Concomitant Malignant Mesothelioma of the Pleura, Peritoneum, and Tunica Vaginalis Testis

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We describe the cytohistological, immunohistochemical and ultrastructural findings in a 55-yr-old-man with history of asbestos exposure and diffuse malignant mesothelioma (DMM) of the pleura, peritoneum, and tunica vaginalis presenting with chest pain and scrotal swelling. Pleural fine-needle aspiration (FNA) revealed mesenchymal elements and spindle-shaped epithelial-like cells, while biopsy showed pure sarcomatous tumor invading lung parenchyma. In both samples tumor cells coexpressed cytokeratin and vimentin. Peritoneal and hydrocele effusions contained aggregates of malignant mesothelial cells. Electron microscopy showed intermediate filaments, rare desmosomes and sparse microvilli. Morphological findings were consistent with a DMM, with a biphasic pattern in the pleura and an epithelial one in the peritoneum and tunica vaginalis. Although the possibility of a multicentric origin cannot be ruled out, clinical chronologic sequence suggests that the pleura was the primary involved site, followed by spread to peritoneum and tunica vaginalis. Diagn Cytopathol 1996;14:243–248.

Key Words: Cytokeratin; Vimentin; Sarcomatoid-mesothelioma; Electron microscopy; Effusion; Fine-needle aspiration biopsy

Diffuse malignant mesothelioma (DMM) arises most frequently in the pleura, less often in the peritoneum, and rarely in the tunica vaginalis testis. It is generally uniserosal at presentation;[1] synchronous multiserosal involvement may occasionally occur at an early stage.[2,3] Direct spread of pleural DMM through the diaphragm into the peritoneal cavity is a common late event in the course of disease or an autopsy finding.[3]

Although multiple tissue biopsy or open surgery are usually required to establish a definitive diagnosis of DMM, some investigators claim that it is possible by using limited tumor sampling such as fluid cytology[4] or fine-needle aspiration,[4,5] which is particularly useful for lesions without effusions or when thick adhesions prevent an endoscopic approach.

We report a case of DMM with unusual concomitant involvement of pleura, peritoneum, and tunica vaginalis testis as initial manifestation of disease, in a patient with asbestos exposure. The diagnosis was established on minimal cytohistological sampling and confirmed by immunohistochemistry and electron microscopy (EM).

Case Report

A 55-yr-old man, a smoker, was referred to our hospital for chest pain and sudden scrotal swelling. The patient had history of recurrent pleural effusion of 1 yr duration. Pleural fluid had been previously evacuated elsewhere and described to contain "atypical" mesothelial cells. CT scan of the thorax showed evidence of a rind of tissue encircling the right lung and decreasing its volume (Fig. 1A). CT scan of the abdomen demonstrated conspicuous thickened peritoneum and ascites. Ultrasound examination of the scrotum displayed normal testis, bilateral hydrocele and irregular, nodular thickening of the inner surface in the right portion; omolateral patent inguinal canal with tumor implants was also evident (Fig. 1B). Abnormal laboratory values included hemoglobin concentration 11.6 g/dl and platelets count 451,000/μl. The patient had a long history of occupational exposure to asbestos. He had been in contact with thermal insulation materials, as supervisor of the heating power station in a tire manufacturing plant. A pleural FNA biopsy and five additional specimens including bronchial washing (n = 1), peritoneal fluid (n = 1) and hydrocels (n = 3) were submitted for cytologic examination. Correlation of CT scanning with pathological find-

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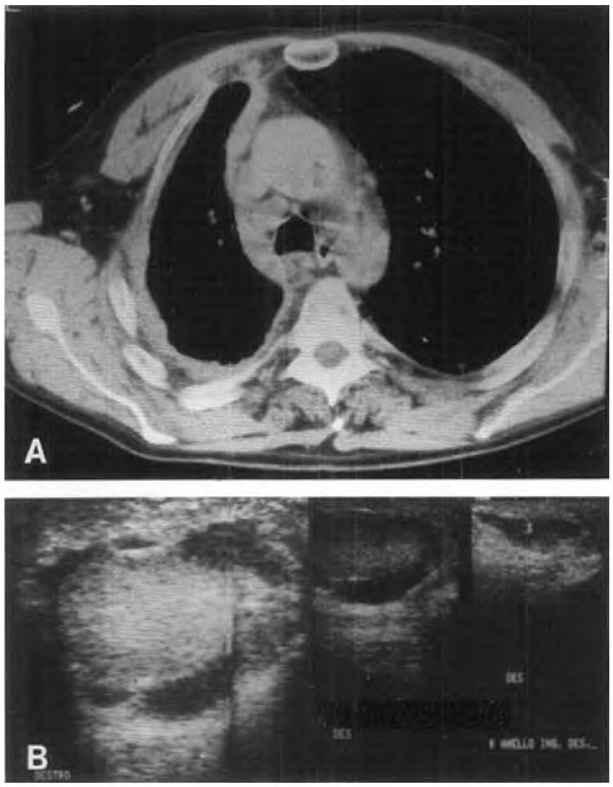

