

Thoracic mass in a dog

Contributors

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Specimen

Fine Needle aspirate biopsy of a thoracic mass

Signalment

12 years old female cross breed dog.

History

The dog was presented on an emergency basis for acute dyspnea following a three-month history of coughing that had shown partial improvement with antibiotic (amoxicillin-clavulanic acid) and corticosteroid therapy. Thoracic radiographs revealed marked pleural effusion, and 1.6 L of fluid were drained by thoracocentesis. The pleural fluid was dark amber in color and moderately turbid. Fluid analysis revealed a total nucleated cell count of 260 cells/ μ L and a total protein concentration of 4.1 g/dL. No neoplastic cells were identified, and the underlying cause of the effusion could not be determined based on fluid analysis alone. The patient was subsequently referred to the oncology service for further investigation of a suspected neoplastic process.

Clinical findings

Initial clinical examination at the referring hospital revealed mild tachypnea, while the remainder of the physical examination was unremarkable. Hematologic and biochemical analyses showed no significant abnormalities.

Thoracic computed tomography (CT) identified an irregular, heterogeneously contrast-enhancing mass within the ventral mediastinum, surrounding the cardiac apex without evidence of diaphragmatic invasion (figure 1).

Fine needle aspiration of the mass was performed for cytologic evaluation (figures 2-3).

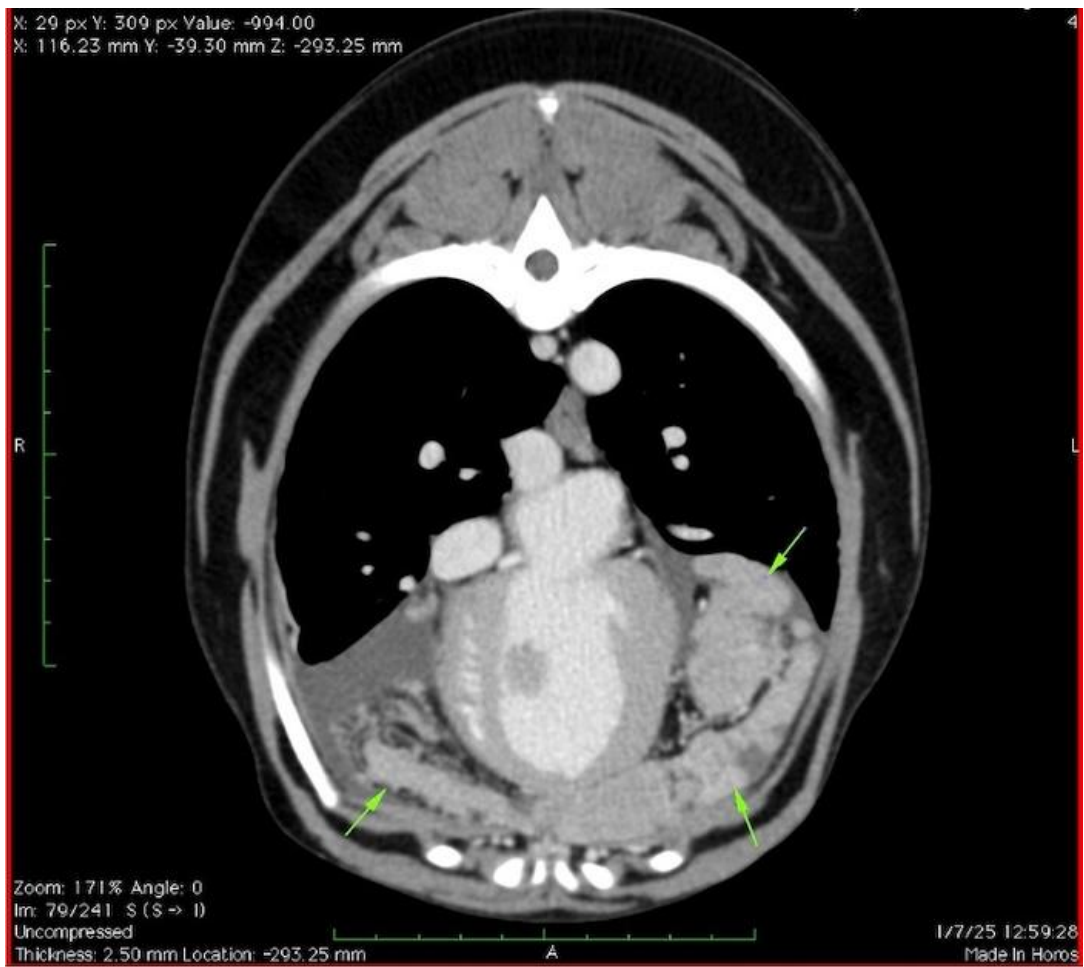


Figure 1. Thoracic CT scan demonstrating a large ventral mediastinal mass (green arrows).

