Bicavitary Effusion in a Horse

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SPECIMEN: Direct smear of thoracic fluid

SIGNALMENT: 9-year-old Missouri Fox Trotter mare

HISTORY:
A 9-year-old Missouri Fox Trotter mare presented to the primary veterinarian for evaluation of respiratory disease and lethargy. Approximately one week prior to presentation, the mare began flaring her nostrils and developed increased abdominal effort while breathing. Physical exam at this time was unremarkable, however, a rebreathing exam revealed increased bronchovesicular sounds and the mare was placed on oral trimethoprim sulpha (10,000mg twice daily). Reportedly, a CBC and biochemistry performed by the primary veterinarian was unremarkable besides a mild eosinophilia (referring veterinary records not available). The mare worsened over the next few days, developing a mild respiratory stertor, and was then referred to the University of Minnesota Equine Center for further investigation.

CLINICAL FINDINGS:
On physical exam, the mare was quiet, alert, and responsive. Temperature was within normal limits, however the horse was mildly tachycardic (56 beats per minute), and moderately tachypneic (32 breaths per minute). Mucous membranes appeared injected and a prolonged capillary refill time (3 seconds) was noted. Diminished bronchovesicular sounds were present on thoracic auscultation and rebreathing exam. The horse also had ventral edema extending from the pectoral muscles to udder and marked muscle wasting of the pectoral and topline muscles. A CBC revealed a mild eosinophilia: 0.47 x 10^3/microL (RI: 0 – 0.3 x 10^3/microL) and a mild hypokalemia: 3.4 mmol/L (RI: 3.6-5.1 mmol/L) and minimally increased bicarbonate: 31.6 mmol/L (RI: 25-31 mmol/L) were noted on serum biochemistry profile. On thoracic radiographs, the horse had pleural effusion, an alveolar pattern in the ventral lung fields, and a moderate unstructured interstitial pattern in the remainder of the lung fields. Endoscopy of the respiratory tract was unremarkable, although a mild eosinophilic inflammation was found on bronchoalveolar lavage. Thoracic ultrasound showed moderate amounts of pleural fluid in the ventral lung field and thickening of the pleural surface. Abdominal ultrasound

identified small amounts of increased peritoneal fluid. Six liters of fluid were removed from the right side of the thorax, and one liter was removed from the left side of the thorax via thoracocentesis. Samples of the thoracic fluid and abdominal fluid were submitted for analysis and appeared similar cytologically.

CYTOLOGIC FINDINGS:
Cytology – Specimen Description
Color: Yellow
Turbidity: Slightly Turbid
Viscosity: Low
Cytology – Numeric Results
Volume: 4.0 ml
Nucleated Cells: 5010 cells/µL
Total Protein: 5.2 g/dL

CYTOLOGY:

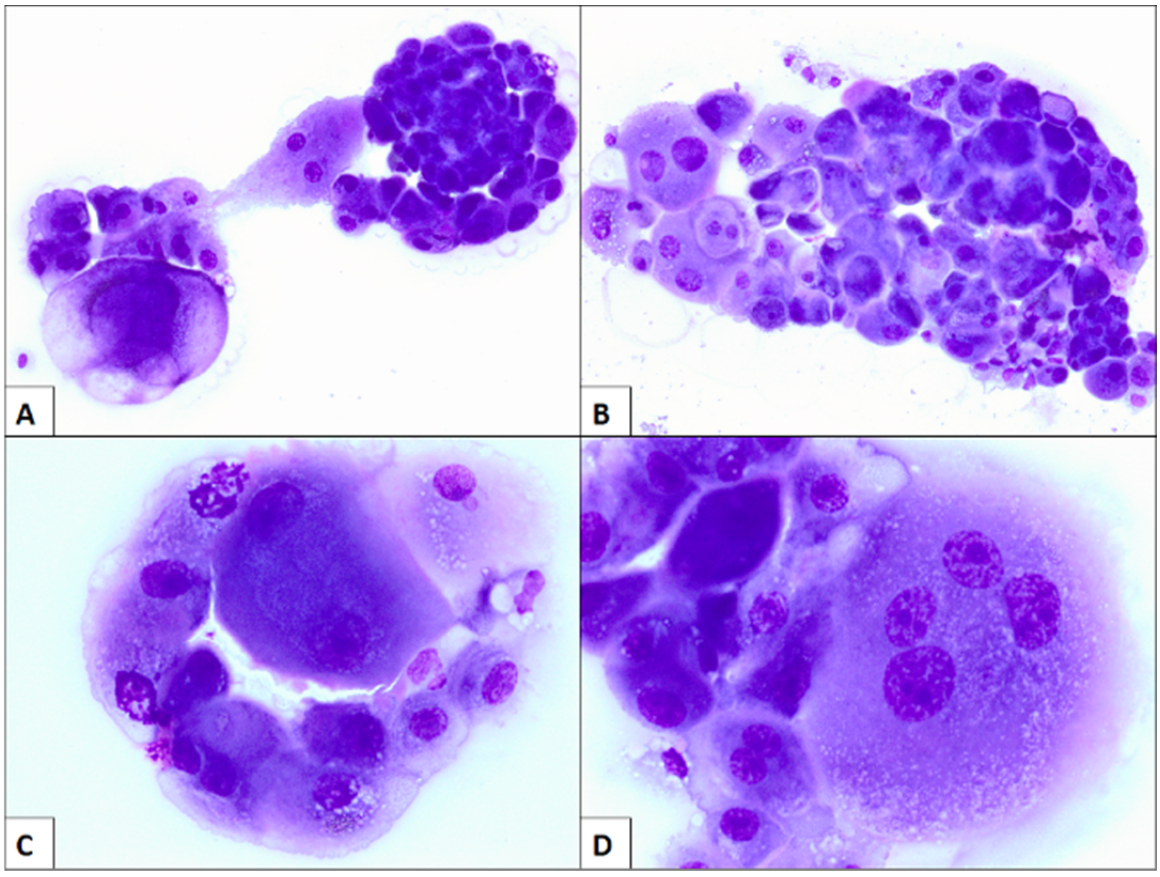

Figure 1. Thoracic fluid from a horse. Wright-Geimsa. **(A)** and **(B)** x20 objective. **(C)** and **(D)** x50 objective.

QUESTIONS:
1. Provide a cytologic description and potential differentials
2. What additional tests could be performed to further evaluate the diagnosis?

CYTOLOGIC DESCRIPTION:
Samples were moderately increased in cellularity and consisted primarily of non-degenerate neutrophils with lesser numbers of large mononuclear cells and occasional small mononuclear cells. Notably, a distinct population of markedly atypical epithelioid appearing cells occurring in cohesive but markedly disorganized sheets and clusters were concentrated along the feathered edge. These cells had cuboidal to polyhedral cell margins, many of which had a pale pink corona. Cells contained variable amounts of vacuolated basophilic cytoplasm with one, to occasionally two, nuclei with prominent nucleoli. Numerous criteria of malignancy were noted, including frequent microsatellite nuclei, multiple nucleoli, marked anisocytosis and anisokaryosis, a high nuclear to cytoplasmic ratio, rare signet rings, and rare mitotic figures. (**Figure 1 A-D**)

CYTOLOGIC INTERPRETATION:
Neoplastic effusion. Probable carcinoma, rule out mesothelioma

CLINICAL OUTCOME:
Despite administration of fluids and electrolytes via a nasogastric tube and twice daily intravenous Flunixin (1.1 mg/kg), the ventral edema worsened, the patient showed signs of pain on palpation of the cranial thorax, and became increasingly depressed. Due to the poor prognosis the owners elected humane euthanasia three days after initial presentation.

GROSS NECROPSY FINDINGS:
Grossly the thoracic cavity contained a small amount of amber-colored, transparent, watery fluid. Widespread on both the parietal and visceral pleura were multifocal to coalescing brown to tan, irregular, soft, minimally raised foci along with multifocal tan, stringy, friable, and easily removed material (fibrin). The pericardial sac was diffusely thickened and there was a large amount of adipose tissue adhered to the pericardial sac. Similar multifocal to coalescing brown to tan, irregular, soft, minimally raised foci were scattered over the entire surface of the pericardial sac and adhered adipose tissue. An approximately 15 cm x 8 cm area of white to tan, semi-firm, string-like material (fibrous adhesion) was firmly adhering the left caudal lung lobe to the left thoracic wall and parietal pleura of the diaphragm. Multifocally on the parietal pleura and peritoneum covering the diaphragm were yellow to tan, irregular, minimally raised, semi-firm, plaques. The abdominal cavity contained a moderate amount of fluid similar in color and consistency to the thoracic cavity. The omentum was diffusely dark red to black with widespread pinpoint, white, nodules arranged in a linear fashion. Samples of the multifocal plaques on the pericardium, omentum, diaphragm, pleura, intercostal muscle, and surrounding connective tissue were routinely collected and processed for histopathology. (**Figure 2A&B**)