Fig. 1. A: Thoracic CT scan showing a rind of tumor tissue extending around the right lung. Contraction of the ipsilateral hemithorax; drawing of the mediastinum to the affected side is evident. There is also mediastinal adenopathy. **B:** Ultrasound scan of the right scrotum demonstrating diffuse, nodular thickening related to the scrotal sac lining (left and center). Note multiple small masses of increased echogenicity corresponding to tumor implants along the inguinal canal (right).

ings of both the aspirate and effusions led to a diagnosis of DMM (Stage IV).[6] The pleural tumor was typized as biphasic, while both peritoneal and tunica vaginalis samples were typized as epithelial. The patient rapidly deteriorated and died at home, 6 mo following hospital admission. An autopsy was not performed.

Materials and Methods

Transthoracic FNA (20-gauge) and core needle biopsy (Tru-Cut 14-gauge) were made through the pleural lesion under CT scan guidance, and routinely processed for cytology, histology, and EM. Smears and cell blocks were prepared from the cell pellet obtained from fresh effusions. Since not enough deposit was available for EM, previously Papanicolaou-stained smears were used to recover material, applying a modification of the cell transfer technique.[7] Briefly, after removing coverslips, smears were covered with mounting medium (Clearium, Surgipath, Grayslake, IL) and heated at 60°C for 1 hr to harden the resin. The mounting medium with attached cells was removed from the slides and placed into a glass tube containing xylene, and progressively rehydrated, fixed in 4% formaldehyde and processed for EM.

Smears were stained by conventional Papanicolaou and May-Grünwald-Giemsa techniques, and sections with hematoxylin and eosin (H&E), periodic acid Schiff-diastase (PAS-D) and hyaluronidase-colloidal iron methods. Immunoperoxidase studies were performed on sections and previously-stained FNA smears.[7] A standard peroxidase anti-peroxidase technique and the following battery of antibodies applied at standard dilutions were employed: polyclonal anti-carcinoembryonic (CEA), and monoclonal anti-cytokeratin MNF-116, Leu-M1 (CD-15), BerEP4, EMA, vimentin, actin. All antibodies were supplied by Dakopatts, Glostrup, Denmark, except anti-Leu-M1 supplied by Becton-Dickinson, Mountain View, CA.

Cyto-Histological, Immunohistochemical, and Ultrastructural Findings

Pleural DMM

Two main cell components were evident in FNA smears: clear-cut mesenchymal cells and epithelial-like elements (Fig. 2). The mesenchymal component consisted of tightly cohesive, multiple-cell thick fragments with peripheral finger-like projections composed of elongated fibroblastic cells (Fig. 2A,B). The epithelium-like cells consisted of monomorphous, medium-size, polygonal elements, occurring mainly in a loose flat arrangement or rarely in papillary fragments (Fig. 2C). The nuclei showed single or multiple, prominent nucleoli. Tumor cells of both components were strongly reactive for cytokeratin (Fig. 2D) and vimentin (Fig. 2E), and negative for other antigens. Occasional cohesive groups of irritated, hyperplastic alveolar cells with foamy cytoplasm were also encountered (Fig. 2F). The bronchial washing showed no evidence of asbestos bodies and contained only normal bronchial cells and macrophages.

Histologically, the specimen was mainly composed of densely collagenized fibrous tissue. However, at one edge more cellular foci of obviously neoplastic, spindle-shaped cells penetrating into lung were evident (Fig. 3). Cytokeratin-vimentin coexpression was demonstrated in these sarcomatous elements. Actin was focally immunodetected in the same cells. EMA was expressed only by alveolar pnemocytes. CEA, BerEP4, and Leu-M1 were negative.

