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CASE REPORT
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# Undifferentiated Pulmonary Adenocarcinoma of Clear Cells Associated to Hypertrophic Osteopathy in a Dog

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**ABSTRACT**

***Background*:** Most of the primary pulmonary tumors in dogs are malignant and from epithelial origin, being bronchioalveolar tumors more prevalent. Adenocarcinoma of clear cells, however, is a very rare pulmonary tumor and its origin is still unknown. It is related to several clinical abnormalities, including hypertrophic osteopathy, an unusual paraneoplastic syndrome characterized by a periosteal reaction along the shaft of long bones. Because of the unusual presentation of the pulmonary adenocarcinoma, the aim of this study was to describe the radiographic, histopathological, and immunohistochemical findings of a dog afflicted with hypertrophic osteopathy secondary to an undifferentiated pulmonary adenocarcinoma of clear cells.

***Case*:** A 12-year-old, 45 kg, not castrated male Great Dane dog was presented with painful swelling of all four limbs and moderate respiratory distress. Radiographic examination and computed tomography of the limbs showed palisade-like periosteal bone proliferation involving radius, ulna, femur, patella, tibia, fibula, tarsus, metacarpal, metatarsal and digits, suggesting hypertrophic osteopathy. Radiographic examination and computed tomography of the lungs also showed a round mass well delimited localized in the right diaphragmatic lobe. A lobectomy of the right diaphragmatic lobe and partial lobectomy of accessory lobe were performed. A poorly differentiated clear squamous cell carcinoma was diagnosed by histological examination. An immune-panel of CK5/CK6, CK7, p63 and TTF-1 was used for immunophenotyping. Immunostaining was weakly positive for CK5/CK6 and negative to all others. Therefore, the diagnosis was poorly differentiated clear cell adenocarcinoma. The dog showed improvement in clinical signs seven days after surgery. One month postoperatively, radiographic examination of the limbs showed less intense periosteal reaction and initiation of bone remodeling.

***Discussion*:** Primary pulmonary tumors are considered very infrequent in small animals, but its true incidence rate is difficult to establish in animal populations. The histological origin of the tumor in the present case, as verified in the literature, is not well established by histological analysis. In these situations, the immunohistochemistry panel may be useful. The modification of the diagnosis between histological analysis and by immunohistochemistry, among other factors, might be due to transdifferentiation from one phenotype to another at various stages in the neoplastic process. The clear cell appearance observed in this case may be verified in all types of carcinoma due to intracellular accumulation of glycogen, most of which is dissolved during the preparation of paraffin sections. This uncommon neoplasm apparently did not influence the radiographic or tomographic findings of the hypertrophic osteopathy in the present case. The frequency of metastases depends on the histological type of the tumor, being common in the pulmonary adenocarcinoma and usually to tracheobronchial lymph nodes and pulmonary parenchyma. Although in this case the imaging studies did not show metastases to other pulmonary lobes, the histological exams showed metastatic lesions that may be associated to the dog's death after the surgery.

**Keywords:** dog, lung, pulmonary primary tumor.

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## INTRODUCTION

Most of the primary pulmonary tumors in dogs are malignant and from epithelial origin [3,7], being bronchioalveolar tumors more prevalent, while adenocarcinomas, adenosquamous and squamous cell carcinomas are less common, representing 13% to 15% of primary lung tumors [12,13]. It's presence, as others thoracic masses, can result in several clinical abnormalities, including hypertrophic osteopathy [1,11,18], a paraneoplastic syndrome reported in less than 3% of primary lung tumors [18,26] and characterized by a periosteal reaction along the shaft of long bones [24].

Because the unusual presentation of the pulmonary adenocarcinoma, the aim of this study is to describe the radiographic, histopathologic and immunohistochemical findings of a dog suffering from hypertrophic osteopathy secondary to an undifferentiated pulmonary adenocarcinoma of clear cells.

## CASE

A 12-year-old, 45 kg, intact-male Great Dane dog was presented for evaluation due to progressive weight loss, difficulty standing up, decreased activity, hyporexia, nonproductive cough, and dyspnea for a period of 30 days. Physical examination revealed lameness, swelling of all four limbs and moderate respiratory distress. Decreased breath sounds were observed on thoracic auscultation. The complete blood cell count revealed 29.3 x $10^3$ leucocytes/μL (9-15 x $10^3$) with neutrophilia (9.3 x $10^3$ neutrophils/μL) and monocytosis (4.7 x $10^3$ monocytes/μL). Abnormalities on the serum biochemical profile included decreased total serum calcium (7.5 mg/dL; 8-12 mg/dL), decreased serum albumin (2.1 g/dL) and increased alkaline phosphatase (237.6 UI/L). Other hematological and biochemical parameters were normal.