HISTOPATHOLOGIC FINDINGS:
Within the pericardium was an unencapsulated mass consisting of small aggregates and densely cellular sheets of anaplastic neoplastic cells. The neoplastic cells were round to spindle shaped with indistinct cell borders and contained a moderate to large amount of pale, eosinophilic, foamy cytoplasm. Nuclei contained one to multiple round to oval vesiculated nuclei with finely stippled chromatin, and one to multiple, prominent round to oval nucleoli. There were numerous bi- and multinucleated cells, along with a high degree of anisocytosis, anisokaryosis, and karyomegaly. There were scattered, rare mitotic figures (an average of <1 per 10 high power fields). Aggregates of neoplastic cells were found within the lumina of numerous blood vessels and lymphatics and there

was scattered individual neoplastic cell degeneration and necrosis/apoptosis. Other sections collected contained a similar population of neoplastic cells. (**Figure 3**).

IMMUNOHISTOCHEMISTRY:
In the section of pericardial adipose tissue, approximately 90-95% of the neoplastic cells were strongly immunoreactive for broad-spectrum cytokeratin and cytokeratin 5/14 and approximately 80-90% of the neoplastic cells were moderately immunoreactive for vimentin (**Figure 4**). Neoplastic cells were negatively staining with calretinin (not shown).

TRANSMISSION ELECTRON MICROSCOPY:
The most important characteristics of the anaplastic cells included the presence of undulating microvilli surrounding large segments of the cell surface that formed dilated intercellular spaces. Intercellular attachments were frequent and were identified as zonula and macula adherens. Occasionally, there were intracytoplasmic lumina, which were occupied by a profusion of microvilli. The cytoplasm exhibited large amounts of intermediary filaments arranged in bundles **(Figure 5**).

MORPHOLOGIC DIAGNOSIS:
Widely disseminated pleomorphic epithelial type mesothelioma of the pericardial sac and associated adipose tissue, omentum, lung, diaphragm, thoracic and abdominal cavity pleura.

CASE SUMMARY:
Bicavitary mesothelioma in a horse

DISCUSSION:
The presentation of a bicavitary effusion in this case was interesting, given the rarity of this finding in horses in which it is much more common to see solitary pleural or abdominal effusion. In horses, the most the common causes of pleural effusion are bacterial pneumonia and lung abscess, whereas ascites most often results from peritonitis or abdominal neoplasms such as lymphoma, squamous cell carcinoma, mammary adenocarcinoma, and mesothelioma[1-3]. Although cytology can be helpful in discriminating between some of these neoplastic and inflammatory conditions, it is often difficult to differentiate between reactive and neoplastic mesothelial cells, as well as between carcinoma, mesothelioma, and adenocarcinoma.

Similar to veterinary species, distinguishing between reactive and neoplastic mesothelial cells often poses a diagnostic challenge in human medicine. Several clinical, cytologic and histologic characteristics have been proposed to aid in evaluation including the amount of fluid accumulation, degree of concurrent inflammation, presence of necrosis, and most importantly, evidence of stromal invasion on histopathology[4]. Unfortunately, cytologic atypia and mitotic activity alone are often not reliable for definitive diagnosis, as there is significant overlap between cytologic features of reactive and neoplastic cell populations, especially in cases of well-differentiated neoplasia[1,4]. In our case, based on cytology alone, we could not fully rule-out a reactive cell population, however, we thought this unlikely considering the bicavitary location and amount of effusion, relatively minimal concurrent inflammation, and extreme cellular atypia. This was further supported by the degree of tumor-affected tissue noted on gross histopathology as well as invasiveness into surrounding tissue and presence of lymphatic and vascular emboli.