EM study revealed a population of spindle-like shaped cells with elongated nuclei and prominent nucleoli. The cytoplasm was characterized by aggregates of monoparticulate glycogen and abundant perinuclear intermediate filaments, including tonofilaments (Fig. 4). Tumor cells were connected to each other by rudimentary intercellular junctions and desmosomes (Fig. 4, inset).

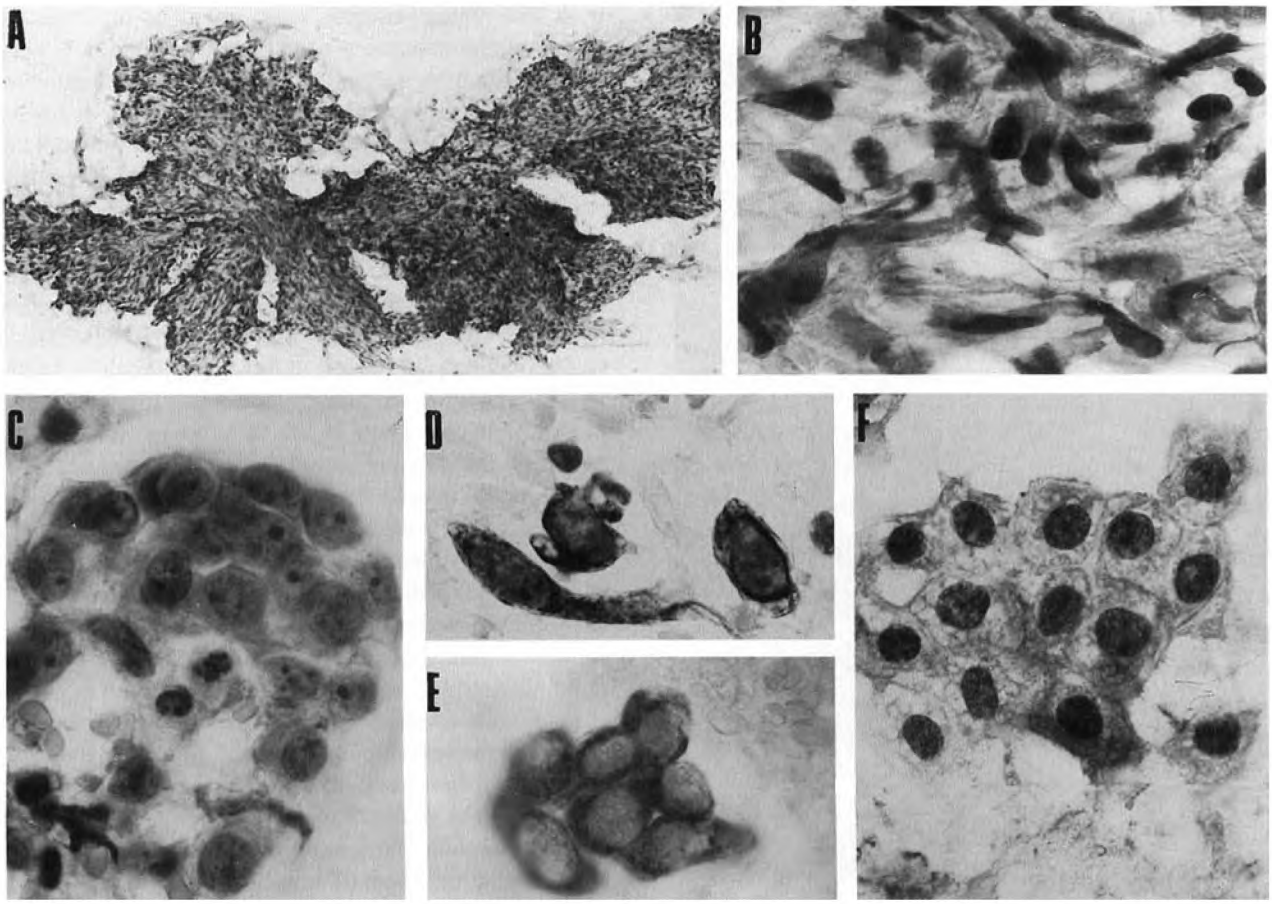

Fig. 2. Pleural FNA (smears). **A:** Wide tissue fragment of connective tissue cells with interweaving pattern (Papanicolaou stain, ×50). **B:** At higher magnification, haphazardly oriented spindle cells with elongated nuclei and pale cytoplasm (Papanicolaou stain, ×525). **C:** Loose papillary fragment of polygonal epithelial-like cells with dense cytoplasm. Nuclei have coarse granular chromatin and prominent nucleoli. Note distinct cell borders (Papanicolaou stain, ×525). **D:** Cytokeratin-positivity in spindle-shaped epithelial-like cell (immunoperoxidase stain, ×525). **E:** Vimentin-positivity in a cluster of polygonal tumor cells (immunoperoxidase stain, ×525). **F:** Flat sheet of benign, irritated alveolar cells (Papanicolaou stain, ×525).