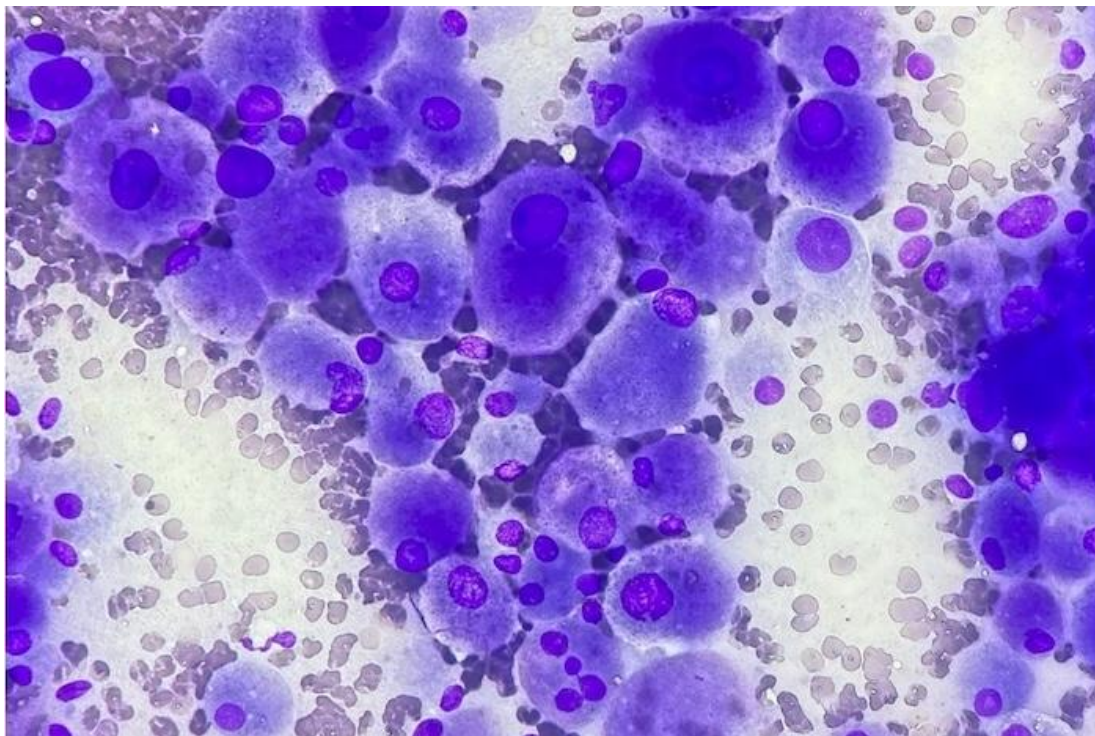


Figure 2. Fine needle aspiration of the thoracic mass (Modified Wright Giemsa) (40x).

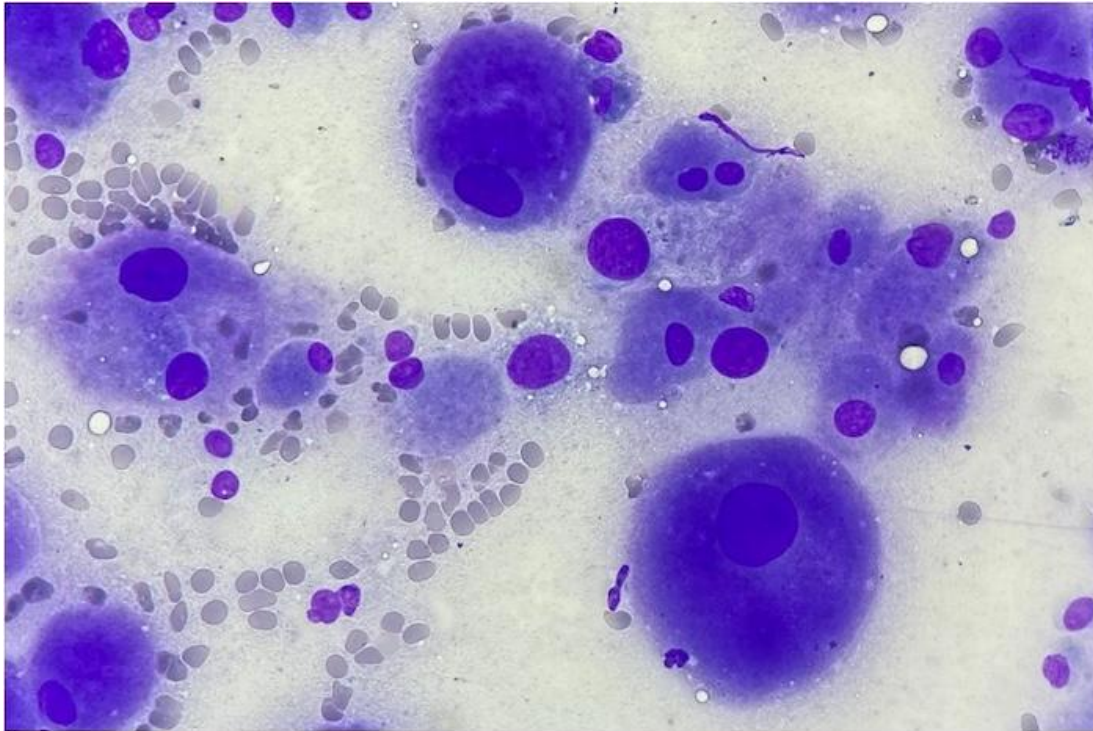


Figure 3. Fine needle aspiration of the thoracic mass (Modified Wright Giemsa) (40x)

Questions

1. What is the cytological description of this lesion?
2. Based on the cytological findings and the location of the mass, what are the differential diagnoses for this lesion?
3. What would be the recommended next steps to further investigate the origin of the mass?

