Immunocytochemistry as a Diagnostic Procedure of Pleural Mesothelioma

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Abstract

Background: Mesothelioma is a primary malignant tumor arising from the mesothelial surface of the pleura, peritoneal, tunica vaginalis, and pericardium. Most cases of mesothelioma originate from the pleura. Most patients have a history of asbestos exposure. A common diagnostic problem is distinguishing mesothelioma from adenocarcinoma since both tumors invade the pleura. Immunocytochemistry of calretinin and TTF-1 can be used to establish the diagnosis of mesothelioma.

Case Report: Male, 56 years old presented with chest pain, shortness of breath, cough, and weight loss since 5 months before hospitalization. The patient had a history of occupational exposure to asbestos for 30 years. The movement and breath sounds were decreased as well as dull upon percussion at the right chest. A chest X-ray revealed a right lung tumor with pleural effusion. Thorax CT scan suggested pleural mass in right hemithorax, infiltration to intercostal muscles, and destruction of the 7th right rib, right perihilar lymphadenopathy, right pleural effusion, and liver nodules according to mesothelioma T4N1M1 Stage IV. Infiltrative stenting of the right and inferior lobe of the right lung, infiltrative and obstructive stenting of the medius lobe suggestive of a chronic malignancy and inflammation were found on FOB. Cytologic examination of pleural fluid, sputum, and Washing-and-brushing of FOB were a class II (no malignant cells). USG-guided transthoracic FNAB revealed adenocarcinoma with differential diagnosis of mesothelioma. Immunocytochemistry with calretinin showed positive results and TTF-1 showed a negative result. These confirmed the diagnosis of pleural mesothelioma T4N1M1 Stage IV. The patient showed a stable response from carboplatin/gemcitabine treatment.

Keywords: calrenitin immunocytochemistry, mesothelioma, TTF-1

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1. Introduction

The most common primary malignant tumors of the pleura are malignant mesothelioma, a dangerous neoplasm with a poor prognosis arising from the mesothelial surface of the pleural and peritoneal cavities, as well as from the tunica vaginalis and pericardium. Eighty percent of all mesothelioma cases originate in the pleura[1].

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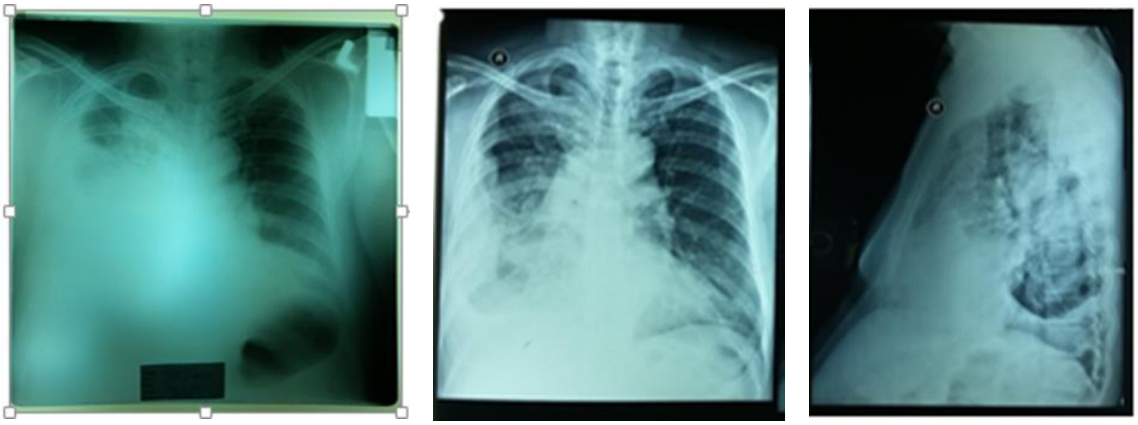
Malignant pleural mesothelioma (MPM) is considered a rare tumor. In the UK, the incidence in men is 3.4/100,000, in France, it is 2.3/100,000 and in the Netherlands, it is 3.2/100,000. In the past 10 years, the incidence of MPM has increased slightly, mainly due to 30-50 years after exposure to asbestos[2]. The World Health Organization (WHO) estimates that asbestos-related disease (ARD) accounts for 92,250 deaths per year globally[2]. Occupational exposure to asbestos accounts for more than 80% of cases, and that makes MPM a preventable disease[1]. An accurate and rapid diagnosis of malignant mesothelioma is important for therapeutic and medico-legal reasons. A common diagnostic problem is distinguishing mesothelioma from adenocarcinoma, particularly because both kinds of tumors might invade the pleura[3]. Mesothelioma can be distinguished from adenocarcinoma with specific antibodies, namely calretinin as a positive marker of mesothelioma and TTF-1 as a positive marker of adenocarcinoma[4]. Here we describe a case report applying immunocytochemistry to confirm the diagnosis of pleural mesothelioma.

