Neoplastic pleural effusion and intrathoracic metastasis of a scapular osteosarcoma in a dog: a multidisciplinary integrated diagnostic approach

Running header: Neoplastic effusion due to osteosarcoma in a dog

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Email: mpiviani@liverpool.ac.ukABSTRACT

A 10-year-old, female spayed cross-breed dog was referred to the Small Animal Teaching Hospital of the University of Liverpool due to tachypnea, dyspnea and pleural effusion not responding to diuretics and antibiotics. The chest was drained and cytology of the pleural fluid was consistent with a modified transudate with presence of atypical cells initially attributed to mesothelial hyperplasia and dysplasia. Computed tomography detected, in addition to the bilateral pleural effusion, diffuse pleural thickening, multiple pleural and pulmonary nodules, and a mineralized and lytic mass in the left scapula. Imaging findings were suggestive of a primary bone tumor with intrathoracic metastasis. Cytology of the left scapular and pleural masses revealed a malignant neoplasm highly suggestive of osteosarcoma. The diagnosis was confirmed by demonstration of a positive cytochemical reaction for alkaline phosphatase on pre-stained cytology slides. This finding prompted review of the initial interpretation of the pleural effusion cytology. The presence of neoplastic osteoblasts in the thoracic fluid was identified by a combination of cytochemistry, cell pellet immunohistochemistry and transmission electron microscopy findings. In this report a multidisciplinary integrated diagnostic approach was used to diagnose and confirm a neoplastic pleural effusion due to osteosarcoma metastasis in a dog.

KEYWORDS: bone tumor, computed tomography, dog, fluid cytology, cell pellet immunohistochemistry, transmission electron microscopy

CASE PRESENTATION

A 10-year-old, female spayed, cross-breed dog was admitted by the Internal Medicine Service of the Small Animal Teaching Hospital (SATH) of Liverpool University for further investigation into a recently developed tachypnea, dyspnea, and anorexia. Clinical signs appeared 10 days prior to referral when a bilateral pleural effusion was detected on thoracic radiographs and drained by the referring veterinarian. After initial improvement, clinical signs reoccurred and oral furosemide (2.8 mg/Kg, orally twice a day) and amoxicillin-clavulanate (8.75 mg/Kg, subcutaneously twice a day) were administered; however no clinical improvement was observed. An automated CBC (LaserCyte Dx, IDEXX Laboratories, Westbrook, ME, USA), performed prior to the referral, revealed a mild leukocytosis (17.76 x109/L; Reference Interval (RI) 5.5-16.90), due to mild neutrophilia (14.08x109/L; RI 2-12) and mild monocytosis (2.30x109/L ;RI 0.30-2); a mild thrombocytosis (688 x109/L; RI 175-500) was also present. This pattern was compatible with chronic inflammation but the blood smear was not reviewed. The biochemistry profile (Catalyst Dx, IDEXX Laboratories, Westbrook, ME, USA) revealed mild hypokalemia (3.2 mmol/L; RI 3.5-5.8) and hypochloridemia (103 mmol/L; RI 109-122), and a mild proportional increase of urea (12.6 mmol/L; RI 2.5-9.6) and creatinine (169 mmol/L; RI 44-159). The mild azotemia and electrolyte disturbances were attributed to the recent administration of diuretics and subsequent dehydration. There was also an increased ALP (406 IU/L; RI 23-212), which could be due to cholestasis, enzymatic induction or bone remodeling.

 On presentation to the SATH, the dog appeared markedly dyspneic and had a restrictive respiratory pattern but was alert and responsive. The body weight was 21.4 Kg with a normal body condition score (BCS 4/9). Mucous membranes were pink, dry and had a prolonged capillary refill time (3 seconds). Thoracic auscultation revealed muffled heart sounds.