1. What is the cytological description of this lesion?

Cytological evaluation revealed moderate to high cellularity with good preservation of cellular morphology. The background contained abundant blood and frequent ruptured cells.

A population of markedly atypical neoplastic cells was observed, distributed individually and in variably sized, loosely cohesive groups. The cells were round to polygonal with distinct cell borders and a moderate to abundant amount of blue to amphophilic granular cytoplasm, sometimes containing small clear vacuoles/globules. Nuclei were round to oval with finely stippled chromatin and occasionally prominent nucleoli. The nucleus-to-cytoplasm ratio was variable, generally moderate. Moderate to marked anisocytosis and anisokaryosis were present. Bi- and multinucleated cells, occasional macronuclei and rare erythrophagia were identified, as well as occasional mitotic figures.

2. Based on the cytological findings and the location of the mass, what are the differential diagnoses for this lesion?

The cytological features were most consistent with a granular cell neoplasm. Differential diagnoses included both benign and malignant neoplasms with granular cell appearance, such as granular cell tumor, oncocytoma, rhabdomyosarcoma and carcinomas (i.e.

hepatocellular). Granular cell mesothelioma, although rarely described in dogs, was also considered. Histiocytic sarcoma was considered less likely based on the overall cytomorphology.

3. What would be the recommended next steps to further investigate the origin of the mass?

Given the cytological and CT findings, surgical biopsy was recommended to further characterize the lesion and determine its tissue of origin.

An intercostal thoracotomy through the left 6th intercostal space was performed to obtain incisional biopsy samples. Intraoperatively, a large grape-like mass involving the ventral and caudal mediastinum was identified, with extension to the pericardium. Marked serosanguineous pleural effusion was also present, and a pleural port was placed.

Histopathological examination revealed a neoplasm composed of solid areas and sheets of large polygonal to round cells with minimal fibrovascular stroma. The cells had abundant granular cytoplasm, and pleomorphic nuclei with a prominent nucleolus and nuclear pseudoinclusions. Based on the histomorphologic features, a granular cell tumor was initially considered the most likely diagnosis. However, other neoplasms with granular morphology could not be completely excluded, including a round cell neoplasm or a neoplasm of neuroendocrine origin (figure 4).

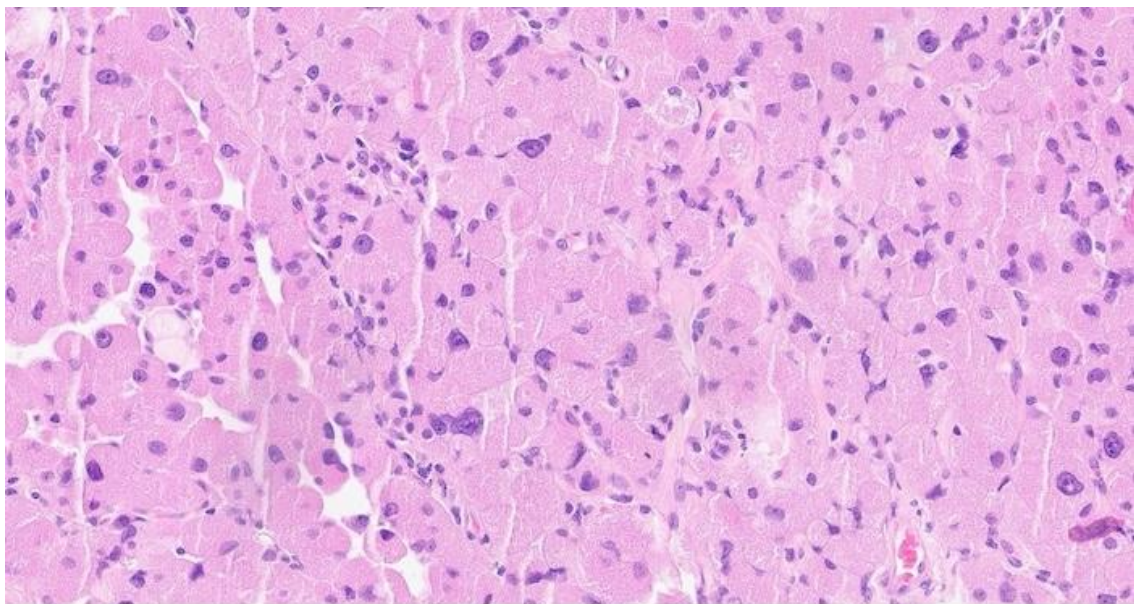


Figure 4. Histologic section of the mass (Hematoxylin-eosin) (40x).

Periodic acid–Schiff (PAS) staining demonstrated granular cytoplasmic positivity in approximately 50% of the neoplastic cells (figure 5A). Toluidine blue staining was negative (figure 5B).

