CASE REPORT

Pleural effusion of malignant aetiology: cell block technique to establish the diagnosis

Rafael García Carretero,¹ Manuela Manotas-Hidalgo,² Marta Romero Brugera,³ Liliam El Bouayadi Mohamed⁴

¹Hospital Universitario de Mostoles, Mostoles, Madrid, Spain

²Hospital de Día Oncológico, Doce de Octubre University Hospital, Mostoles, Madrid, Spain

³Department of Internal Medicine, Hospital Universitario de Mostoles, Mostoles, Madrid, Spain

⁴Department of Pathology, Hospital Universitario de Mostoles, Mostoles, Madrid, Spain

Correspondence to

Dr Rafael García Carretero, rgcarretero@salud.madrid.org

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SUMMARY

We describe cases of two previously healthy women presenting with progressively worsening breathlessness for 1–2 months. In both cases, physical examination was suggestive of a left-sided pleural effusion, confirmed by chest X-ray. Analysis of aspirated fluid showed a lymphocytic exudate, but cytological analysis was negative for malignancy in both patients. CT scan revealed malignancies as the underlying cause of the effusions. Both patients were managed with intercostal drainage in order to collect a sufficient amount of pleural fluid to perform a new technique in our hospital: cell block. This proved to be extremely useful in assessing the definitive diagnosis and management of both women. We briefly discuss the approach to a malignant pleural effusion and the aid of this not-so-new technique.

BACKGROUND

Assessment of a pleural effusion can be diagnostically challenging. Aspiration is a simple technique to achieve diagnosis, as information provided by body fluid analysis can assist clinicians in formulating and pinpointing the aetiology of effusion. It can also help in the differential diagnosis. Cytological examination, as one of the most commonly performed investigations, can help to identify malignant or reactive mesothelial cells,¹ but conventional cytological smears can be a diagnostic problem, as differentiation between benign and malignant cellular changes may be elusive when performing the conventional cytological smear method, given its lower diagnostic yield.

Although the cell block technique is one of the oldest methods for assessment of body cavity fluids, it may increase the sensitivity of detecting malignancies.¹ There are some helpful advantages, such as preservation of tissue architectural patterns or acquisition of multiple sections, so that pathologists can use special stains and immunohistochemistry.^{1 2}

CASE PRESENTATION

Case 1

A 74-year-old woman was admitted to the hospital with malaise, weight loss, cough and dyspnoea. Physical examination revealed an impaired general condition, and pulmonary auscultation showed hypoventilation in the basal and middle fields. The rest of the clinical examination was normal. The patient's blood pressure was 144/80 mm Hg, heart rate 90 bpm and oxygen saturation 95% on room air. She had type 2 diabetes and her medication

consisted of metformin 850 mg twice a day and insulin glargine 12 IU in the evening.

Case 2

A 35-year-old woman presented with a 2-month history of progressive dyspnoea, oppressive chest pain and difficulty breathing when lying flat (orthopnoea), as well as night sweats, fever and weight loss. Physical examination revealed decreased breath sounds on the left side. She had swelling in the ankles, feet and legs. Her blood pressure was 120/70 mm Hg, heart rate 92 bpm and oxygen saturation 92% on room air. She had no medical history of interest and was not taking any medication.

INVESTIGATIONS

Case 1

The routine blood tests were normal, but chest X-ray revealed massive left-sided pleural effusion, collapse of the left lung and a nodule near the right root of the lung (figure 1). Thoracentesis was performed and analysis revealed an exudate (fluid protein 3.6 g/dL; serum protein 6.9 g/dL; fluid glucose 123 mg/dL; fluid lactate dehydrogenase 335 U/L; serum lactate dehydrogenase 199 U/L; and fluid adenosine deaminase 1.6 U/L). Cytology of the fluid was negative for malignancy. Thoracic CT scan showed massive left-sided pleural effusion, which pushed the mediastinum towards the right; a lung mass in the left lower lobe (6.9×4.5×7.6 cm), which suggested malignancy; complete collapse of the left lower lobe and partial collapse of the left upper lobe; multiple pulmonary nodules, within both lungs, suggesting metastatic lesions; and several lymph nodes within the mediastinum.

As the massive pleural effusion was causing dyspnoea and discomfort, we decided to drain it through a pleural tube. By doing so, we were able to collect a considerable amount of pleural fluid in order to establish the final diagnosis. The cell block technique was therefore performed to achieve a suitable smear on which pathologists could see malignant cells, suggesting adenocarcinoma. Immunohistochemistry stains were used to rule out mesothelioma and to confirm lung adenocarcinoma. The cells were positive for the following stains: Ber-EP4, TTF-1 and CEA (figure 2); and negative for: WT-1 and calretinin. MicroRNA techniques established epidermal growth factor receptor mutation (exon 19 deletion confirmed).

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Figure 1 Chest X-ray at hospital admission showed the presence of left pleural effusion (A). CT scan showed complete collapse of the left lower lobe and partially of the left upper lobe (B). Mediastinum is deviated to the right.