Thoracic ventrodorsal, right lateral and left lateral radiographs revealed a solitary nodular density measuring approximately 10 x 9 cm in diameter and located in the right diaphragmatic pulmonary lobe (Figure 1). Abnormalities in mediastinal region were not identified. Radiographic examination of the limbs showed palisade-like periosteal bone proliferation involving radius, ulna, femur, patella, tibia, fibula, tarsus, metacarpal, metatarsal and digits of both forelimbs and hind limbs. In addition, a laminar periosteal bone proliferation was observed at the cranial cortical in both femurs. The soft tissue swelling was evident in those areas.

Abdominal ultrasonography did not show the presence of masses or other abnormalities.

Computed tomography (CT) examination of the thorax was performed with the dog placed in dorsal recumbency and demonstrated a round solid mass localized in the right diaphragmatic pulmonary lobe. The mass was in contact with mediastinal structures, but without invasion (Figure 1). Abnormalities in mediastinal region were not identified, as lymph nodes involvement. At the same time CT examination of the limbs was also performed showing palisade-like periosteal bone proliferation involving the bones above-mentioned, and a laminar periosteal bone proliferation especially on the humerus. A diagnosis of hypertrophic osteopathy secondary to primary pulmonary tumor was performed.

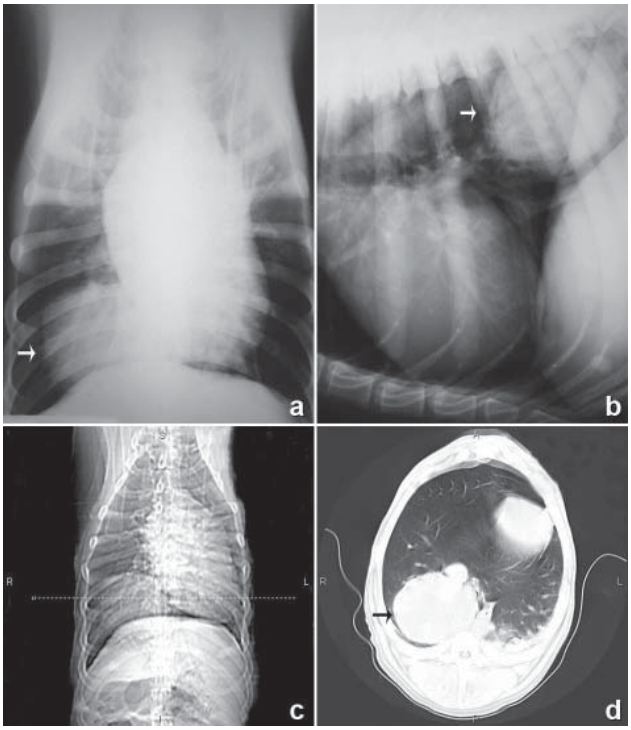


**Figure 1.** Ventrodorsal (a) and right lateral (b) radiograph views of the thorax showing a well-delimited mass localized in the right diaphragmatic pulmonary lobe. Coronal and transverse CT views at the level of the diaphragmatic pulmonary lobe (c, d). Note the round solid mass localized in the right diaphragmatic pulmonary lobe.

A complete lobectomy of the right diaphragmatic lobe and partial accessory lobe were performed by intercostal thoracotomy. For this, after premedication with fentanyl[1] (5 mg/kg, IM), anesthesia was induced with propofol[1] (5 mg/kg, IV), and maintained with isoflurane under controlled ventilation.

Macroscopically the right diaphragmatic lobe had an 8 cm diameter mass with a whitish, smooth

external surface and some brownish areas. The cut surface of the mass measured 8 x 7.5 cm and had a whitish color and yellowish-white necrotic center. Other irregular shaped tumoral masses of approximately 1.5 cm in diameter were distributed through the pulmonary parenchyma. In addition, the bronchi were filled by mucous and the pulmonary parenchyma had firm consistency and white-brown color suggesting lipoid pneumonia. The accessory pulmonary lobe had a condensed area with small whitish areas indicating metastases.

