

Suspected malignant mesothelial cells in bronchoalveolar lavage fluid of a dog with respiratory distress

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Abstract

An 11-year-old neutered female Labrador Retriever presented with a 1-week history of respiratory distress, lethargy and anorexia. On physical examination, the dog exhibited dyspnea with a rapid and shallow breathing pattern. Thoracic radiography showed diffuse nodular or miliary interstitial patterns, which raised suspicion of bacterial, fungal and parasitic infections and metastatic tumors. Bronchoalveolar lavage (BAL) was performed and the culture yielded no growth of infectious agents, including bacteria and fungi. A presumptive diagnosis of pulmonary mesothelioma was made based on malignant mesothelial cells on BAL fluid cytology. Therefore, this is a report identifying suspected pulmonary mesothelioma using BAL fluid in a dog.

Keywords: Canine, dyspnea, miliary interstitial pattern, mesothelioma, bronchoalveolar lavage

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Introduction

Mesothelioma is a rare neoplasm arising from the lining cells of the serous membranes in the body (Reggeti *et al.*, 2005). Primary mesothelial tumors affecting the pleura, peritoneum, pericardium and tunica vaginalis of the testes have been reported in dogs (Yap *et al.*, 2017). The pathogenesis of mesothelioma involves multiple factors. Exposure to asbestos may be an important causative environmental carcinogen for mesothelioma in humans and dogs (Glickman *et al.*, 1983). The major clinical sign is dyspnea from pleural effusion or a distended abdomen from peritoneal effusion. Exudation can occur via occlusion of lymphatic vessels due to tumor invasion or by the tumor itself, which leads to extensive effusion. Fluid accumulation in the body cavity promotes the desquamation and implantation of mesothelial cells, resulting in mesothelioma seeding in the body cavity. Therefore, mesothelioma should be suspected in adult dogs with a history of chronic nonspecific clinical signs and fluid accumulation in any body cavity (Vail *et al.*, 2019).

Bronchoalveolar lavage (BAL) is a minimally invasive technique that involves instillation of sterile saline into an airway in sufficient volumes to soak the alveoli dependent on that airway. The instilled saline is immediately aspirated and retrieved fluid analysis provides valuable diagnostic information on viral, bacterial, protozoal and fungal infections, as well as neoplasia (Burton, 2017). Establishing a definitive diagnosis of malignant mesothelioma may require adequate sampling of the body cavity lining; however, this procedure requires an open biopsy, which is invasive (Vail *et al.*, 2019). When malignant mesothelial cells are suspected of spreading to the tracheal tree, BAL may be a less invasive diagnostic option for diagnosing pulmonary mesothelioma (Akinfolarin *et al.*, 2008).

To the best of our knowledge, this is the first report of a suspected mesothelioma diagnosed via BAL in a dog. Additionally, this report shows the possibility of lung parenchymal involvement of mesothelioma and shedding of cells to the tracheal and bronchial tree.

Case Report

An 11-year-old neutered female Labrador Retriever dog was referred for severe respiratory distress, lethargy and anorexia for 1 week and had a history of periodically going to the swimming pool. The dog had been regularly vaccinated. On physical examination, the dog showed tachypnea (respiratory rate, 72 breaths per minute), purpura on the left thorax and petechiae on the abdomen. The most prominent sign was tachypnea. A complete blood count showed moderate anemia (hematocrit, 25.9%; reference interval (RI): 37.7-61.7%), neutrophilia ($16.09 \times 10^3/\mu\text{L}$; RI: 2.95-11.64 $\times 10^3/\mu\text{L}$), monocytosis ($1.47 \times 10^3/\mu\text{L}$; RI: 0.16-1.12 $\times 10^3/\mu\text{L}$), and thrombocytopenia ($17 \times 10^3/\mu\text{L}$; RI: 148-484 $\times 10^3/\mu\text{L}$). Changes in the chemistry profile included hyperalbuminemia (3.4 g/dL; RI: 2.6-3.3 g/dL) with increased alkaline phosphatase activity (635 IU/L; RI: 29-97 IU/L), alanine aminotransferase activity (270 IU/L; RI: 21-102 IU/L), aspartate aminotransferase activity (120 IU/L; RI: 23-66 IU/L)

and C-reactive protein (90.68 mg/L; RI: 0-10 mg/L). The SNAP® 4Dx Plus test (IDEXX Laboratories, Westbrook, ME, USA) was negative for vector-borne diseases. Hemostatic abnormalities included prolonged activated partial thromboplastin time (>200 s; RI: 75-105 s) and increased D-dimer concentration (>10,000 ng/mL; RI: 0-250 ng/mL).