Case 2

Chest X-ray revealed a massive left-sided pleural effusion and a widened mediastinum. CT scan revealed a large mass with a maximum diameter of $17 \times 13 \times 12$ cm within the mediastinum, squeezing the trachea, and both the left pulmonary artery and vein; bilateral mediastinal lymph nodes; massive left-sided pleural effusion; and complete collapse of the left lower lobe and partial collapse of the left upper lobe (figure 3). Thoracentesis was performed; the fluid was an exudate (fluid protein 4.1 g/dL; fluid glucose 84 mg/dL; lactate dehydrogenase 619 U/L; adenosine deaminase 37 U/L).

Bronchoscopy showed tracheal and left bronchial stenosis, and several samples were collected. Neither bronchial nor pleural fluid smears revealed conclusive results. Therefore, when the patient underwent intercostal drainage to relieve the dyspnoea, another sample of pleural fluid was collected so that the cell block technique could be performed. It revealed a non-germinal centre B-cell lymphoproliferative disorder. Later, when flow cytometry analysis of the fluid was performed, it showed a diffuse large B-cell lymphoma and detected the following CD markers: CD19+, CD20+, CD10–, CD15– and CD38–.

DIFFERENTIAL DIAGNOSIS

Pleural effusion suggests the presence of pulmonary, pleural or extrapulmonary disease. The differential diagnosis of pleural

effusion is wide but a systematic approach allows a definitive aetiology to be established.

The most common causes of pleural effusions are cardiac failure, pneumonia and malignant tumours. In order to establish a diagnosis, it is essential to obtain the patient's medical history and to perform a physical examination. Chest X-ray and analysis of pleural fluid are always required, and, if necessary, the diagnosis can be achieved with further investigations: CT, pleural biopsy, thoracoscopy or bronchoscopy.³

Medical history and physical examination can often be helpful in indicating appropriate investigations. Dyspnoea, cough, or pleuritic chest pain can be non-specific symptoms, but a history of cardiac, renal or liver disease can suggest a transudative effusion. Similarly, a history of cancer, weight loss or haemoptysis can be suggestive of a malignant pleural effusion, but haemoptysis can also indicate pulmonary embolism or severe empyema. Recent leg swelling or deep vein thrombosis may result in an effusion related to pulmonary embolism.⁴ Certain medications such as amiodarone or methotrexate, as well as conditions such as rheumatoid arthritis and other autoimmune disorders, can also result in effusions. If associated with fever, tuberculosis, empyema or pneumonia must be suspected.⁵

With regard to malignant conditions, lung cancer is the most common cause of a malignant pleural effusion (40%), followed by breast cancer (25%), ovarian and gastric cancer (5% each), and lymphoma (10%). Likewise, exudative effusions are a

Figure 2 Glandular structures formed by the malignant epithelial cells, which suggested adenocarcinoma of the lung. (A) H&E, $\times 20$ magnification. (B) Cell block section, stained with TTF-1, $\times 20$ magnification.

Figure 3 Left massive pleural effusion (A). Complete collapse of the left lower lobe and partial collapse of the left upper lobe, and large mediastinal mass (B).

common complication of lymphomas and neoplasms. Thoracic duct obstruction, impaired drainage, alteration of the permeability of the mesothelium or direct pleural infiltration appear to be the primary mechanism for pathogenesis of pleural effusion.^{6 7}

When the pleural effusion is suspected of being malignant, thoracentesis is the initial diagnostic—and often therapeutic—method of choice in these patients, especially when the patient is presenting with respiratory distress.

As previously mentioned, analysis of the fluid can quickly narrow down the list of differential diagnosis of an effusion. Although most cases of aspirated fluid are straw-yellow in colour, other appearances can be helpful: bloodstained, milky white, turbid, etc. Once aspirated, the fluid must be sent for biochemical, microbiological and cytological analysis. It is important to determine protein, pH, lactic dehydrogenase, glucose and albumin levels, as it is helpful to differentiate transudates from exudates.³

TREATMENT, OUTCOME AND FOLLOW-UP

Case 1

After the diagnosis was established, oncologists were asked for assessment. They considered the patient was only suitable for palliative and supportive care, as opposed to active chemotherapy treatment.

Case 2

After the diagnosis of a diffuse non-germinal centre B-cell lymphoma was established, bone marrow biopsy, cerebrospinal fluid (CSF) analysis and positron emission tomography CT (PET-CT) scan were performed to determine the stage of the disease. Bone marrow and CSF were normal, and the PET-CT showed the mediastinal mass, supradiaphragmatic lymphadenopathy, and atrial, pleural and pericardial involvement. Our patient underwent chemotherapy treatment with rituximab, cyclophosphamide, hydroxydaunorubicin, oncovin and prednisone (R-CHOP) and is currently in remission with regular visits with a haematologist–oncologist.

DISCUSSION

Cytological examination of serous effusions is essential in clinical medicine, as a positive diagnosis is often considered the definitive test in malignancies and several inflammatory and

non-inflammatory lesions and it obviates the need for exploratory surgery or further investigations.