2. Case

Mr. J, a 56-year-old male, Asian, was presented to a tertiary hospital with shortness of breath, right chest pain, and cough that was sometimes accompanied by blood spots. He also experienced nausea, decreased appetite, and unexplained weight loss since 5 months before hospital admission. The patient had a history of working as a construction worker and in other fields related to asbestos exposure for more than 30 years.



A B C

Figure 1. A. AP CXR (February 2nd, 2017) showing right pleural effusion and suspected right lung tumors. B. AP CXR after thoracentesis with pleural effusion fluid aspiration (February 20th, 2017) showing a more visible right lung tumor and decreased volume of pleural effusion. C. Right lateral CXR (February 20th, 2017) showing some opacity on the anterior side with a spiculated edge. Conclusion: right pleural effusion and right lung tumor.

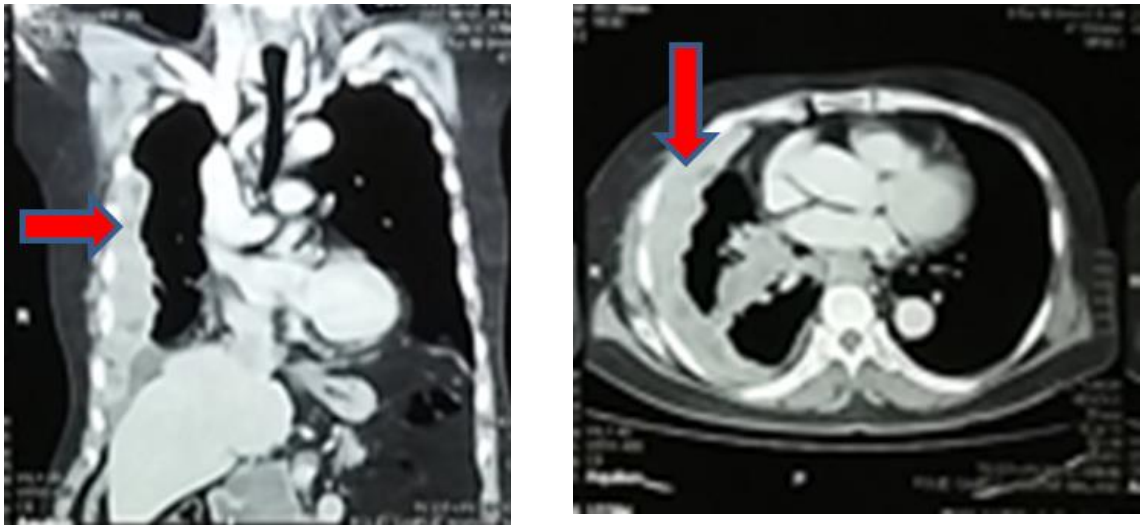


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Figure 2. Figure 2 Thorax CT scan + contrast (April 29th 2017); A. Coronal view, B. Axial view Pleural mass in right hemithorax, to musculus intercostalis, destruction of 7th right rib, right perihilar lymphadenopathy, right pleural effusion, and liver nodules according to mesothelioma T4N1M1 Stage IV

Physical examination showed that the right chest appears to be more static than the left chest. There was also an increase of breathing frequency, dullness upon percussion at the lower right chest, and a decrease in breathing sound accompanied by friction rub on expiration at the lower right chest. Blood gas analysis results were hyperoxemia and metabolic acidosis with respiratory alkalosis compensation. Laboratory workup revealed an increased number of neutrophil (81%), monocyte (8,0%), lactate dehydrogenase (1671), Carcinoembryonic antigen (234,4) and Neuron-specific enolase (48,25). A decreased number of lymphocyte (8,6%) and albumin (3,39 g/dl) were also found.