Thoracic radiography showed miliary nodular interstitial patterns throughout all the lung lobes (Figure 1) but abdominal radiography and ultrasonography were unremarkable. Based on radiographic findings, bacterial, fungal and parasitic infections and metastatic neoplasia were considered differential diagnoses; hence, BAL was performed under general anesthesia to identify the underlying infectious causes and neoplastic disease within the respiratory tract. General anesthesia was induced with midazolam (0.2 mg/kg, IV; midazolam; Bukwang Pharm, Seoul, Korea) and alfaxalone (1 mg/kg, IV; Alfaxan®, Jurox Pty Ltd, Rutherford, NSW, Australia). Anesthesia was maintained with 2.0-2.5% isoflurane (Terrell, Piramal Critical Care, Bethlehem, PA, USA) in oxygen. The recovered fluid culture revealed an absence of bacterial agents. A fungal culture on Sabouraud's dextrose agar was also negative. Cytology of the BAL fluid showed polygonal to round-shaped cells with anisocytosis, anisokaryosis, multinucleation, coarse chromatin pattern and marked nucleoli, which are suggestive of malignancy (Figure 2). Macrophages were also identified. Unfortunately, the dog was euthanized with the owner's consent because of the expectation of a poor prognosis.

Discussion

A diagnosis of pulmonary mesothelioma by the BAL procedure has been reported in a human patient (Akinfolarin *et al.*, 2008); however, it has not yet been described in dogs. In this report, a Labrador Retriever dog showed infiltration of malignant mesothelial cells in BAL fluid, although a definitive diagnosis of pulmonary mesothelioma may require histological examination.

The mesothelium is a monolayer of specialized flattened cells lining the body's serous cavities and vital organs such as the lungs and heart (Mutsaers, 2004). Inflammation or irritation of the mesothelium causes mesothelial cells to proliferate and exfoliate from the serous membrane into the serosal fluid (Vail *et al.*, 2019). When regeneration ability becomes uncontrolled, malignant transformation of mesothelial cells results in mesothelioma (Paintal, 2016). Mesothelioma has distinct histological patterns, such as epithelial, mesenchymal or biphasic, as determined by the World Health Organization classification (Travis *et al.*, 2015). The epithelial form is the most common form in small animals and resembles carcinoma or adenocarcinoma (Nind *et al.*, 2003; Reggeti *et al.*, 2005). Although cytology alone makes it difficult to distinguish mesothelioma from carcinoma, the presence of cell balls, binucleation, giant atypical mesothelial cells and a uniform cell population may be useful cytological criteria for human mesothelioma cases. Binucleation and a uniform cell population were identified in this BAL fluid sample, indicating that cells

are more likely to be mesothelioma, which is not generally observed in BAL fluid (Cakir *et al.*, 2009; D'Angelo and Di Francesco, 2014). It is also important that mesothelioma be differentiated from reactive mesothelial cells. Serous effusion, particularly longstanding effusions, could be associated with the reactive mesothelial cells. Mesothelial cells could also become reactive when there is pathologic process such as infection, trauma, or metastatic tumor in a body cavity (Shidham, 2021). In the present case, pleural

effusion and metastatic tumor were not detected by thoracic radiography and infection was excluded by BAL fluid culture and SNAP® 4Dx Plus test. No other traumatic events were identified; therefore, the cells were less likely to be reactive mesothelial cells. Although the published sensitivity of cytology for the mesothelioma is variable, a study enrolling 6,285 human patients has shown the cytologic diagnosis of mesothelioma can be made with 73% sensitivity and 99.9% specificity (Segal *et al.*, 2013).