Regarding malignant effusions, cytology alone is diagnostic in 33–72% of cases.^{3 8} However, in up to 74% of effusions, cytological results can be negative for malignant cells.⁹ With respect to lymphomas, there is a wide variation in the rate of positive cytological findings in pleural effusion (22–94%).⁶

One of the most prevalent issues in conventional cytology is the need to draw a distinction between reactive mesothelial cells and metastatic or primary cells.² There is a highly heterogeneous cell population when compared with that obtained by needle biopsy. There are plenty of reactive mesothelial cells and inflammatory cells, but a paucity of representative cells; lack of tissue architecture contributes to the considerable difficulties faced while making conclusive diagnosis. We must bear in mind that appreciation of tissue architecture makes diagnosis easier, and overcrowding of cells and processing artefacts do not help.² These issues are probably related to the low sensitivity for identifying malignant cells.¹

The cell block technique was introduced in 1896, and it has since been in use for processing fluids.² This technique is clearly not new, but it has a major advantage over the cytological method: it can avoid loss of valuable tissue particles. There are many methods for cell block preparation such as the plasma thrombin clot method, agar method or formalin method. The principle is that a gel would be formed due to the cross-linking of proteins. Since this gel could not be dissolved in any material during processing of fluid specimens, cell loss is avoided. These fixatives can better preserve the antigenicity and cytomorphological features, as they would produce a clot suitable for histological rather than cytological techniques, by obtaining a satisfactory specimen for sectioning or special, immunohistochemical stains.¹⁰ On the contrary, in conventional cytological smears, tissue particles cannot be properly examined on a smear because they do not provide sufficient material for histological sections.

The main benefit of the cell block technique is the recognition of histological patterns of disease that sometimes cannot be reliably identified in smear preparations, such as glandular structures, papillary structures or the demonstration of mucin in cytoplasm,¹ since the cell block method provides high

cellularity, better architectural patterns, morphological features and additional data of malignant cells, and increases the sensitivity of the cytodiagnosis when compared with the conventional smear method.² Similarly, glandular forms are more reliable in cell block. Therefore, in addition to increased cellularity, better morphological details are also obtained with the cell block technique, which includes preservation of the tissue architecture.

This material contains valuable diagnostic evidence and tissue fragments that cannot be processed by a cytological technique. Malignant cells not present on the smears are often found within the cell block and cases that are suspicious or elusive on the smears can be diagnosed with the aid of a cell block preparation.¹¹

Clot preparation can be used in conjunction with the smear to establish a more definitive cytopathological diagnosis. It may improve the accuracy and yield of the cytological diagnosis and can also greatly help special studies such as immunohistochemistry staining.¹² A cell block made from cell or tissue remnants can also be used for immunohistochemistry of specific biomarkers for accurate diagnosis of malignancy, such as calretinin, WT-1, etc.¹ The technique is simple, safe, cost-effective and reproducible, and should be used for assessment. The cell block allows morphological interpretation, as well as special staining and immunohistochemistry.¹

All these features increase the sensitivity of the diagnosis of malignancies by this method. Multiple sections could be obtained if required for special staining or immunohistochemistry study. Therefore, along with the routine conventional smear cytology, the cell block technique should be considered as a useful evaluation method for a final cytodiagnosis,² and it bridges the gap between both methods (cytology and hist-

ology).² Some studies^{1 13} confirm this technique may be useful in exudative pleural effusions caused by malignant conditions.¹⁴

As pointed out by Kalhor and Wistuba,¹⁵ the methodologies, practices and required tissue preparation may be different depending on the laboratory. Clinicians as well as pathologists should also be aware of the advantages and drawbacks of these two different techniques, in order to optimise diagnosis and avoid subjecting the patient to additional unnecessary sampling.

In our patients, the cell block technique was not prepared the first time the pleural fluid was sampled, as it is not a routine test in our hospital unless requested. The cell block technique was only requested from our laboratory when routine cytological examination of pleural effusion was negative.

Although cell block is not a new technique, we as clinicians must be aware of it. As previously mentioned, this technique may improve the performance of any sample obtained through thoracentesis, and clinicians should therefore be encouraged to use it. For this reason, we would like to emphasise its benefits and promote the use of this technique.

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Learning points

- The development of a malignant pleural effusion is a common complication and indication of advanced stages of cancers such as lung, breast or lymphomas. Examination of fluids from serous cavities of the body is an essential component of management in adult patients.
- Although the preparation of conventional smears is a much simpler procedure than that of paraffin sections, it has limitations due to the lack of tissue architecture, and this is why the cell block technique may be helpful.
- The cell block technique may increase diagnostic yield; it is not a new technique as such, but it can be an important complementary method for evaluation of body cavity fluids. It uses histological techniques for processing and therefore offers a valuable advantage, since multiple sections may be processed for both, routine stains and immunohistochemistry.
- Clinicians must be aware of this technique, the appropriate type of tissue needed for this specific test and the size of tumour needed for any particular methodology, as long as it may improve the performance of any pleural liquid sample obtained. Although it is not available in all laboratories, we would like to promote its use and to emphasise the educational purpose of this paper.

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