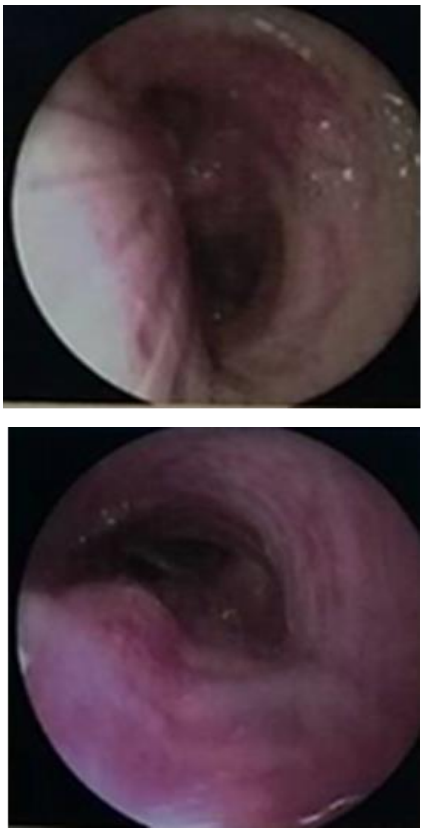

Figure 3. Fiberoptic bronchoscopy (March 7th, 2017); A. The main right bronchus: lumen narrowing, mucosal hypervascularization and edema, B. Right bronchus medius lobes; lumen narrowing, mucosal hypervascularization and edema, and uneven surface.

The pleural fluid analysis showed an exudative pleural effusion dominated by mononuclear (MN) leucocyte (93%), total protein 5,02 g/dl, glucose 58 g/dl, 1865 iu/l, and tested positive for Rivalta test. The result of spirometry suggested a mild restriction in which vital capacity (VC) was 59.8%, and mild obstruction in which the forced expiratory volume 1/Forced Vital Capacity (FEV1/FVC) was 67,48 %. Cytologic examination of Pleural fluid and sputum showed a class II which means no malignant were found, only mesothelial and epithelial cells with inflammatory changes. Washing and brushing of FOB also showed a class II.