Peritoneal and Tunica Vaginalis Testis DMM

The samples from both serosal cavities were similar in morphology and cell arrangement, showing many spherical clusters, tubular structures, and huge cell balls of mesothelial cells (Fig. 5). Cell-in-cell engulfment, peripheral cytoplasmic blebbing, and numerous abnormal mitoses were frequently noted. Isolated, binucleated or multinucleated neoplastic mesothelial cells with large nuclei, conspicuous nucleoli and compact cytoplasm were also a prominent feature. Colloidal iron and PAS-positive material was respectively removed by prior hyaluronidase and diastase digestion. Strong perinuclear immunoreactivity was observed with anti-cytokeratin in all tumor cells. In addition, anti-EMA revealed distinct "thick" cell membranes[8] (Fig. 5, right). Staining for BerEP4, CEA, and LeuM1 gave negative results.

On EM examination, the cells had polygonal contours with roundish nuclei and prominent nucleoli; desmosomes were occasionally encountered and long, slender microvilli were present on the cell surface.

Discussion

In this case report, the diagnosis of DMM was made with minimal tissue sampling, appropriate clinical findings[9–11] and confirmed by the gross appearance of the tumor, as assessed by CT scanning.[12] In the pleural cavity, the diagnosis of DMM was based on: a) the correlation of cytologic and histologic features, b) the coexpression of cytokeratin and vimentin with focal immunolabeling for actin, and c) the retention of some subcellular characteristics of mesothelial differentiation.[13] The case was further subclassified by the overwhelming pattern[2] as biphasic, predominantly sarcomatoid. By contrast, cytologic examination of serous effusions coupled with consistent EM findings and immunohistochemical tests provided the base

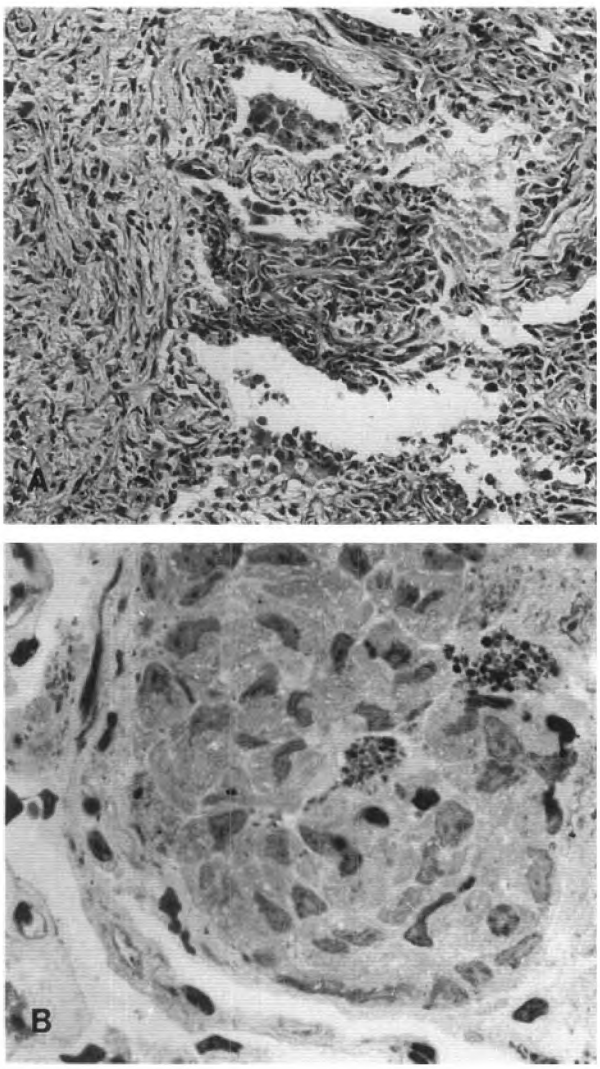

Fig. 3. Pleural biopsy (histologic sections). **A:** Advancing margin of sarcomatous growth. Several spaces lined by bronchioloalveolar epithelium containing macrophages are surrounded by spindle-shaped neoplastic elements (H&E stain, ×100). **B:** Note extrinsic compression of the interalveolar septum by a proliferation of polygonal cells with elongated, irregular nuclei (semithin section, toluidine-blue stain, ×525).

for the diagnosis of epithelial DMM in the peritoneum and tunica vaginalis.