Due to the unusual morphology and inconclusive histopathologic findings, the samples were submitted to a second laboratory for further evaluation and immunohistochemical characterization. On review, the morphology was considered most consistent with a granular cell neoplasm. However, considering the anatomical location and histologic appearance, additional differential diagnoses included thymoma with granular cell morphology and rhabdomyosarcoma.

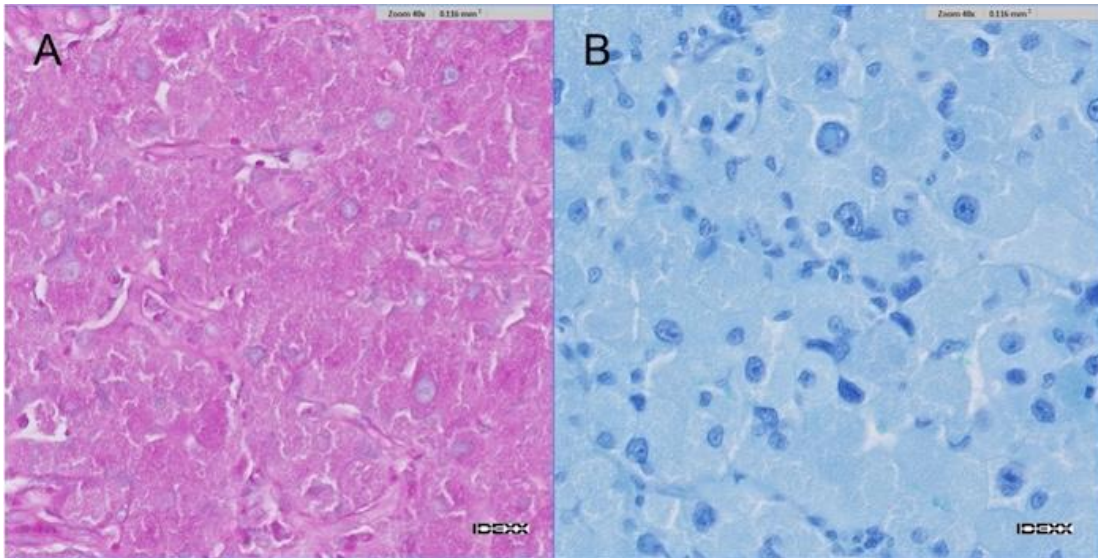


Figure 5. Histologic section of the mass (40x). A: PAS stain in an area of high positivity. B: Toluidine blue stain.

Immunohistochemically, the neoplastic cells showed positive labeling for cytokeratin (fig. 6A), vimentin (fig. 6B), desmin (fig. 6C), and Wilms'tumor 1 (WT1) (fig. 6D) whereas MyoD1, myogenin, chromogranin, synaptophysin, S100, and Nerve Growth Factor Receptor (NGFR) was negative (not depicted) (table 1).