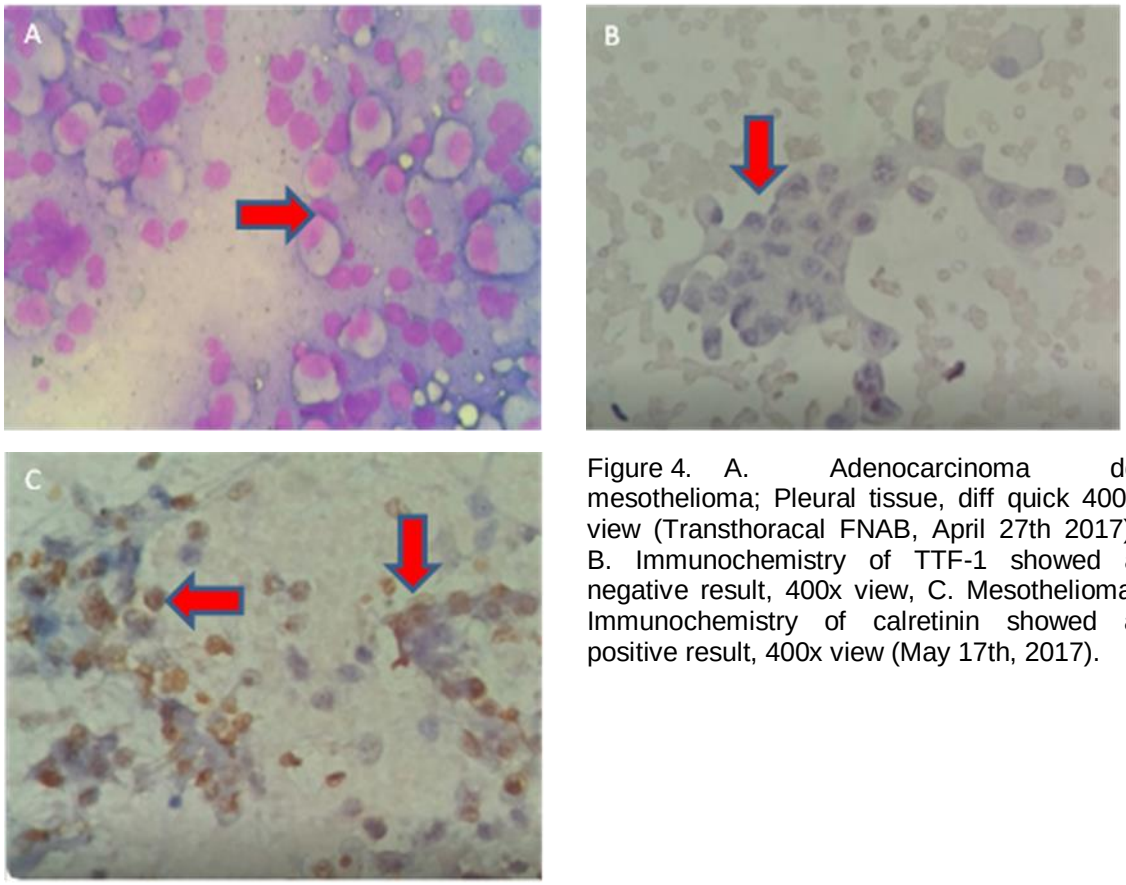

Figure 4. A. Adenocarcinoma dd mesothelioma; Pleural tissue, diff quick 400x view (Transthoracal FNAB, April 27th 2017), B. Immunochemistry of TTF-1 showed a negative result, 400x view, C. Mesothelioma; Immunochemistry of calretinin showed a positive result, 400x view (May 17th, 2017).

Based of findings above, patient was diagnosed with Right Pleural Mesothelioma T4N1M1 stage IV (with bone and liver metastases). The patient was then treated with Carboplatin/Gemcitabine chemotherapy and 3 out of 6 cycles were completed. The current result is a stable disease.

3. Discussion

Pleural mesotheliomas are considered relatively rare tumors. Eighty percent of all mesothelioma cases originate in the pleura.[1] In most epidemiologic surveys, mesotheliomas are more common in men (the typical male / female ratio is 5/1) and some conclude sex-related vulnerability[5].

From the history obtained, the main complaints from the patient were right chest pain and shortness of breath, and cough that is sometimes accompanied by blood spots. The most common symptoms appearing in mesotheliomas are chest pain and shortness of breath, usually due to massive pleural effusion[6.] Pleural mesothelioma patients can present with chest pain (pleuritic or non-pleuritic), shortness of breath, coughing and decreased breath sounds, weight gain, or a combination of these symptoms[7]. In our patient, systemic symptoms including nausea, decreased appetite and unexplained weight loss are also present. This is in accordance with clinical symptoms that can occur in mesotheliomas such as weight loss, chills, sweating, weakness, fatigue and anorexia, myalgia, aphonia, dysphagia, abdominal distension, nausea and discomfort in the mouth[6].

The patient had a history of working as a construction worker and in other fields related to asbestos exposure for more than 30 years. The relationship between

mesotheliomas and asbestos exposure is clear, with asbestos being the cause factor above 80%. Mesothelioma events rarely occur before asbestos is widely produced[8]. Over the past decade, there have been reported changes in exposure to mesothelioma cases. Asbestos processing work is divided into 2 which are primary asbestos workers (handling of asbestos raw materials) and secondary asbestos workers such as construction workers, electricians, plumbers, and heating workers. The highest risk of mesothelioma is in the first group[9]. After initial exposure to asbestos, the development of mesotheliomas until the onset of the disease can take 20-60 years. Although 70-80% of pleural mesothelioma is caused by occupational asbestos exposure, only about 5% of those exposed to asbestos develop into pleural mesothelioma[10].

