosed as desmoplastic mesothelioma based on a histopathological examination with H&E staining, immunohistochemistry, and fluorescence in situ hybridization (FISH) (Fig. 3f). Of these five specimens, when ECS findings were compared with histopathology, four were consistent with malignancy, but one was inconsistent (Table).

**Patient 3**

An 80-year-old man visited the hospital with a chief complaint of dyspnea. He had never smoked. He had a history of asbestos inhalation when he was working at an oil company. Chest X-ray showed right pleural effusion (Fig. 4a). Chest CT showed right dorsal pleural thickening with contrast enhancement (Fig. 4b). Pleural fluid cytology yielded no significant findings. We suspected malignant mesothelioma of the pleura based on the history of asbestos exposure and enhanced pleural thickness on chest CT. Medical thoracoscopy showed flat reddish lesions in the right parietal pleura (Fig. 4c). NBI showed dilated vessels in the flat reddish lesions (Fig. 4d). Nine pleural biopsies of the flat reddish lesions were performed. ECS of the second biopsy specimen showed irregularly shaped nuclei on the screen (Fig. 4e). Histopathological findings of the second biopsy specimen showed irregularly shaped nuclei with increased spindle-shaped cells (Fig. 4f). In four specimens, a high density of nuclei and irregularly shaped nuclei on ECS were considered indicative of malignancy, consistent with the histopathological diagnosis of malignancy. However, five specimens with a low nuclei density and regularly shaped nuclei, which were assessed as non-malignant based on ECS, were diagnosed as malignant based on histopathological findings (Table). The histopathological diagnosis with H&E staining, immunohistochemistry, and FISH was desmoplastic mesothelioma.

Table shows the results of the ECS evaluation and pathological diagnosis for each biopsy specimen in this study. Representative ECS images of nuclei with a high density and irregularly shaped nuclei are shown (Fig. 3e, 4e).

Nineteen specimens, which excluded 3 unevaluated specimens out of 22 specimens, were used for the comparison of ECS and histopathological findings (Fig. 5). In these 19 specimens, the 7 with a high density of nuclei and irregularly shaped nuclei are shown (Fig. 3e, 4e). Nineteen specimens, which excluded 3 unevaluated specimens out of 22 specimens, were used for the comparison of ECS and histopathological findings (Fig. 5). In these 19 specimens, the 7 with a high density of nuclei and irregularly shaped nuclei were evaluated as malignant based on ECS. All seven of these specimens were diagnosed as malignant based on histopathology. The remaining 12 of the 19 total specimens with a low density of nuclei consisted of 3
Table. Results of the ECS Evaluation and Histopathological Diagnosis of Biopsy Specimens.

| Biopsy no. | Density | Irregularly shaped nuclei | ECS | Histopathological diagnosis |
|------------|---------|---------------------------|-----|------------------------------|
| Patient 1  |         |                           |     |                              |
| 1          | Low     | ×                         | -   | -                            |
| 2          | High    | ○                         | +   | +                            |
| 3          | High    | ○                         | +   | +                            |
| 4          | Low     | ×                         | -   | -                            |
| 5          | Low     | ×                         | -   | -                            |
| Patient 2  |         |                           |     |                              |
| 1          | High    | ○                         | +   | Not evaluable                 |
| 2          | High    | ○                         | +   | Not evaluable                 |
| 3          | High    | ○                         | +   | +                            |
| 4          | Low     | ○                         | +   | +                            |
| 5          | High    | ○                         | +   | Not evaluable                 |
| 6          | Low     | ×                         | -   | +                            |
| 7          | Low     | ○                         | +   | +                            |
| 8          | Low     | ○                         | +   | +                            |
| Patient 3  |         |                           |     |                              |
| 1          | Low     | ×                         | -   | +                            |
| 2          | High    | ○                         | +   | +                            |
| 3          | High    | ○                         | +   | +                            |
| 4          | High    | ○                         | +   | +                            |
| 5          | Low     | ×                         | -   | +                            |
| 6          | Low     | ×                         | -   | +                            |
| 7          | High    | ○                         | +   | +                            |
| 8          | Low     | ×                         | -   | +                            |
| 9          | Low     | ×                         | -   | +                            |

○: present
×: absent
+: Malignant
●: Non-malignant
■: specimens evaluated as malignant based on ECS or histopathology
Not evaluable: specimens too small to slice for histopathological diagnosis

specimens with irregularly shaped nuclei interpreted as malignant based on ECS and 9 with regularly shaped nuclei interpreted as non-malignant based on ECS. These nine specimens interpreted as non-malignant based on ECS consisted of six specimens diagnosed as malignant and three diagnosed as non-malignant based on histopathology. The three specimens with a low density of nuclei and irregularly shaped nuclei that were interpreted as malignant based on ECS were all diagnosed as malignant based on histopathology.