After a reactive mesothelial cell population has been ruled-out, the next diagnostic challenge is discriminating between a mesothelial and epithelial origin[1,4]. Grossly, mesotheliomas often have a widely disseminated, multifocal, nodular to papillary appearance associated with serosal surfaces and lack the presence of a primary mass[4]. In humans it is reported that histologically, and occasionally cytologically, mesotheliomas tend to display a papillary structure, whereas carcinomas are arranged in spheres and acini[4]. Additionally, mesotheliomas can appear epithelial, mesenchymal, or mixed and are subclassified into epithelial, sarcomatoid, and biphasic subtypes. In people, the recommendation is to determine the histologic subtype and then choose a panel of immunohistochemical markers depending on the primary differential diagnoses for the location and tumor type[4]. Given the substantial development of neoplastic surface markers in human medicine, the staining pattern often leads to a definitive diagnosis. In cases where immunohistochemistry does not yield a clear diagnosis, electron microscopy is used for definitive diagnosis[4]. Unfortunately, in veterinary medicine the availability of such specific cell markers is lacking, and therefore immunohistochemistry is usually limited to staining with broad-spectrum cytokeratin and vimentin[3,5]. Although mesotheliomas tend to stain positive for broad-spectrum cytokeratin and vimentin, in contrast to carcinomas, which stain positive for broad-spectrum cytokeratin but negative for vimentin, highly anaplastic tumors may stain aberrantly. Therefore, evaluation of ultrastructural features via electron microscopy is often recommended for definitive diagnosis in veterinary medicine with the defining features of mesotheliomas being long microvilli, desmosomes, and tonofilaments[4-6]. Ultimately, in veterinary medicine, the diagnosis of mesothelioma is made by a combination of clinical presentation, cytologic findings, histopathology, IHC, and ideally ultrastructural features[5]. Since neoplasia was suspected in our case, a mesothelioma was favored on histopathology due to the absence of a primary mass and dual immunopositivity for broad-spectrum cytokeratin and vimentin. Furthermore, immunopositivity for cytokeratin 5/14 is supportive for mesothelioma over carcinoma, where in humans it is recommended as one of the most specific and sensitive immunohistochemical markers for diagnosing mesothelioma[4]. The diagnosis was then confirmed on TEM given the finding of long microvilli, desmosomes, and intermediate filaments.

Interestingly, calretinin, which is one of the other highly sensitive and specific immunohistochemical markers used for diagnosis of mesothelioma in humans, was negative in this case[4]. This is in contrast to a recent case report of equine mesothelioma, which stained positive to calretinin. The negative staining in our case may have been due to aberrant receptor expression considering the highly anaplastic nature of the tumor, variability in expression by equine mesotheliomas, or suboptimized staining technique.

Mesothelioma is uncommonly reported in veterinary species, however there are small numbers of case reports in the literature and it is reported as the second most common primary thoracic tumor in horses, with lymphoma being the most common [1-3,5-12]. Older horses tend to be affected, although it has been seen in horses from 2-13 years of age[2]. In contrast to humans, a predisposing factor is unknown, as asbestos exposure has not been identified in reported equine cases. Clinical signs are often vague, but respiratory distress is the presenting complaint in some patients, similar to the case reported here[2]. Similar to the case reported here, the primary site of origin is sometimes difficult to establish, as pleural and peritoneal mesothelioma can be multicentric in origin, and transcoelomic metastasis can occur[1]. Due to the poor prognosis, affected horses are often euthanized without treatment.