Thoracocentesis was performed and 810 mL of sero-hemorrhagic pleural fluid were removed from a hemithorax on the right side and 600 mL from the left side. Total nucleated cell and erythrocyte counts were 2.9x109/L and 0.09x1012/L, respectively (Advia 2120 Hematology System; Siemens Healthcare Diagnostics, Deerfield, IL, USA). Total protein concentration was 25 g/L (Konelab 30i; Thermo Clinical Labsystems, Vantaa, Finland). Direct and sediment smears prepared from the fluid submitted in EDTA were air-dried and stained with Wright-Giemsa (Wescor Inc., Logan, UT) using an automated stainer (Aerospray 7150 Hematology Slide Stainer-Cytocentrifuge, Wescor Inc., Logan, UT). A board-certified clinical pathologist (MP) examined all the slides. The smears contained moderate numbers of vacuolated macrophages with occasional erythrophagocytosis, fewer small lymphocytes, and occasional neutrophils, admixed with moderate numbers of atypical cells amid a background of moderate numbers of erythrocytes. Atypical cells were round to vaguely polygonal, mostly individualized but also occurring in loose aggregates occasionally surrounding scant pink material. Nuclei were round, central to paracentral, with granular chromatin and one to multiple variably prominent nucleoli. These cells had moderate to abundant blue cytoplasm with distinct vacuoles and occasional peripheral blebs. A small proportion of these cells had a pericellular pink fringe. Anisocytosis and anisokaryosis were moderate with frequent binucleation and occasional multinucleation. Mitoses were frequent, including atypical figures (Figure 1). The cytologic interpretation was modified transudate with low-grade chronic inflammation and mild hemorrhagic component with proliferation of atypical cells interpreted as reactive and dysplastic mesothelial cells, although neoplasia was not completely ruled out.

To further characterize the cells in the effusion, an aliquot of the fluid was placed in a plastic 1.5 mL eppendorf tube (Eppendorf, Hauppauge, NY, USA), centrifuged, the supernatant removed and 0.5 mL of 10% neutral buffered formalin added. The cell pellet was paraffin embedded and processed for immunohistochemistry (IHC) using commercially available antibodies for vimentin (monoclonal, clone V9, Dako, Glostrup, Denmark), pancytokeratin (monoclonal, clone AE1/AE3, Dako, Glostrup, Denmark), Iba1 (polyclonal, LS-B2402, LifeSpan BioSciences, Seattle, WA), CD18 (clone CA16.3C10, Peter Moore, University of California - Davis, CA), MUM1 (clone MUM1, Dako, Glostrup, Denmark), and S100 (polyclonal, Z0311, Dako, Glostrup, Denmark). Another pellet was re-suspended in a solution of 2.5% glutaraldehyde in 100 mM phosphate buffer at pH 7.0 and submitted for transmission electron microscopy (TEM, Phillips EM 208, FEI UK, Cambridge, UK). Immunohistochemistry of the pellet of the pleural effusion was examined by a board-certified pathologist (LR) and revealed that the large atypical cells were positive for vimentin and negative for pancytokeratin (Figure 2A-2C), thus consistent with mesenchymal cells. Immunohistochemistry for all the other markers was negative. On TEM, atypical cells had abundant rough endoplasmatic reticulum and a hyperplastic Golgi apparatus (Figure 2D).

A computed tomography (CT) was performed with a Toshiba Aquilion Prime 80 slices Computed Tomography scanner (Toshiba Medical Systems, Tokyo, Japan) with the patient under sedation. Images of the thorax and abdomen were acquired pre and post-contrast medium administration (Iobitridol 600mg/kg, IV). The CT revealed a rounded, mineralized mass centered on the dorsal part of the left scapular spine measuring 1.7 cm (width) x 1.3 cm (high) x 4 cm (long) with destruction of the cortical and medullary bone and an ill-defined transition zone consistent with a primary bone tumor (Figure 3). There was also bilateral pleural effusion with diffuse pleural thickening and multiple pleural and pulmonary nodules and masses (Figure 4) compatible with metastatic disease or a mesothelioma. The sternal lymph nodes were mildly enlarged with areas of mineralization (data not shown). The CT of the abdomen revealed no abnormalities.

Fine-needle aspiration (FNA) of the left scapular and pleural masses was performed and squash smears made. Slides contained many neoplastic cells, seen mostly individualized and occasionally embedded in scant light pink material reminiscent of osteoid, intermingled with frequent osteoclasts and few macrophages. Several fragments of mineral material were noted in the background. Neoplastic cells were round to oval with an eccentric oval to round nucleus, stippled chromatin, single to multiple prominent nucleoli and abundant blue cytoplasm with a paracentral clearing (Figure 5). Criteria of malignancy included moderate anisocytosis and anisokaryosis, rare binucleation, prominent nucleoli and occasional mitoses (5 per 10 fields at 400x magnification). The cytologic findings were highly suggestive of an osteosarcoma.