This case represents an unusual DMM for concomitant involvement of three serosal cavities and morphological features of both epithelial and biphasic-type. Synchronous pleural and peritoneal DMM may occur at an early stage of disease, in which it is impossible to find out which cavity is primarily involved and the possibility of a multicentric origin arises.[3] Single cases of primary DMM of the tunica vaginalis with a possible independent pleural[14] and peritoneal[15] DMM have been rarely reported. On the other hand, we are unaware of previously documented cases of concomitant pleural, peritoneal, and tunica vaginalis involvement as initial manifestation of the disease. An exception is a case of pleural DMM which at autopsy was found to have penetrated the diaphragm, spread to the peritoneal cavity and extended around the spermatic cord to the tunica vaginalis.[16] The site of origin of the DMM in the present case is difficult to determine, because either pleural, peritoneal, or even tunica vaginalis testis source is possible. However, clinical chronologic sequence suggests that the pleural cavity be the primary serosal site involved, followed by the peritoneum with subsequent extension to tunica vaginalis. Usually, contiguous spread of pleural DMM other than lung, mediastinum, controlateral pleura, and chest wall is the diaphragm, with potential extension into the peritoneal cavity.[1-3] Peritoneal DMM may in turn invade the tunica vaginalis and present as inguinal mass,[17] as it occurred in the present case. Although in the pleura the biphasic growth was predominantly of sarcomatoid-type, only epithelial aggregates were found in the other two cavities. This discrepancy can be explained by the fact that DMMs are known for their phenotipic variability within a given tumor[1-3] and that the two components of DMM with a biphasic pattern may metastatize separately.[2,18] In our case, it is most likely that only the epithelial component of the pleural DMM diffused to the other serosal cavities. In order to explain the morphological differences between pleural and peritoneal/tunica vaginalis tumors, it can also be hypothesized that multiple independent DMMs have concomitantly developed. In any event, since neither extensive sampling to show the tumor appearance throughout nor an autopsy has been performed, we cannot figure out with certainty whether the peritoneal and/or tunica vaginalis DMMs were secondary to pleural DMM or independent tumors.

The presence of atypical mesothelial cells was apparently undervalued at the time of the first cytological examination; thus, the recurrent pleural effusion 1 yr earlier, was probably mesothelioma-related. On the other hand, the effusion could have been benign and related to asbestosis.[19] Patients with DMM usually have no specific biochemical and hematological findings, except a number of them who have high platelet count,[20] like in the case under discussion. Since certain occupations carry a high risk of DMM, notably heating trades,[21] in our patient direct exposure to asbestos used as thermal insulating material in confined spaces[21] might have determined the development of multiple mesotheliomas. Additional identifiable asbestos exposure included working in the tire manufacturing industry, where talc powders that can be contaminated with asbestos are widely used.[22]

In conclusion, the reported case highlights the importance of a watchful technical approach to the handling of