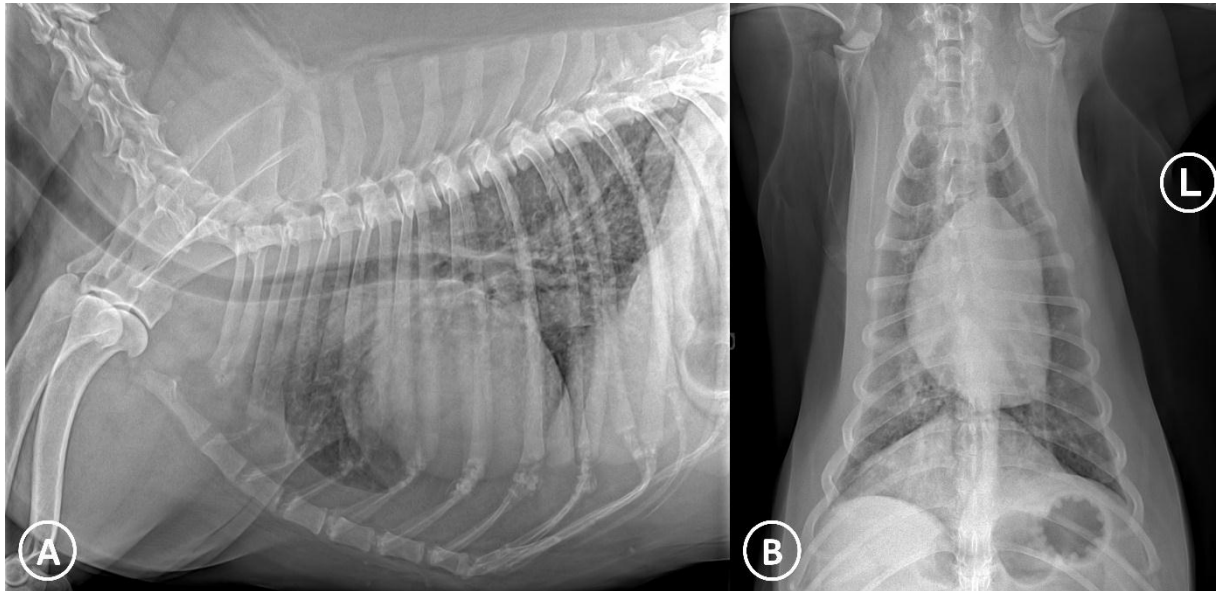


Figure 1 Radiographic findings of suspected mesothelioma in a dog. There were diffuse miliary interstitial patterns in all lung lobes in right lateral (A) and ventrodorsal (B) views.