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FIGURES WITH LEGENDS

Figure 2A

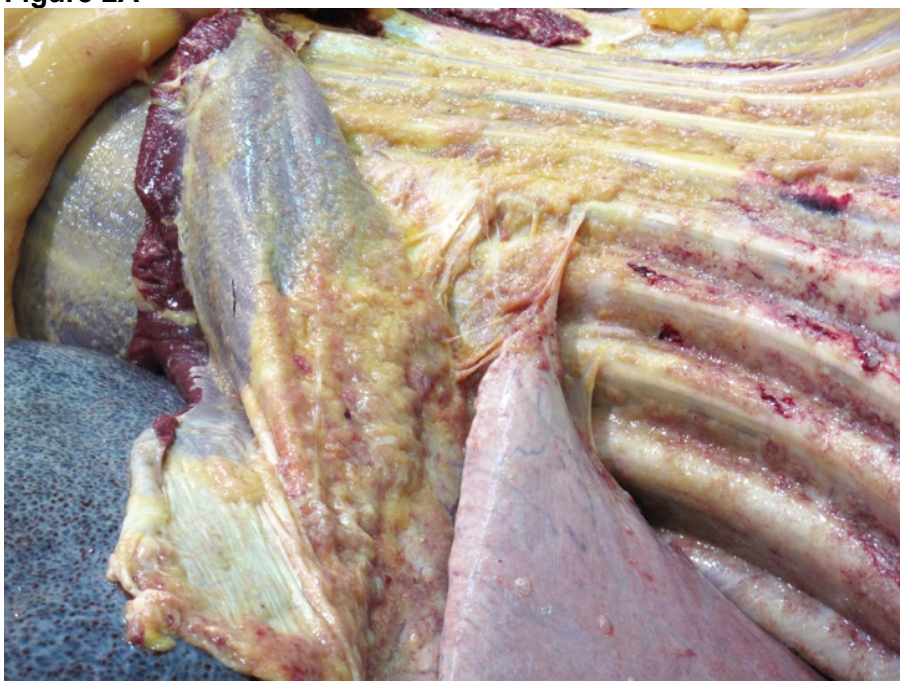

Figure 2B

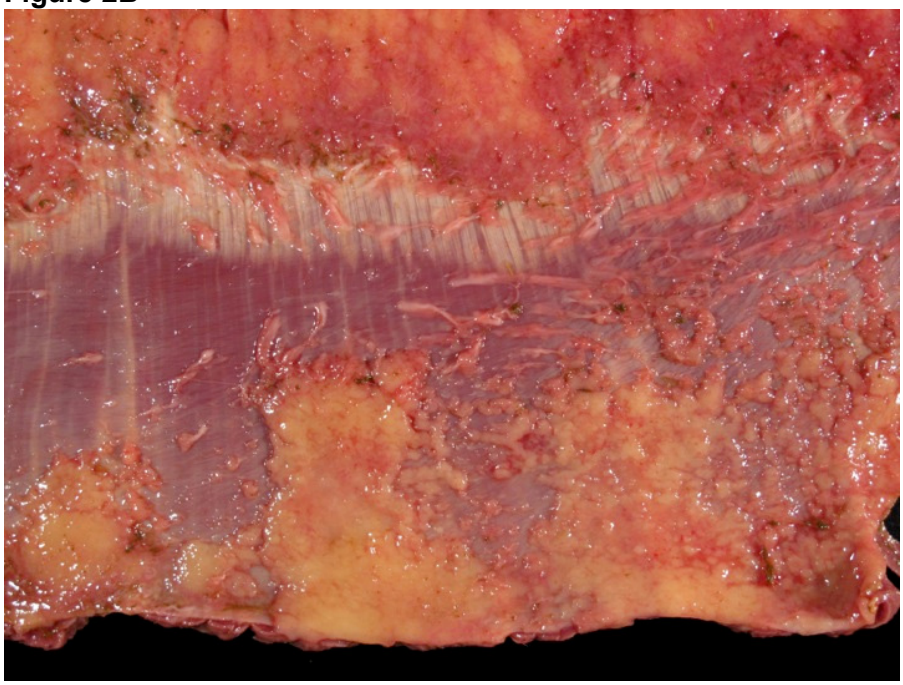

Figure 2: Photograph of the thoracic wall (rib cage), left caudal lung lobe, diaphragm, and spleen of a 9-year-old Palomino, Missouri Fox Trotter horse. **A**. The left caudal lung lobe is firmly adhered by fibrous material to the left thoracic wall and the parietal pleura of the diaphragm. Multifocally the parietal and peritoneal pleura of the diaphragm is covered by yellow to tan, irregular, minimally raised, semi-firm plaques. **B.** Close up photograph of the pleura of the diaphragm covered in plaques as described in Figure 2A.