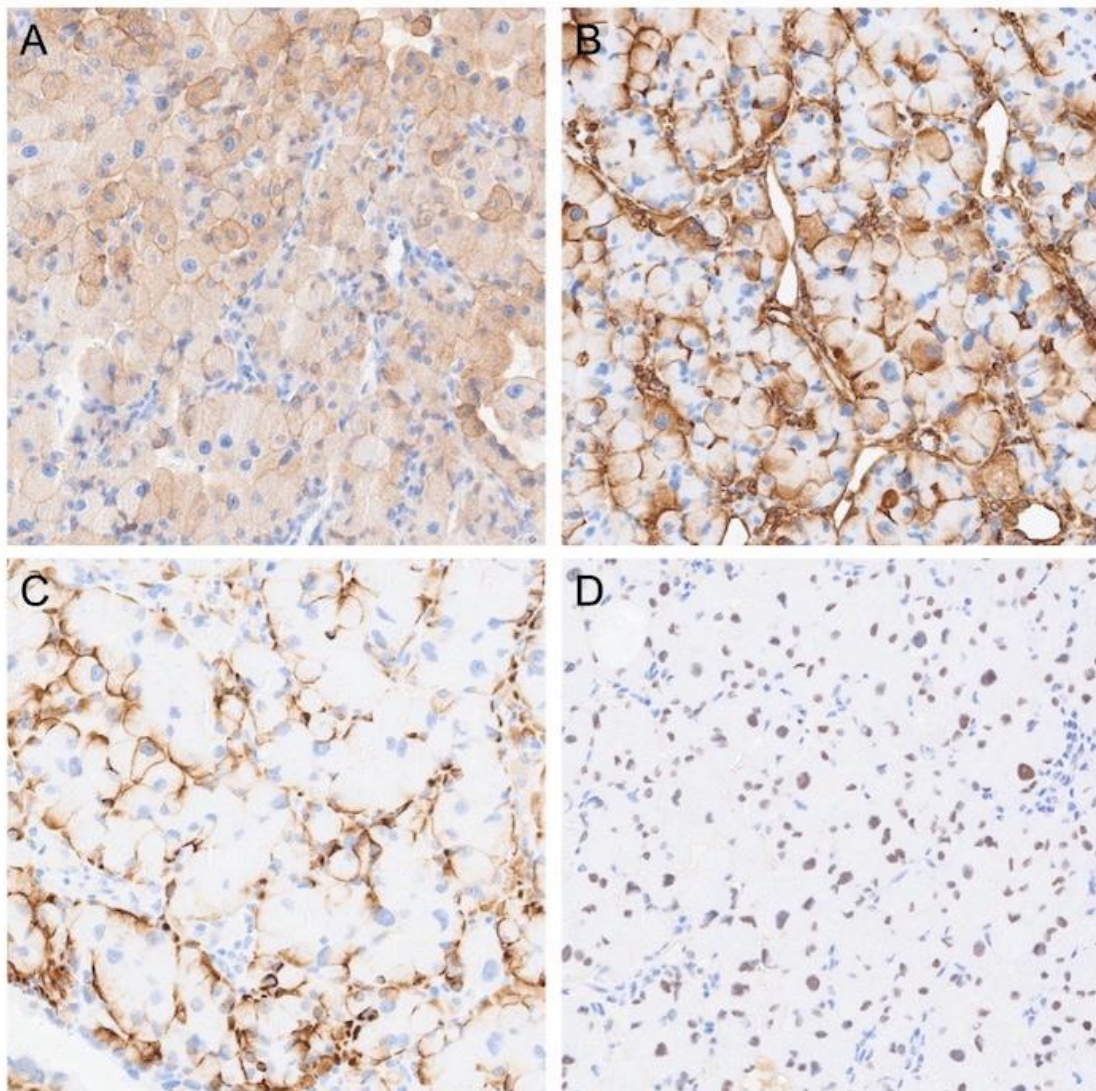


Figure 6. Immunohistochemical staining of the neoplastic cells. A: Cytokeratin, B: vimentin, C: desmin, D: WT1. (20x)

Marker	Clone	Control	Result	Normal labeling
PAS	NA	NA	+ (cytoplasmic)	Glycogen, mucin
Toluidin blue	NA	NA	-	Mast cells
Cytokeratin	AE1/AE3	Normal skin (epithelial structures)	+ (cytoplasmic)	Epithelial cells
Vimentin	V9	Normal skin (dermis)	+ (membranous and cytoplasmic)	Mesenchymal cells
Desmin	DE-R-11	Intestine	+ (membranous and cytoplasmic)	Muscle cells
WT1	WT49	Ovary	+ (nuclear)	Mesothelial cells
MyoD	5.8A	Rhabdomyosarcoma	-	Skeletal muscle
Myogenin	F5D	Rhabdomyosarcoma	-	Skeletal muscle
Chromogranin	5H7	Adrenal gland	-	Neuroendocrine cells
Synaptophysin	27G12	Adrenal gland	-	Neuroendocrine cells
S100	EP32	Normal nerve	-	Neurons, glial cells
NGFR	ME20.4	Intestine (myoenteric plexus)	-	Perineural cells

Table 1. Immunohistochemical findings and diagnostic significance of the evaluated markers

Interpretation/diagnosis

Granular variant of mesothelioma.

The diagnosis was based on the combined histomorphologic features and immunohistochemical profile, including positive labeling for CK, WT1, desmin and vimentin together with exclusion of skeletal muscle, neuroendocrine and neural differentiation.

Additional information

Following the diagnosis of mesothelioma, immunocytochemistry was performed on the cytological slides using the same panel of markers. The neoplastic cells showed positive immunoreactivity for cytokeratin (not depicted), vimentin (figure 7), desmin (figure 8), and WT1 (figure 9), consistent with the immunohistochemical findings.

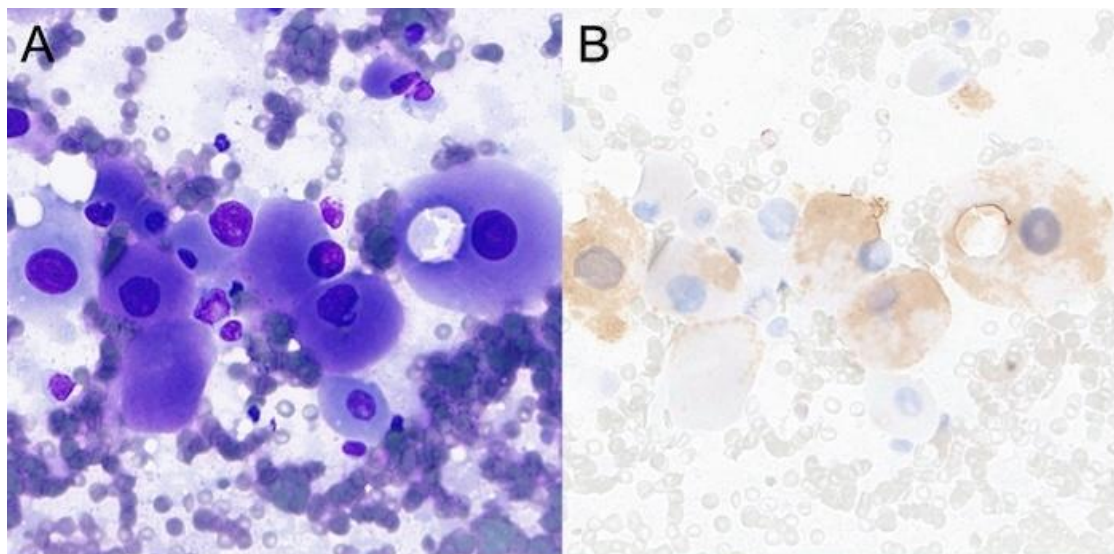


Figure 7. Comparison of the same cytological field stained with modified Wright Giemsa (A) and vimentin immunocytochemistry (B) (40x).