To corroborate the cytologic interpretation of scapular osteosarcoma with pleural metastasis, the substrate 5-bromo, 4-chloro, 3-indolylphosphate/nitroblue tetrazolium (BCIP/NBT, Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD, USA) was applied for 60 minutes to one prestained slide of FNA from each site and to one archived canine liver FNA cytology slide used as positive control (data not shown), following published guidelines.1 Slides were then counterstained with a rapid romanowsky staining kit (TCS Biosciences Ltd, Botolph Claydon, UK) for one second in each solution cup, and rinsed with distilled water. A brown granular material, indicative of alkaline phosphatase activity, was evident within the cytoplasm of neoplastic cells (Figure 6) and along the cytoplasmic membrane of the hepatocytes but absent in leukocytes. The BCIP/NBT substrate was also applied to one of the prestained sediment smears of pleural fluid available and revealed alkaline phosphatase activity in the majority of the atypical cells (interpreted as neoplastic osteoblasts) but, as expected, not in erythrocytes and leukocytes, including macrophages (used as internal negative control). Few atypical cells (those that were more cohesive and often showed a peripheral pink fringe) did not show ALP activity and were interpreted as reactive mesothelial cells (Figure 7).

Based on the combination of imaging, cytology, cytochemistry, fluid pellet IHC and TEM findings, the dog was diagnosed with a neoplastic pleural effusion because of intrathoracic metastasis of a scapular osteosarcoma. Given the poor prognosis, owners elected euthanasia. A post-mortem examination was not authorized.

DISCUSSION

The main finding of this case report was the presence of neoplastic osteoblasts in the pleural effusion. Cavitary effusions may be caused by disturbances of hydrostatic or oncotic pressure, inflammation, impaired lymphatic drainage, hemorrhage, organ rupture or neoplasia.2 Laboratory evaluation of cavitary fluid, including cell counts, total protein and cytology, is useful in determining the cause of the effusion and to identify neoplastic cells in many cases.3 In this case routine fluid cytology alone did not allow a confident interpretation of neoplasia as the atypical cells were still in the morphologic spectrum of reactive and dysplastic mesothelial cells. The reported sensitivity of cytology for the diagnosis of malignant tumors in canine and feline effusions is 60%.4 Achieving a definitive diagnosis using a fluid sample harvested during therapeutic thoracocentesis, avoiding invasive procedures such as thoracotomy and pleural or pulmonary biopsy, would be ideal but it is often not possible. With long-standing effusions the mesothelium lining the body cavities often becomes hyperplastic and cells exfoliating into the fluid may mimic neoplasia as increased nuclear size, multiucleation and mitotic figures may be seen. Thus, an interpretation of malignancy requires caution. Mesothelial cells often have a peripheral pink fringe but this feature is not consistent. In addition, mesothelial and epithelial cells exfoliating into an effusion often lose their cell-to-cell adhesion and can mimic round cells. This further limits the ability of a definitive identification of the cell phenotype. 5Immunocytochemistry, flow cytometry and cell pellet IHC are all useful tools to characterize cells present in effusions and to refine the cytologic interpretation. The latter is a simple, fast and effective diagnostic tool, which can be performed in most histopathology laboratories with a wider panel of validated markers compared to that available for the 2 former techniques.6 In this case, atypical cells were vimentin positive but pancytokeratin negative. These findings were consistent with a mesenchymal proliferation, refuting the initial interpretation of mesothelial hyperplasia and excluding the possibility of carcinoma and mesothelioma, as epithelial and mesothelial cells are expected to be pancytokeratin positive. The negative result for CD18 and Ionized calcium-binding adapter molecule 1 (Iba1) excluded a histiocytic proliferation. Ionized calcium-binding adapter molecule 1 is a widely used marker for microglial cells which has recently been recognized as a 'pan-macrophage marker' expressed by all cells of the monocyte/macrophage lineage.7 Plasma cell tumor and melanoma were excluded based on negativity for MUM1 and S100, respectively. A lymphoid origin was unlikely given cell morphology and negative immunostain for CD18. Cell pellet IHC findings were consistent with a neoplastic effusion due to a sarcoma. Transmission electron microscopy confirmed that the atypical cells in the effusion were not mesothelial as they lacked the densely stippled, regular, long microvilli typical of mesothelial cells, while a myocyte or endothelial origin was ruled out based on the absence of contractile myofilaments and Weibel Palade bodies, respectively.