From a physical examination, it was found that the frequency of breathing increases, dull on percussion in the lower right chest and a decrease in breathing sound in the lower right chest. The signs and symptoms discovered on this patient are similar to the previously known literature and upon further investigation, right pleural effusion was found. Evacuation of pleural fluid in the right chest proves the presence of right pleural effusion. Sixty percent of patients have right-sided lesions, historically related to gravitational tendencies wherein inhalation of asbestos fibers and dust travel directly to the right lower airway[1].

Radiological examination done in patient are chest Xray and Thorax CT Scan with contrast. Chest XRay showed a reduced amount of pleural fluid effusion and a clearer picture of what was previously suspected to be a right lung tumor, and CT Scan showed right hemithorax pleural mass, the impression of infiltration of intercostalis muscles, with the destruction of the 7th rib on the right

chest wall, accompanied by right perihilar lymphadenopathy, right pleural effusion, and liver nodules (T4N1M1 mesothelioma). This findings are in accordance to literatures in which was said that mesotheliomas can be present as thickening of the unilateral, concentric, plaque, or nodular pleura, but effusion can obscure the underlying pleural thickening. These tumors often extend into fissures with irregular contours[1]. Suspicion of CXR must be followed by contrast chest thoracic CT, with findings of "skin-like pleural involvement", "mediastinal pleural involvement", "pleural nodularity" and "pleural thickness of more than 1 cm" which potentially differs the malignant disease from benign pleural disease[11].

The bronchoscopic examination was carried out as one of the investigations since initially the patient was suspected as a right lung tumor with right pleural effusion. The results of bronchoscopic examination suggested infiltrative stenting in the superior and inferior lobes of the right lung, infiltrating, and obstructive stenting in the medial lobe suggestive of malignancy and chronic inflammation. But from the results of anatomic pathology examination on biopsy, washing-brushing preparations taken during bronchoscopy, cytologic sputum and pleural fluid cytology only obtained class II which meant no malignant cells were found. It is known that the diagnosis of malignant mesothelioma is difficult to establish, with symptoms and clinical findings that can be mimicked and imitated by other diseases[3,7]. The diagnosis of malignant mesotheliomas in the majority of cases is based on an evaluation of effusion. So that effusion cytology treatment can begin immediately.. However, if effusion cytology is inconclusive for the diagnosis of malignant mesotheliomas, a tissue core biopsy must be performed

according to previous recommendations, in this scenario, the two techniques complement each other[4]. In many centers, a tissue biopsy is the main examination for diagnosis, but some patients are in the poor physical condition and cannot tolerate surgical procedures. Various biopsy procedures might be used such as 'blind' percutaneous needle biopsy, fine needle aspiration (FNA) biopsy, imaging-guided core biopsy, video-assisted thoracoscopy (VAT)-guided biopsy, and thoracotomy[7].

USG-guided transthoracic FNAB was performed based on the result of the previous chest x-ray and CT scan. Samples obtained from FNAB showed a possibility of adenocarcinoma with a differential diagnosis of mesothelioma, so it was recommended to proceed with immunohistochemical staining with calretinin and TTF 1. Calretinin was tested positive in cells and TTF-1 was tested negative supporting the diagnosis of mesotheliomas. From the literature, two main differential diagnoses of mesotheliomas (MM) are adenocarcinoma and benign mesothelial cells. MM is distinguished from metastatic carcinoma with the help of ICC (Immunocytochemistry) or IHC (Immunohistochemistry). Metastatic adenocarcinoma reacts with BerEp4, B72.3, CEA and/or CD15. Malignant mesotheliomas are distinguished from adenocarcinomas by the use of specific antibodies. Malignant mesotheliomas are characterized by staining for EMA, calretinin, WT1, cytokeratin 5/6, HBME-1 (anti-mesothelial cell antibodies), or mesothelin (more than 85 percent of malignant epithelioid malignancies are positive for mesothelin) and the absence of antigen staining in antigens carcinoembryonic; Thyroid Transcription Factor-1 (TTF-1); and Ber-EP4[3,11]. Based on the

immunohistochemical examination of calretinin and TTF-1, in this case, it was concluded that reactive or malignancy could not be ruled out. It is more difficult to distinguish reactive mesothelial cells from those that are malignant based on immunohistochemistry, and at least two antibodies must be used in the panel[4].