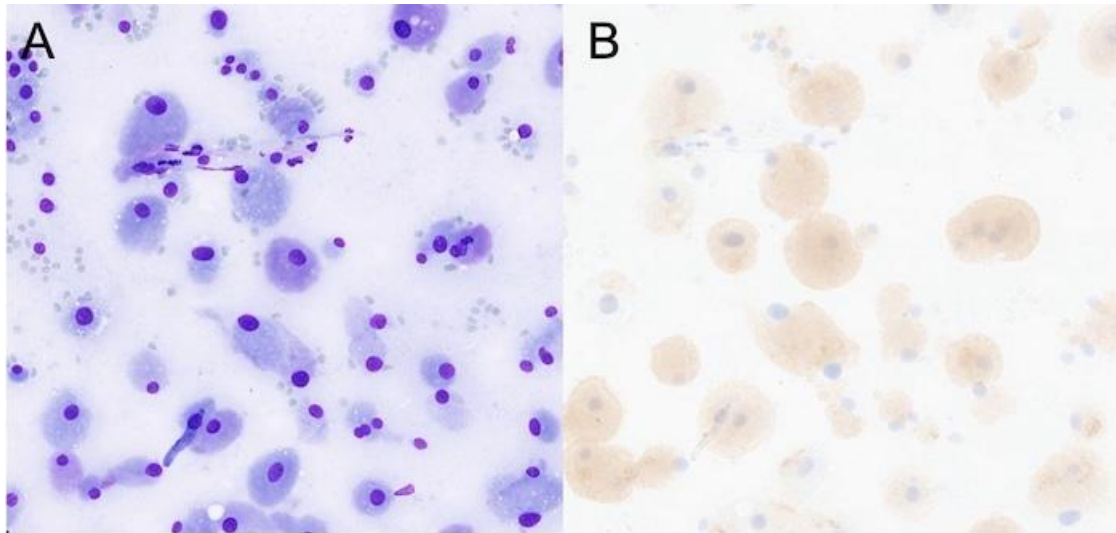


Figure 8. Comparison of the same cytological field stained with modified Wright Giemsa (A) and desmin immunocytochemistry (B) (20x).

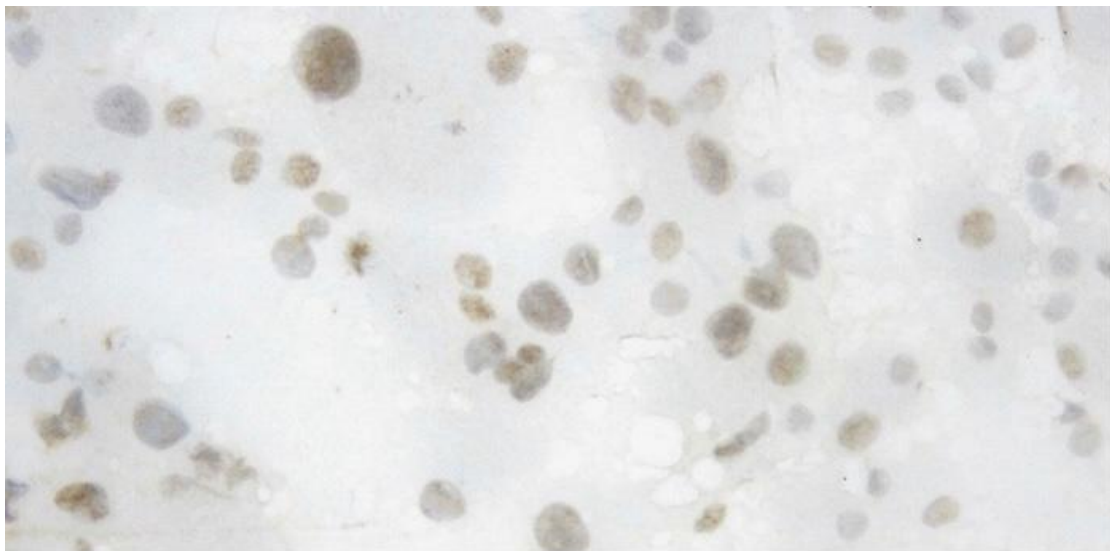


Figure 9. Positive nuclear staining for WT1 in the neoplastic cells (40x).

Follow up and clinical outcome

Repeated thoracocenteses were subsequently required due to marked pleural effusion, with up to 1050 mL drained during a single procedure. Intracavitary chemotherapy with 5-fluorouracil (150 mg/m²) was administered through the pleural port five days after collection of the incisional biopsy samples. Despite treatment, recurrent pleural drainage continued to be necessary. The owners declined systemic chemotherapy with carboplatin, and prednisolone therapy (1 mg/kg PO SID) was initiated.