Figure 3

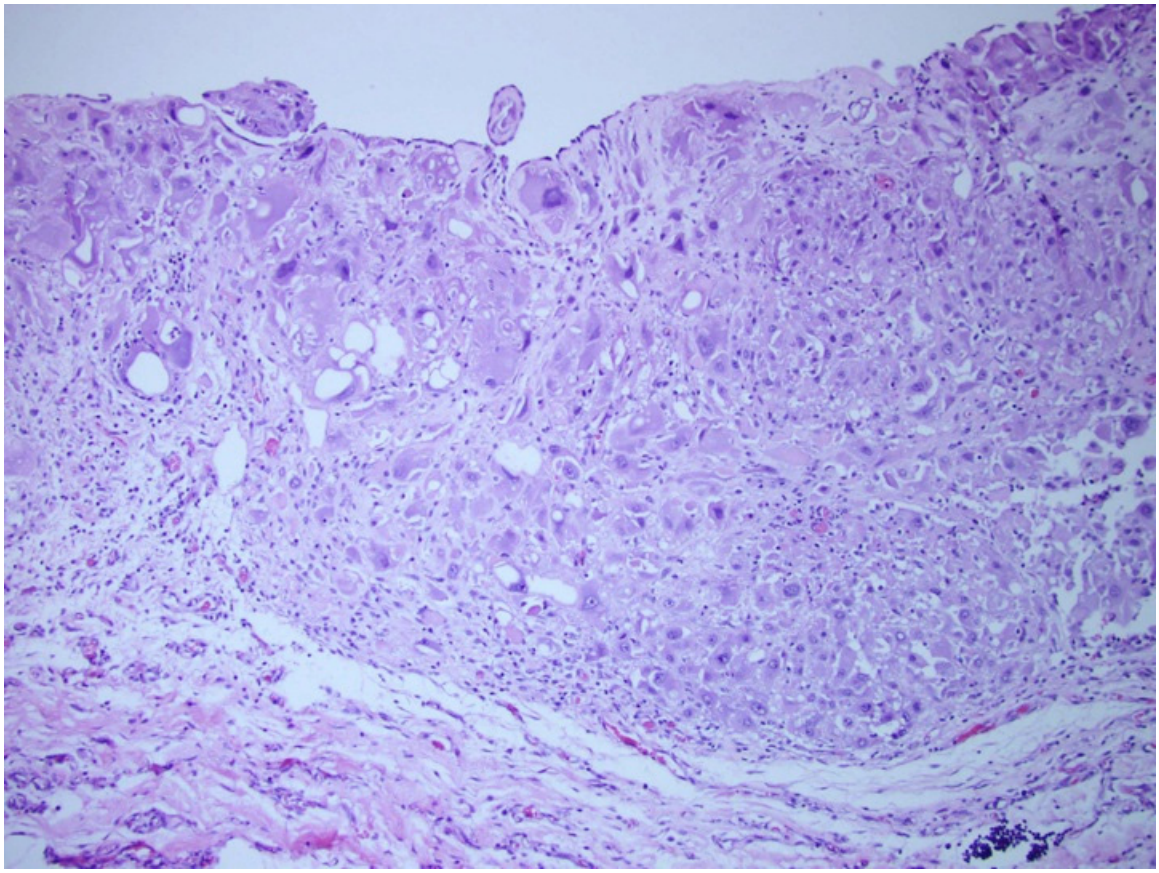

Figure 3. Photomicrograph of a section of pericardium with plaques seen grossly in Figure 2. The neoplastic cells are aligned in a plaque-like arrangement along the connective tissue of the pericardium. Note the neoplastic cells demonstrating a high degree of pleomorphism, anisokaryosis, and karyomegaly. H&E stain x10 objective.

Figure 4

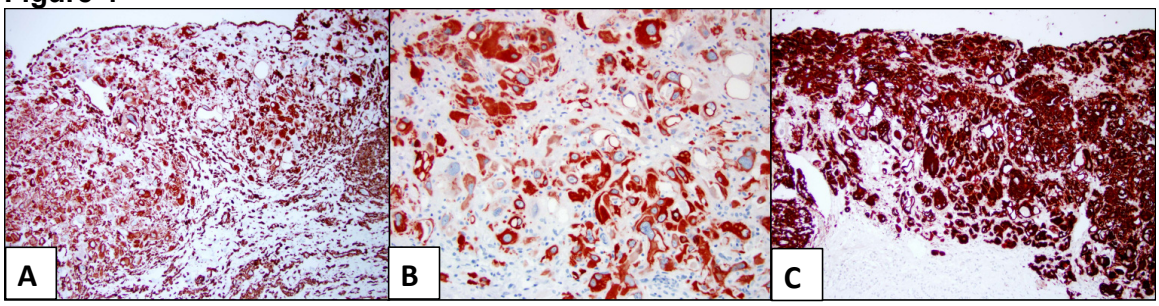

Figure 4. Positive immunohistochemical staining of neoplastic cells in a section of pericardium for (A) broad-spectrum cytokeratin, (B) cytokeratin 5/14, and (C) vimentin. x20 objective.