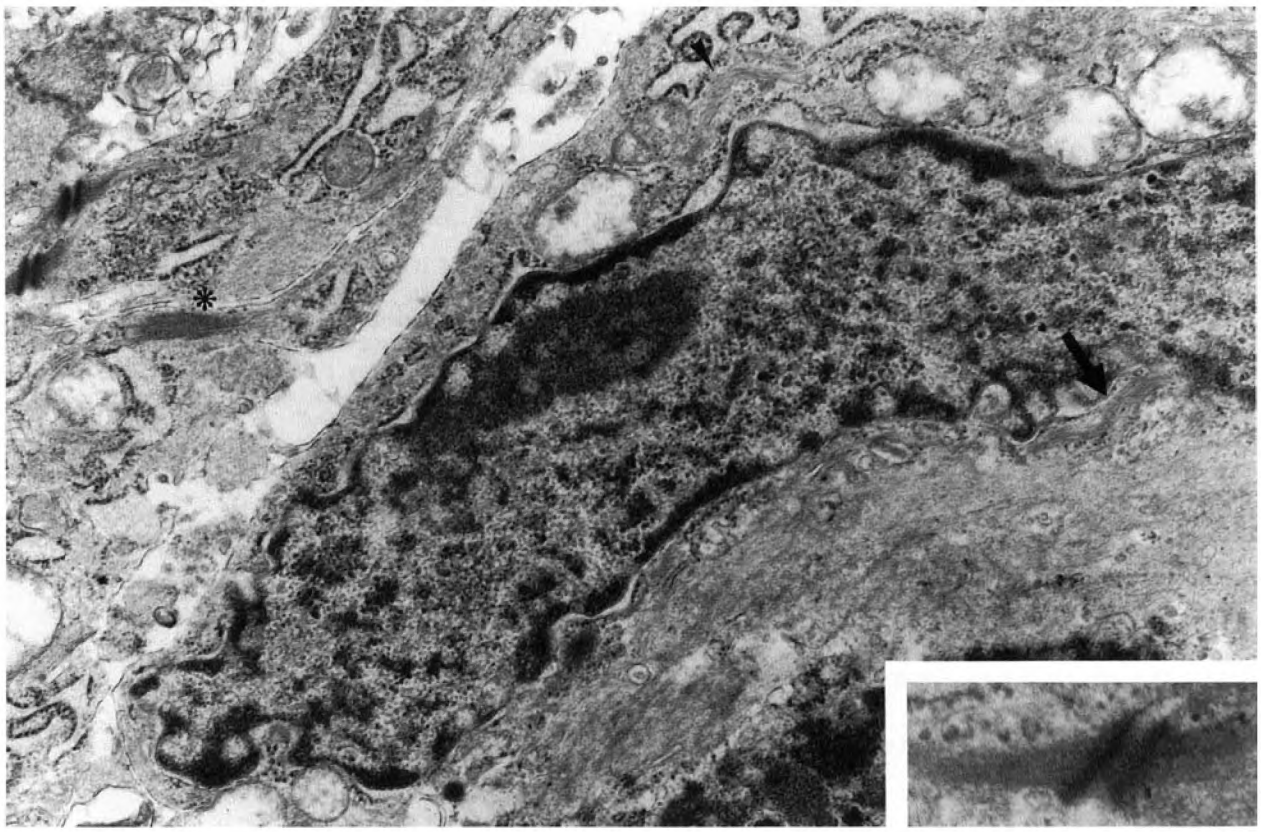

Fig. 4. Ultrastructural micrographs of the pleural sample. The tumor cell has a large nucleus with a prominent nucleolus; the cytoplasm contains several bundles of intermediate filaments. Note perinuclear filaments (arrow), randomly dispersed intermediate filaments (arrowhead) including tonofilaments (asterisk) and desmosomes **(inset)** typical of mesothelial differentiation (uranyl acetate-lead citrate stain, ×24,500; inset ×69,000).

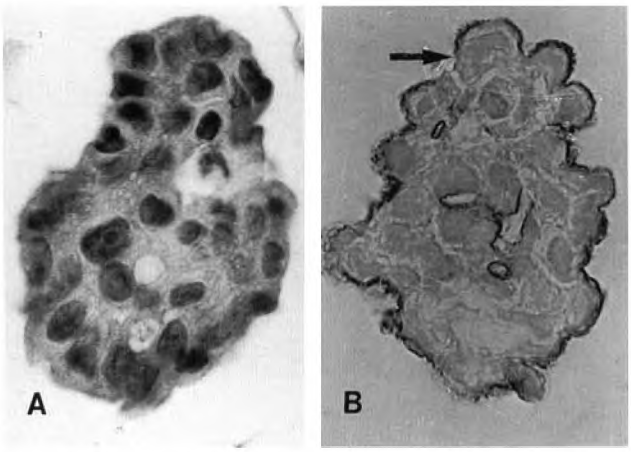

Fig. 5. Peritoneal fluid (cell block sections). **A:** Large cell aggregate of mesothelial cells with a knobbly external contour (H&E stain, ×525). **B:** Thick cell membrane immunolabeling with anti-EMA is striking (long arrow) (immunoperoxidase stain, H&E counterstain, ×525).

limited cytologic specimens, cell recovery for EM and immunostain testing coupled with clinico-radiological correlation to provide a definite diagnosis of DMM and eliminate unnecessary open biopsy.

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