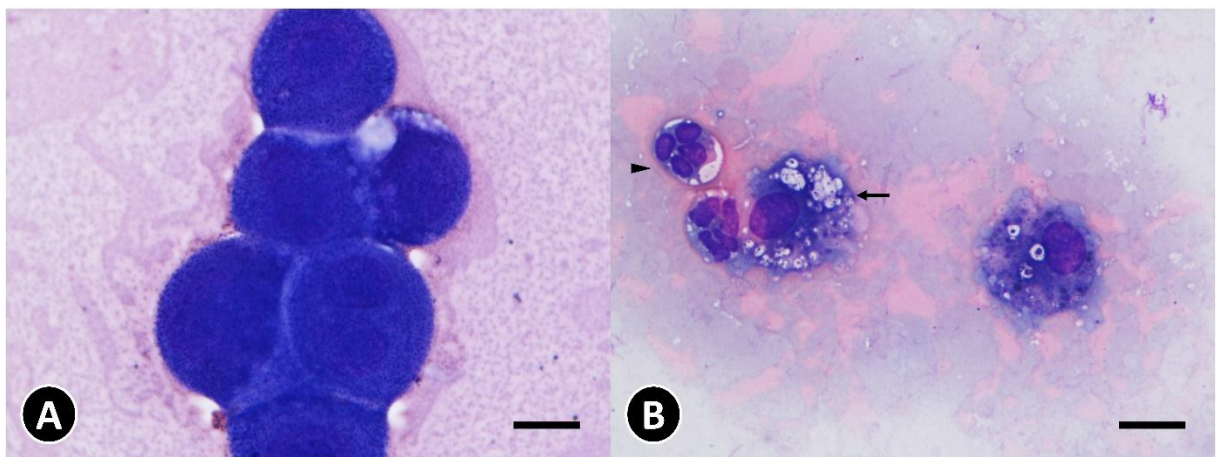


Figure 2 Cytologic findings of bronchoalveolar lavage fluid in a dog with suspected mesothelioma. (A) Polygonal to round-shaped cells with anisocytosis, anisokaryosis, multinucleation, coarse chromatin pattern, and marked nucleoli are present (Wright-Giemsa stain; scale bar: 20 µm). (B) Macrophages (arrow) and degenerated neutrophils (arrowhead) were also identified (Wright-Giemsa stain; scale bar: 20 µm).

Although, in this case, the dog had no pleural effusion, pleural effusion is a common finding in mesothelioma and is the cause of dyspnea, as mentioned above. However, the dog showed a short, rapid and shallow breathing pattern, which raised the suspicion of pleural effusion, diaphragmatic hernia, pneumothorax and bacterial and mycotic pneumonia. These differential diagnoses can be ruled out using radiology and BAL fluid culture. Instead, poor healing of the mesothelium could result in fibrous serosal

adhesions between the organs and body walls, interfering with the lung's ability to expand (Mutsaers, 2004). Therefore, a mesothelioma may be a cause of the restrictive breathing pattern.

Although mesothelioma is considered malignant due to its high potential for metastasis, distant metastasis is rare and is confined to serosal surfaces (Vail *et al.*, 2019). Therefore, pleural mesotheliomas usually extend along the fissural plane. However, in more advanced cases, the mesothelioma can spread to

the subjacent pulmonary parenchyma by transcoelomic implantation (Machida *et al.*, 2004; Nind *et al.*, 2003; Vail *et al.*, 2019). Conceivably, the penetration of pulmonary alveoli through the pores of Kohn, which are alveolar pores that allow the communication of two adjacent alveoli, might be involved in the shedding of malignant mesothelial cells in the bronchial tree.

Overwhelming evidence has been identified for the relationship between the development of mesothelioma and exposure to asbestos. The chronic inflammatory response is one of several underlying mechanisms of mesothelioma, which is initiated by activated macrophages attempting to phagocytose asbestos fibers (Glickman *et al.*, 1983; Vail *et al.*, 2019; Yap *et al.*, 2017). Even if there is no exposure to asbestos, chronic inflammation can lead to neoplastic transformation in canine mesothelioma (Machida *et al.*, 2004). The chronic inflammatory response leads to an immunosuppressive environment characterized by the infiltration of immune suppressor cells, such as myeloid-derived suppressor cells, tumor-associated macrophages and regulatory T cells, which then attract fibroblasts and induces angiogenesis, which facilitates tumor progression (Yap *et al.*, 2017). Macrophages were detected in the BAL specimen, which supported the presence of some degree of chronic inflammation. Macrophages also directly inhibit antitumor T-cell immune responses (Lievense *et al.*, 2016). In the present case, no exposure to asbestos was observed. However, chronic irritation could have occurred because of a history of frequent swimming. Seawater is more hyperosmolar than plasma; hence, inhalation could cause severe damaging stimuli, such as apoptosis, inflammation and DNA damage, in the lungs and alveoli (Jin and Li, 2017).

The primary limitation of this case report is that pleural biopsy was not available for the owner's refusal. Making a definitive diagnosis of mesothelioma via cytomorphology alone is challenging and requires adequate tissue biopsy. Nevertheless, the obtained cells would be mesothelioma because mesothelial cells usually cannot be found in the tracheal tree and show malignant features with no pleural effusion. In addition, because they revealed binucleation and one cell population, it appeared to be mesothelioma rather than carcinoma which is an important differential diagnosis. Hence, a tentative diagnosis of pulmonary mesothelioma was made in the case reported here because of malignant changes in mesothelial cells on cytologic findings of BAL material. Another limitation of this case report is that viral diseases are not excluded by targeted serologic or PCR tests. Considering that the dog had been regularly vaccinated, morbidity through viral pneumonia might be low.

This report describes a case of suspected pulmonary mesothelioma diagnosed via BAL. Therefore, clinicians should be aware that mesothelioma could be involved in the tracheobronchial tree.

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