Figure 5

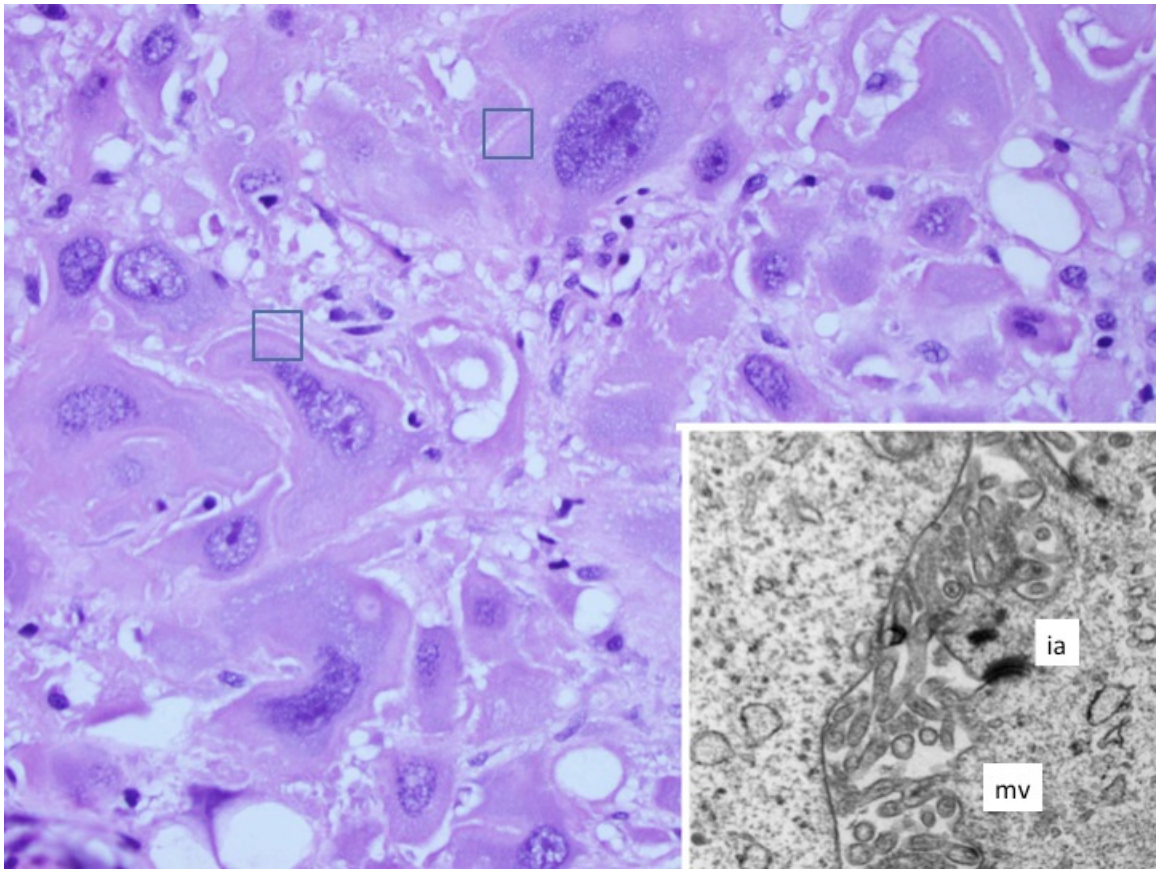

Figure 5. Close up photomicrograph of a section of a section of pericardium as described in Figure 3 (x40 objective). Inset: Transmission electron microscopy of parietal pleura. mv = microvilli and ia = intercellular attachments (x20,000 objective).