To evaluate the ability of ECS to diagnose mesothelioma, we calculated the sensitivity, specificity, and diagnostic accuracy of ECS in five specimens from Patient 1, five from Patient 2, and nine from Patient 3 for which the ECS and histopathology results could be compared. The sensitivity, specificity, and diagnostic accuracy for the specimens from Patient 1 were 100%, 100%, and 100%, respectively; those for the specimens from Patient 2 were 80%, not applicable, and 80%, respectively; and those for the specimens from Patient 3 were 44%, not applicable, and 44%, respectively. Not applicable was used when the variables were insufficient. The overall sensitivity, specificity, and diagnostic accuracy were 63%, 100%, and 68%, respectively.

Discussion

In this study, we encountered three patients in whom ECS findings of pleural specimens biopsied during medical thoracoscopy were able to be evaluated based on the density of the nuclei and irregularity in the size of nuclei. The endocytoscope used in this study was a catheter type with a diameter of 3.2 mm, which was used to observe the biopsy specimens while holding them in contact with the endocytoscope. In the field of esophageal endoscopy and colonoscopy, the endocytoscopic classification was based on the shape of epithelial cell nuclei, similarly to atypia in the histopathological diagnosis (11, 12). Kumagai et al. reported that esophageal lesions could be classified as type 1 (non-malignant), type 2 (borderline lesions), or type 3 (malignant) based on ECS findings. They compared this classification system with the pathological diagnosis. Classification by an endoscopist had a sensitivity of 93.6% and specificity of 94.6% (8). We re-
Figure 3. Patient 2: desmoplastic mesothelioma. (a) Chest X-ray showed left pleural effusion. (b) Contrast-enhanced computed tomography showed left pleural thickening and enhancement with contrast (yellow arrow). (c) The left parietal pleura was reddish and had multiple white plaques. (d) Narrow-band imaging showed vessels with abrupt caliber changes (red arrows). Biopsy specimens were collected from these areas. (e) Endocytoscopy of the third biopsy specimen showed irregularly shaped nuclei. (f) A histopathological examination of the third biopsy specimen also showed irregularly shaped nuclei (Hematoxylin and Eosin staining, ×200).

In pulmonology, Shibuya et al. reported that ECS with in vivo spraying of methylene blue on the bronchial epithelium could help discriminate normal bronchial epithelium cells from dysplastic or malignant cells in real time. In this study, we used the same type of prototype ECS that Shibuya et al. used. Shibuya et al. mentioned that it was difficult to observe the upper lobe bronchus with the mother bronchoscope, which was 6.9 mm in diameter. Further investigation will be needed to reduce the diameter of the mother bronchoscope and develop an integrated endocytoscope with a conventional endoscope (7). Nosaka et al. reported that ECS could be used as a substitute for a rapid pathological diagnosis during the intraoperative ex vivo evaluation of bronchial margins for squamous cell carcinoma (5). Shah et al. compared ECS and endomicroscopy findings in conventional biopsies obtained from the same area of the airway. They stated that ECS was technically more difficult than confocal endomicroscopy, but ECS was able to distinguish normal epithelium from dysplasia and carcinoma (13).

ECS is used for the in vivo and ex vivo evaluation of bronchial lesions. The advantage of in vivo ECS is that it can evaluate living cells in the human body, but the disadvantage of in vivo ECS with methylene blue staining is the risk of DNA damage from methylene blue staining (14). The advantage of ex vivo ECS is that it can evaluate nuclei without waiting for the pathological diagnosis to be made. The rapid on-site evaluation (ROSE) of stamp tissue using cytology might be less expensive than this ECS method, but the ROSE requires help from the pathology department.