In this case further immunohistochemical examination to differentiate malignant and reactive mesothelial cells was not carried out. Based on the consideration that from the results of a CT scan that have been done previously showed metastases to the 7th rib on the right wall and metastasis to the liver (liver nodules), right perihilar lymphadenopathy, right pleural effusion and infiltration to the intercostals muscle. So that it is concluded that the mesothelioma, in this case, is in fact a malignancy. Also, histopathological examination to identify the type of malignant mesothelial cells (epithelioid, sarcomatoid, desmoplastic, or biphasic) was not carried out as this will cause a delay for the patient to start treatment. Based on the latest data the patient can begin treatment as a malignant pleural mesothelioma T4N1M1. According to the International Mesothelioma Interest Group (IMIG) Stage IV Staging System, T4 is defined as mesotheliomas spread to the chest wall, peritoneum, spine, mediastinal organs, contralateral pleura, the internal surface of the pericardium or myocardium. N1 is defined as metastasis in the bronchopulmonary lymph nodes or lymph nodes ipsilateral. Lastly, M1 is defined as distant metastases.[1]

Three combination regimens are recommended as first-line treatment options for patients with untreated clinical IV stage, sarcomatoid type histology, or malignant pleural mesotheliomas that are medically inoperable or for those who refuse

surgery. 3 combination regimens include (1) cisplatin/pemetrexed (category 1), (2) carboplatin/pemetrexed, and (3) cisplatin/gemcitabine. Pemetrexed is the usual regimen used, whereas gemcitabine is only recommended for patients who cannot receive pemetrexed. These three combination regimens can also be used as adjuvant therapy for patients as part of multimodality therapy[12]. Our patient received chemotherapy treatment with a choice of regimen according to first-line therapy, namely carboplatin, and gemcitabine. But the combination of the two carboplatin and gemcitabine regimens does not match the 3 recommended first-line chemotherapy regimens, because of the limited regimens available at the hospital.

The patient was planned to receive carboplatin and gemcitabine chemotherapy as per the The Indonesia Society of Respirology (ISR) guidelines for lung cancer for non-small cell carcinoma lung cancer. Generally, chemotherapy can be given up to 4-6 cycles/sequence, or after the patient shows an adequate response. Treatment results for 4-6 cycles are not significantly different but giving 6 cycles can prolong the progression of the disease (time to progression = TTP). Evaluation of the therapeutic response is done by looking at the change in tumor size on the chest X-ray after the second chemotherapy cycle and if possible using a chest CT-scan after 3 times of administration. The 2009 Bangka Consensus recommends 4 cycles if it shows a permanent outcome (stable disease)[13]. Until now, the patient has completed 3 cycles of chemotherapy, and the chest X-ray had been evaluated after the 2nd chemotherapy cycle. The objective response obtained in this case was a relatively stable tumor size (stable disease). For the evaluation of objective responses after the 3rd cycle chemotherapy with CT-Scan could not be scheduled on time because the number of devices was not proportional to the number of queues of patients in the regional referral hospital. Leukopenia and anemia were found after chemotherapy which were known side effects of carboplatin/gemcitabine

chemotherapy for malignant pleural mesotheliomas[14]

4. Conclusion

Pleural mesothelioma is a rare malignancy related to chronic exposure of Asbestos. The diagnosis of this disease, however, remains a challenge because the clinical manifestation is very similar to adenocarcinoma since both diseases might invade the pleura. The patient was tested positive calretinin, negative for TTF-1 thus a diagnosis of Mesothelioma was established. Bone and liver metastases were found on Thorax CT-scan confirming a malignancy. The patient received treatment of Carboplatin/Gemcitabine. The patient showed a stable progression of disease in response to chemotherapy.

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