Based on the preliminary histopathological findings and the possibility of a neuroendocrine origin, toceranib phosphate (Palladia®; 50 mg administered Monday, Wednesday, and Friday) was added to the treatment protocol in combination with corticosteroids. Nine days after initiation of therapy, the patient maintained a good general condition, and a marked reduction in pleural effusion was observed, with only 40 mL drained during thoracocentesis. One month after starting Palladia®, the patient remained clinically stable and no pleural effusion was detected, allowing reduction of prednisolone dosage to 0.5 mg/kg PO SID. Three months later, corticosteroid therapy was further tapered to 0.5 mg/kg PO every other day.

Eight months after initiation of Palladia® therapy, the patient continued to show a good general condition. Follow-up CT examination demonstrated partial remission of the mass, with only minimal residual pleural effusion (figure 10).

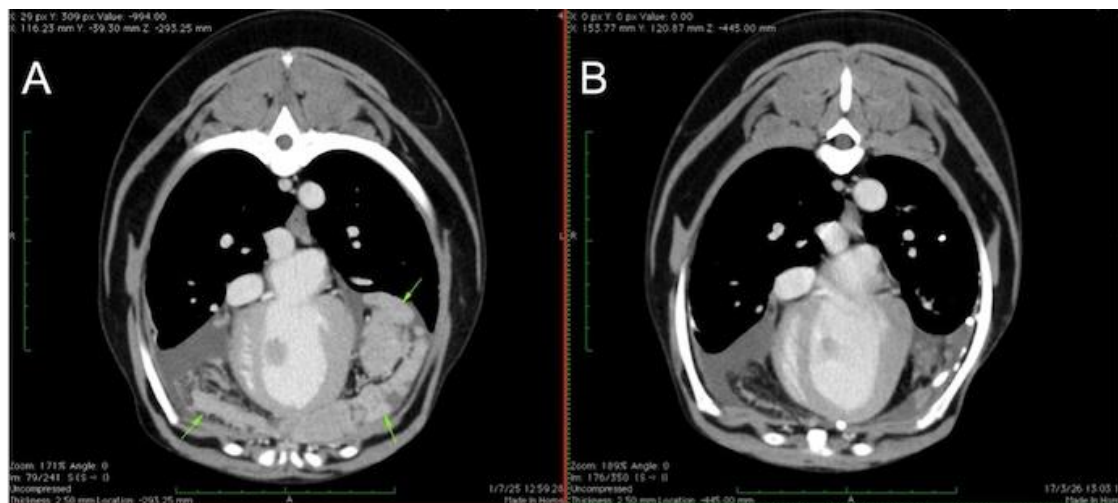


Figure 10. Comparison of the initial CT scan (A) and the follow-up CT scan performed 8 months later (B), demonstrating partial remission of the mass.

At the time of writing, 11 months after initiation of Palladia® therapy, the dog is alive and continues to maintain a good quality of life.

Discussion

Mesothelioma is a well-recognized neoplasm affecting dogs, cats, humans, and other species, and has been described involving the pleura, peritoneum, pericardium and tunica vaginalis of the testicle.^{1,2} Its cytological diagnosis represents a challenge for veterinary clinical pathologists due to its marked pleomorphism and morphological overlap with other neoplasms, particularly carcinomas. Histologically, mesotheliomas are classified as epithelioid, sarcomatoid (fibrous), or mixed, with additional histological subtypes also described.¹ In addition, granular cytomorphology is exceptionally uncommon, substantially complicating both cytologic and histopathologic diagnosis. The granular cytologic appearance of neoplastic mesothelioma cells has only rarely been reported in veterinary medicine, both cytologically and histopathologically.^{3,4}

Most common clinical signs in dogs with mesothelioma include tachypnea, dyspnea, and lethargy, followed by exercise intolerance, hyporexia, abdominal distension, and coughing. Similar clinical findings were observed in the present case, as the patient presented with tachypnea, dyspnea, and chronic coughing.⁵ In addition, cavitory effusions are reported in the vast majority of dogs diagnosed with mesothelioma.^{5,6}

Although diffuse serosal involvement is most frequently described, focal mass formation may occasionally occur. In the study by Lajoinie et al. (2022)⁶, discrete mass formation represented only a small minority of cases.

In the study by Moberg et al. (2022)⁵, cytological evaluation of cavitory effusions was available in 31 dogs, and mesothelial cells were identified in 30 cases; however, only 9 dogs (29%) were diagnosed with a neoplastic effusion on cytology, while the remaining samples were classified as other type of effusions. In contrasts, in the study by Lajoinie et al. (2022)⁶, cytopathological examination was considered suggestive of mesothelioma in most of the cases, 36 of 40 dogs (90%). Three of the other cases were inconclusive because of poor cellularity, and in the last one only a proliferation of well-differentiated mesothelial cells without clear malignant criteria was identified cytologically. In our case,

no mesothelial cells were described in the cytological report, which may have been related either to suboptimal sample processing or to a lack of neoplastic exfoliation into the pleural fluid.

On cytopathology, mesothelioma is extremely challenging to differentiate both from carcinoma and marked reactive mesothelial hyperplasia.⁷ Furthermore, mesothelioma cells may display highly variable cytomorphological features, probably due to the different histological subtypes described, including granular cell morphology. This latter subtype has only been reported in a few cases,^{3,4} in which the neoplastic cells showed cytomorphological features similar to those observed in the present case. The major differential diagnosis in those cases was a granular cell tumor.