When conducting our assessment with ECS in the present study, we used the density of nuclei for simplicity’s sake, as this approach allowed for classification into two categories. We considered a low density of nuclei to correspond to non-malignant specimens. All specimens with a high density of nuclei or irregularly shaped nuclei on ECS, which were considered malignant, were diagnosed as malignant based on histopathology. In the specimens with a low density of nuclei, it was somewhat difficult to evaluate the irregularity of their size due to the small number of nuclei. In our 3 patients, 10 specimens were evaluated as malignant with ECS based on irregularly shaped nuclei. The ECS findings were similar to the histopathological findings, suggesting that ECS can help determine whether or not specimens are appropriate for a histopathological diagnosis. The first, fourth, and fifth biopsy specimens in Patient 1, which had few nuclei and were evaluated as non-malignant based on ECS, had a consistent histopathological diagnosis. An evaluation of ‘no malignancy’ based on ECS indicates that we should perform a biopsy at another site. In Patient 2, three biopsy
Figure 4. Patient 3: desmoplastic mesothelioma. (a) Chest X-ray showed right pleural effusion. (b) Chest computed tomography showed bilateral pleural thickening and calcification. (c) Flat reddish lesions were found in the right parietal pleura. (d) Narrow-band imaging showed increased dilated vessels in brownish areas. (e) Endocytoscopy of the second biopsy specimen showed irregularly shaped nuclei (red arrows). (f) A histopathological examination of the second biopsy specimen also showed irregularly shaped nuclei (Hematoxylin and Eosin staining, ×100).

Figure 5. Tree diagram of the ECS diagnosis.

specimens were too small to slice for the histopathological diagnosis. However, even if the biopsy specimens are too small, the presence of malignant cells in the biopsy specimens based on ECS may motivate the performance of a repeat biopsy in the same area. Shibuya et al. reported that squamous cell carcinoma cells on ECS images have increased cellular density and a high nucleus-to-cytoplasm (N/C) ratio (7). Shah et al. stated that carcinoma cells have an irregular shape, have large amounts of nuclear material, and have high N/C ratios (13). As we gain experience using ECS to evaluate pleural diseases, we intend to revise the criteria for distinguishing between benign and malignant lesions based on cellular density, the N/C ratio, and cellular irregularity in the future.

In Patient 3, five of nine specimens were evaluated as non-malignant because of a low density of nuclei and regu-
larly shaped nuclei, but all specimens were diagnosed as malignant based on histopathology. This discrepancy might have been caused by differences in observation sites. For example, the observation site of ECS was the surface of the specimen, but the observation site for the histopathological specimens was the cut surface at the center of the specimens. In Patient 3, the differential diagnosis of the histopathological examination included fibrosing pleuritis based on the immunostaining results, but the final diagnosis of desmoplastic mesothelioma was based on the results of FISH. It is often difficult to diagnose malignant mesothelioma with H&E staining (15) or frozen sections (16). We anticipate some difficulties in evaluating malignancy with ECS as well.

The main limitation of our study is that we performed ECS for only three patients with mesothelioma. There were no data on other pleural diseases. Thus, it is necessary to accumulate cases of mesothelioma and other diseases, such as pleural metastasis from lung cancer and tuberculous pleuritis, for further investigations.

Regarding suitable sites for a thoracoscopic biopsy, Ishida et al. reported the usefulness of NBI during thoracoscopy. They classified specimens based on vascular patterns and irregularities in the vessel caliber, reporting that the detection rate for malignant lesions with NBI was markedly higher than the rate with white light alone (17). It is difficult to push and make contact with the parietal pleura in the thoracic cavity using an endoscope because it is so flexible. We therefore hope that future versions of the endoscope will be semi-rigid, similar to a pleuravideoscope. Combining NBI with advances in ECS for selecting biopsy sites and evaluating biopsy specimens might reduce the number of biopsies required and shorten the examination time.

Conclusion

This case series suggests that ECS was feasible and had some utility for ultra-magnifying imaging of thorascopic biopsy specimens from patients with mesothelioma.

Author’s disclosure of potential Conflicts of Interest (COI).

Noriaki Kurimoto: Honoraria, Olympus and AstraZeneca. Yukari Tsubata: Honoraria, Chugai Pharmaceutical, Daiichi Sankyo and AstraZeneca.

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