 Radiography is considered the first line diagnostic imaging technique for animals with thoracic disease but findings are often nonspecific or limited by the presence of pleural fluid.8,9 According to the referring veterinarian, the thoracic radiographs revealed only pleural effusion. In addition to the pleural effusion, the thoracic CT also revealed pleural thickening, pleural nodules and masses, pulmonary nodules, possible sternal lymphadenopathy, and a scapular mass. Several studies have demonstrated the utility of CT to identify other abnormalities beyond pleural effusion, including lesions in the pleura, lung and mediastinum, or extra thoracic lesions.9,10,11 The presence of pleural nodules and masses is frequently associated with neoplasia.9,11,12 However, similar lesions may also be present in patients with chylothorax, pyothorax, and foreign body migration, with overlapping CT features.11 The mineralized mass in the left scapular spine detected in the CT was not mentioned in the clinical history provided by the referring veterinarian, although it is uncertain if this anatomical region was included in the chest radiographs performed before referral as the images were not available for review. The CT features of the scapular osteosarcoma described in this case report are consistent with the current published veterinary literature. Compared to radiography, the main advantage of CT is a clearer delineation of the internal and extracortical tumor margins.13,14 Osteosarcoma is the most common neoplasm of the scapula in dogs, followed by soft tissue sarcoma, chondrosarcoma, hemangiosarcoma and histiocytic sarcoma.15 Although less than 15% of dogs with osteosarcoma have radiologic evidence of pulmonary nodules or masses at the time of the diagnosis, more than 85% of patients develop gross metastases despite effective control of the primary tumor, suggesting that micrometastases arise early in the course of the disease.16 Considering just osteosarcoma of extracranial flat and irregular bones, the incidence of thoracic metastases is less defined, although it is thought to be higher than in osteosarcoma of long bones.17 Other reported metastatic sites include bones, visceral organs, lymph nodes and eye.16,18-21 To the authors’ knowledge, despite the high incidence of intrathoracic metastasis, a neoplastic pleural effusion due to osteosarcoma has never been reported before.

The FNA of the scapular mass and one of the pleural nodules harvested a population of cells with features very reminiscent of osteoblasts (eccentric nuclei, abundant deep-blue cytoplasm with paranuclear clearing) and several criteria of malignancy (moderate anisocytosis and anisokaryosis, binucleation, mitoses), leading to an interpretation of osteosarcoma. Histology is considered the gold standard for the diagnosis of osteosarcoma upon demonstration of a neoplastic mesenchymal population producing osteoid. The scant pink material seen in the FNA was highly suggestive of osteoid and, although different types of extracellular matrix cannot be reliably distinguished in cytology, histology has similar limitations, as osteoid may sometimes be difficult to differentiate from fibrin or collagen and its presence may be dependent on tumor subtype, inconspicuous in small biopsies or absent in metastases. In a recent study, preoperative FNA of scapular lesions was performed in 11 of 42 dogs included in the study.15 The cellular yield was high and cytologic interpretation was confirmed by histopathology in all cases. The authors strongly recommended FNA for investigation of bone lesions, including those of the scapula, as a diagnosis may be achieved more quickly with less risk for the patient and lower cost compared to tru-cut biopsy. In our case, obtaining a biopsy sample of the scapular and thoracic masses for histologic examination would have been an unnecessary and invasive procedure considering that cytology, combined with alkaline phosphatase cytochemistry, was already diagnostic.

The presence of a scapular osteosarcoma with intrathoracic metastasis suggested a possible osteoblastic origin for the neoplastic mesenchymal population identified by cell pellet IHC in the effusion. In the diagnosis of osteosarcoma, IHC is generally used to rule out other types of neoplasia rather than definitively confirm osteosarcoma. During the preparation of this manuscript, a study demonstrating the utility of osteocalcin as a marker for osteosarcoma in dogs was published.22 Immunostain for osteocalcin could have been useful to confirm osteosarcoma in our case albeit its specificity is limited by the frequent positivity in chondrosarcomas and the positive cytochemical reaction for alkaline phospahatase was already confirmatory, as bone is the only connective tissue shown to produce alkaline phopshatase in dogs. The BCIP/NBT substrate can be applied to cytology slides, even if prestained, as in this case, with a reported sensitivity and specificity for a diagnosis of osteosarcoma of 88% and 94%, respectively.1 Positive cytochemical reaction for alkaline phosphatase was found in one of 2 and in one of 4 chondrosarcomas in 2 different studies.23,24 In one case the unexpected result was attributed to a possible misdiagnosis of chondroblastic or periosteal osteosarcoma for chondrosarcoma in histology due to the presence of cartilage and lack of convincing osteoid in the section examined, or expression of alkaline phophatase by undifferentiated neoplastic chondroblasts.24 Alkaline phosphatase activity was also detected in one of 2 and in one of 8 cases of amelanotic melanoma.1,23 In our case, the lack of S100 expression in the fluid cell pellet IHC further supports a diagnosis of osteosarcoma versus chrondrosarcoma or melanoma, as this marker is usually positive in the latter 2 types of neoplasia.25,26