Granular cell tumor represented one of the main differential diagnoses in this case too, due to the prominent granular cytoplasmic features observed both cytologically and histologically. Granular cell tumors are uncommon neoplasms in dogs and are most frequently reported in the tongue, although other anatomical locations have been described.⁸ A case of a granular cell tumor involving the pleura was described by Foley et al. (1988)⁹; however, subsequent study by Brower et al. (2006)⁴ suggested that this may actually be a granular variant of mesothelioma.

Given the immunohistochemical staining pattern, the final diagnosis was granular mesothelioma. Mesotheliomas are typically positive for cytokeratin, desmin, and WT1, with variable vimentin expression, as observed in the present case. Additional markers, including IMP3 and GLUT1, have also been described.¹⁰ Positivity for vimentin, desmin, and WT1 supports a mesothelial origin and helps differentiate mesothelial cells from epithelial neoplasms, thereby excluding carcinoma. However, these markers do not reliably differentiate reactive mesothelial proliferations from true mesothelioma. IMP3 has been proposed as a useful marker in canine mesothelioma, as increased expression may be observed in neoplastic mesothelium compared with reactive mesothelial proliferations. However, in the study by Milne et al. (2021)¹⁰, significant overlap between groups was observed, limiting its diagnostic utility, and interpretation should therefore be performed in combination with morphology and additional immunohistochemical markers. In this case, IMP3 was not considered useful for diagnostic purposes as cellular atypia and tissue architecture was already diagnostic of neoplasia.

In contrast, granular cell tumors are typically positive for PAS, S100, Luxol fast blue and ubiquitin, although negative staining for S100 and Luxol fast blue has occasionally been reported. They are generally negative for cytokeratins, muscle-specific actin and lysozyme.^{1,8} In the present case, these markers were not performed due to their limited commercial availability and because the immunohistochemical findings already strongly supported a diagnosis of mesothelioma.

Periodic acid–Schiff (PAS) staining is not routinely performed in mesotheliomas; however, due to the granular morphology observed in the present case, this stain was carried out in the histological sample, and showed positive granular cytoplasmic staining in approximately 50% of the neoplastic cells. In previously reported cases of granular mesothelioma, PAS staining was also performed, yielding positive results in one case and negative results in the other.^{3,4}

Rhabdomyosarcoma was initially considered among the differential diagnoses. Although positive immunolabeling for vimentin and desmin could support this diagnosis, both markers lack specificity for skeletal muscle differentiation and may also be expressed in other neoplasms. MyoD1 and myogenin are considered more useful markers for canine rhabdomyosarcoma. In the study by Tuohy et al. (2021),¹¹ all confirmed

rhabdomyosarcomas showed MyoD1 immunoreactivity, whereas myogenin expression was identified only in a subset of cases. In the present case, the absence of MyoD1 and myogenin labeling, together with cytokeratin positivity, argued against a diagnosis of rhabdomyosarcoma.

Another possible origin considered was a neuroendocrine neoplasm. Synaptophysin and chromogranin immunostaining were therefore performed, as these markers are typically positive in neuroendocrine tumors.⁷ Since both markers were negative in the present case, a neuroendocrine origin was excluded.

S100 is not considered a routine marker for the diagnosis of canine mesothelioma, however it can be positive in some cases and has also been described in human medicine.³ In the present case, S100 immunohistochemistry was primarily performed to further investigate differential diagnoses associated with granular cytoplasmic morphology, including granular cell tumor and neoplasms of neural origin. The absence of S100 immunoreactivity supported exclusion of these entities.

Nerve growth factor receptor (NGFR) immunostaining was performed to evaluate a possible neural origin of the neoplasm; however, as the result was negative, this possibility was also excluded.

Immunocytochemistry was performed on the archived cytological preparations and yielded positive labeling for cytokeratin, vimentin, and desmin, consistent with the immunohistochemical findings and previous reports of canine mesothelioma.¹² WT1 was also positive on immunocytochemistry, as expected given its well-established expression in mesothelial cells, its widespread use as an immunohistochemical marker of mesothelial origin and its previously reported immunocytochemical expression in canine mesothelioma.¹³

Treatment with toceranib phosphate (Palladia®) was initiated after the owners declined carboplatin therapy and because a neuroendocrine origin had initially been considered among the differential diagnoses. The clinical response was favorable, with complete resolution of the pleural effusion, partial remission of the mass on follow-up CT examination and maintenance of a good quality of life 11 months after initiation of treatment. Although toceranib is not routinely described for canine mesothelioma, favorable responses have been reported in a limited number of cases.¹⁴

In conclusion, this case illustrates the marked cytomorphological variability of canine mesothelioma and emphasizes that rare granular variants may closely resemble other granular cell neoplasms, posing significant diagnostic challenges. Integration of cytology, histopathology, and targeted immunohistochemistry was essential for establishing a definitive diagnosis. In addition, the concordance between immunocytochemical and immunohistochemical findings highlights the potential utility of immunocytochemistry in challenging cases.

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