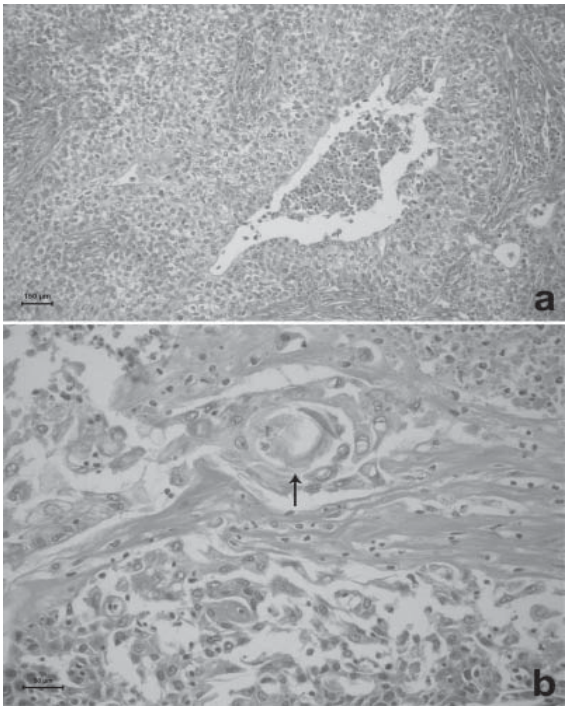


**Figure 2.** Histological diagnosis of pulmonary squamous cell carcinoma in a dog. Neoplastic cells with ample clear cytoplasm, arranged in mantle. Note the central focus of necrosis consisting of cellular debris. HE. Bar = 150 μm (a). Attempted formation of keratin pearl by neoplastic cells (↑). Note in the lower portion of the field, the polygonal to epithelioid neoplastic cells. HE. Bar = 50 μm (b).

Lung samples of the excised mass were fixed in 10% buffered formalin for histopathologic analysis. After embedding in paraffin, 5 μm sections were cut and stained with Hematoxylin and Eosin. Histologically, the tumoral masses were composed of cells with atypical nuclei, coarse chromatin and clear cytoplasm. The neoplastic cells were arranged predominantly in blocks or solid strings contained large areas of necrosis, sometimes infiltrating bronchus. Areas of keratinization characterized by the attempted formation of keratin pearl were observed in some aspects. In other aspects were also observed sparse and rare arrangements of the tumor in acinar structures in the midst of inflammatory infiltrate. The histological examination was compatible with squamous cell carcinoma (Figure 2). The presence of intracytoplasmic glycogen was confirmed by using positive and negative PAS staining, respectively before and after diastase treatment. An immune-panel of CK5/CK6, CK7, p63 and TTF-1 was used for immunophenotyping. Immunostaining was weak positive for CK5/CK6 and negative to all others. Therefore, the diagnosis was poorly differentiated clear cell adenocarcinoma (Figure 3).

The dog showed improvement in clinical signs seven days after surgery. One month postoperatively, radiographic examination of the limbs showed less intense periosteal reaction and initiation of bone remodeling. Adjuvant chemotherapy was recommended, but the owner did not accept it.

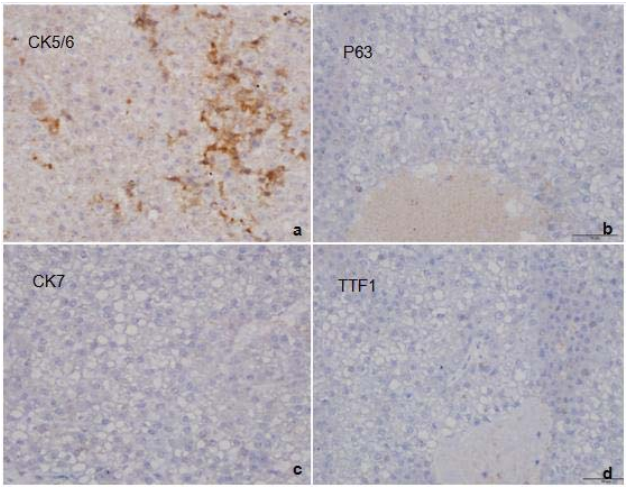


**Figure 3.** Immunohistochemistry of the pulmonary tumor showing weak positive for keratins CK5/6 (a) and negative for P63 (b), CK7 (c) and TTF1 (d). Bar = 50 μm (b, d) and 100 μm (a, c).