In conclusion this case report shows that metastasis of osteosarcoma is a possible consideration for a cavitary effusion containing oval cells with atypia in a dog with a bone tumor, highlights the importance of an integrated interpretation of CT findings and cytology, and further demonstrates the utility of cytochemistry, cell pellet IHC and TEM to refine a cytologic diagnosis when obtaining a histology sample is not feasible.

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FIGURE CAPTIONS

Figure 1: Sediment smear of pleural effusion from a dog with dyspnea and metastasizing oseosarcoma. There are round to vaguely polygonal atypical cells, individualized or arranged in loose aggregates, and few inflammatory cells including vacuolated macrophages (arrow). Atypical cells include forms with a peripheral pink fringe (arrowhead). Note the numerous mitoses in this field. Wright–Giemsa stain, bar 35 µm.

Figure 2: Cell pellet histology (A) and immunohistochemistry, (B, C), bar 50 µm, and transmission electron microscopy (D), bar 5 µm, of a pleural effusion from a dog with dyspnea and metastasizing osteosarcoma. (A). The section contains many round to oval cells with moderate anisocytosis and anisokaryosis, prominent nucleoli and occasional mitoses. H&E. B. Atypical cells are negative for pancytokeratin immunostain (arrow). Scattered cells (likely mesothelial, arrowhead) are positive. Indirect immunoperoxidase, Diaminobenzidine (DAB) and Mayer’s haematoxylin counterstain. C. Atypical cells are diffusely positive for vimentin immunostain. D. Ultrastructure of a single atypical cell characterized by abundant rough endoplasmic reticulum and hypertrophic Golgi apparatus (arrow). Asterisk: nucleus; double asterisk: nucleolus; triple asterisk: cytoplasm.

Figure 3: Computed tomographic transverse pre-contrast image at the level of the scapulae in the bone window in a dog with dyspnea and metastasizing osteosarcoma showing a mineralized mass on the left scapular spine with associated cortical and medullary bone destruction (arrow).

Figure 4: Computed tomographic transverse image pre-contrast (A) and post-contrast (B) of the thorax of a dog with dyspnea and metastasizing osteosarcoma in the soft tissue window. A: Note multiple pleural nodules of mixed soft issue and mineral attenuation (arrow), pleural effusion (arrowhead) and pleural thickening. O: esophagus, D: diaphragm, Ao: aorta, CVC: caudal vena cava. B shows contrast enhancement of a pleural nodule (arrow).

Figure 5: Squash preparation of fine- needle aspiration of a scapular mass from a dog with dyspnea and metastasizing osteosarcoma. There are many oval neoplastic cells occasionally surrounding scant amounts of pink material reminiscent of osteoid (arrow). Neoplastic cells display moderate anisocytosis and anisokaryosis, and multiple prominent nucleoli. Several osteoclasts are also present (arrowhead). Wright–Giemsa stain, bar 50 µm.

Figure 6: Fine-needle aspirate of a scapular mass in a dog with dyspnea and metastasizing osteosarcoma. Neoplastic cells express alkaline phosphatase activity. Blood leucocytes (arrow) served as internal negative control. Positive control not shown. Wright–Giemsa, followed by 5-bromo, 4-chloro, 3-indolyl phosphate/nitroblue tetrazolium (BCIP/NBT) and rapid romanowsky stain counterstaining, bar 50 µm.

Figure 7: Sediment smear of pleural effusion from a dog with dyspnea and metastasizing osteosarcoma. Neoplastic cells express alkaline phosphatase activity. Erythrocytes, , macrophages (arrowhead) and mesothelial cells (arrow) are negative. Wright–Giemsa, followed by 5-bromo, 4-chloro, 3-indolyl phosphate/nitroblue tetrazolium (BCIP/NBT) and rapid romanowsky stain counterstaining, bar 20 µm