## DISCUSSION

Primary pulmonary tumors are considered very infrequent in small animals, but its true incidence rate is difficult to establish in animal populations [13,26]. Dogs necropsied in Veterinary schools from North America and Europe had a frequency of 0.1% and 0.9% respectively of pulmonary cancer [21]. In contrast, because the pulmonary vascular bed, the majority of lung tumors are metastases of primary tumors located in nonpulmonary sites [7,26]. In the present case, no other neoplastic lesions were identified, and according to the owner, the animal had never presented neoplastic lesions before.

Increase in life expectancy of the animals, sensibility and capability for diagnosis, and predis-

posing factors as pollution and passive smoking have been associated with the increased reported cases [18,28]. However, the dog in the present case was from a rural area and had no access to pesticides. In general, the middle aged or older dogs (average age of 10 years) and weighing over than 20 kg are the most commonly affected by primary pulmonary tumors [18, 28], as verified in the present case. There is no sex or breed predilection, but some studies reported higher incidence in females than males in a ratio of 2:1 [17,28].

The histological origin of the present tumor, as verified in the literature, is not well established by histological analysis [17,21]. In these situations, an immunohistochemistry panel may be useful. In the present case, the immunohistochemical diagnosis was compatible with undifferentiated adenocarcinoma since primary or metastatic pulmonary squamous cell carcinoma is usually positive for CK5/CK6 cytokeratin of high molecular weight 34βE12, CK5/CK6 cytokeratin and low molecular weight cytokeratins 35βH11. Additionally, few cases are TTF-1 and 7 cytokeratin positives [23]. In one study, evaluating protein expression in non-small cell lung carcinomas, one case was CK7+ e SP-A+, and 14/18 (77.8%) were p63+. In this same study, all adenocarcinomas were negative for p63; 9/16 (56.2%) were CK5/CK6+, 16/17 (94.1%) were CK7+, and 4/15 (26.6%) were SP-A+ [4]. The expression of p63 protein is very useful to differentiate of both histologic tumors types [5].

The modification of the diagnosis between histological analysis and immunohistochemistry, among other factors, might be due to transdifferentiation from one phenotype to another at various stages in the neoplastic process [26]. According to Carvalho [6], there is a negative correlation of 71.3% between histologic analysis by HE and immunohistochemistry in the pulmonary tumors diagnosis. Although immunohistochemistry be a good complementary method for lung cancer diagnosis, the antigenic markers panel utilized was not able to define histogenetically 6.9% of the cases submitted to immunohistochemistry [6].

The dog in the present case had all the most common primary clinical signs, but also hypertrophic osteopathy. The hypertrophic osteopathy appears not to have racial or sexual prevalence, and affects dogs over eight years of age [2,27]. However, a study evaluating seven dogs with hypertrophic osteopathy secondary to pulmonary neoplasms found that approximately 57% of these were German Shepherds with average of 11 years of age [24], wich is similar to the findings of the present study. The pathogenesis of hypertrophic osteopathy is not well known, but the clinical signs tend to decrease within a few days after removal of the tumor mass [9,16,20], as observed in the present case. In another report case of a Maltese dog with hypertrophic osteopathy secondary to a pulmonary adenosquamous carcinoma, the periosteal reaction of forelimbs showed significant decrease 14 days after lobectomy [15].

Hematological and biochemical abnormalities verified in the present study, as neutrofilia and increased alkaline phosphatase are common findings of hypertrophic osteopathy [15,27] and observed in, respectively, 55% and 61.2% of dogs suffering from hypertrophic osteopathy due to pulmonary masses [27]. These findings may be resulted from stress, inflammation and increased osteoblast activity surrounding the new bone formation and secondary to the neoplastic process [27].

The most common radiographic and tomographic findings of the limbs are diffuse periosteal new bone formation along the diaphyses and metaphyses of certain limb bones, including radius, ulna, tibia, metacarpal and metatarsal [15,16,20,22]. The uncommon neoplasm of the present case apparently did not influence in the radiographic or tomographic findings.

The frequency of metastases depends on the histological type of the tumor, being common in the pulmonary adenocarcinoma. Metastases most often occur in tracheobronchial lymph nodes and pulmonary parenchyma [7,21,26]. Although in this case the imaging studies did not show metastases to other pulmonary lobes, the histological exams showed metastatic lesions that may be associated to the dog's death after the surgery.

SOURCE AND MANUFACTURER

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***Declaration of interest.*** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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