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NOTE

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Squamous cell carcinoma of unknown primary origin in a dog presenting with bone metastasis

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ABSTRACT. A 10-year-old female American Pit Bull dog was diagnosed with metastatic undifferentiated carcinoma of the scapula. Immunohistochemistry showed positive immunoexpression for cytokeratins (AE1/AE3, 34BE12, CK7) and vimentin, confirming squamous cell carcinoma. No evidence of nodules was found in the complete physical examination and imaging procedures conducted. The patient was diagnosed with carcinoma of unknown primary origin. Amputation and adjuvant chemotherapy with doxorubicin and piroxicam were performed, but the patient died of respiratory failure after 737 days of diagnosis. Necropsy confirmed undifferentiated carcinoma infiltrating the lungs and kidneys, and showing the same immunoexpression as the tumor in the scapula. Amputation associated with chemotherapy extended the overall survival time of this patient.

KEY WORDS: canine, carcinoma of unknown primary, metastatic cancer, neoplasm

Carcinoma of unknown primary (CUP) origin constitutes a group of metastatic carcinomas with no identified primary tumor after a complete and thorough examination of the body, including patient anamnesis, physical examination, laboratory tests, and imaging techniques (thoracic radiography, abdominal ultrasound or computed tomography (CT) of the thorax, abdomen, and pelvis), as well as mammography and prostate-specific antigen detection in blood in humans [10]. The veterinary field lacks concise information regarding CUP in dogs or cats. Some case reports have described CUP, but only one study diagnosed CUP after performing a complete patient clinical history recording, physical examination, blood tests, urinalysis, and whole body CT in 21 dogs [12]. However, no clear consensus exists for the diagnosis algorithm of CUP in dogs.

CUP in humans is the seventh most common cancer and constitutes approximately 3–5% of solid tumors [10]. CUP incidence is comparable with that of pancreatic carcinoma, wherein 3,000 new cases are observed every year in the United States [15]. Although the lack of studies in veterinary medicine makes it impossible to define its incidence in pets, it seems less common as compared to the incidence in humans.

Some theories have tried to explain the reason and mechanism of the primary tumor remaining hidden and unidentifiable, but the answer is still unknown [3, 10, 12]. Several immunohistochemistry (IHC) algorithms have been described in human medicine to identify its origin, and they constitute an easy and practical way to detect the primary tissue of origin [13]. Veterinary studies used IHC and seemed to offer valuable results, but IHC algorithms were not fully described. Clinical signs depend on the organs affected by metastasis, but treatment should be aimed at the suspected neoplasm origin and chemotherapy is a part of the treatment in most cases. Prognosis is poor because CUP is described as an aggressive tumor with rapid development of new metastasis in both humans and dogs [1, 8, 12].

This report aims to describe a case of CUP in a dog with a significant survival time treated with surgery and chemotherapy, as well as to describe how the IHC algorithm could help detect the primary tissue of origin and aid in decision-making.

A 10 year-old, spayed, female, American Pit Bull dog was referred to the Oncology Service at the Veterinary Hospital of the

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São Paulo State University, Campus of Jaboticabal, after being diagnosed with a metastatic carcinoma of the left scapula and amputation of the left thoracic limb performed three weeks ago. Owners reported lameness and pain as the first clinical signs observed one month before amputation, and pain was more evident when left scapula was palpated with no response to analgesics reported over time.

Radiographic examination of the left scapula by the referring veterinarian showed a lytic process in its proximal region. A bone fine-needle aspiration was performed but results were inconclusive. Due to worsening of the clinical condition and failure to control pain, the referring veterinarian elected to amputate the left thoracic limb with the owner's consent. After the amputation procedure, the surgically removed left scapula was sent for pathologic evaluation. Histopathologic results described undifferentiated cells, apparently polyhedric-shaped, exhibiting large and rounded nuclei with moderately abundant eosinophilic cytoplasm. Cells were disorganized and formed clusters and coalescent cellular trabeculae with mitotic index of 1 mitosis/high power field. Final diagnosis was undifferentiated carcinoma infiltrating trabecular osseous tissue. Blood test (complete blood count, creatinine, and alanine aminotransferase [ALT]) and thoracic radiography showed no signs of metastatic nodules.

During the initial consultation in our hospital, the owner reported no signs of nodules or skin masses in the anamnesis of the dog. The animal was spayed at 8 months of age, before her first estrous cycle. The physical examination did not reveal any signs of skin nodules or pain in any region of the body. Special attention was focused on examination of oral cavity, cervical region, mammary glands, and anal sacs.

After an unremarkable physical examination, we performed blood tests, including complete blood count, creatinine, ALT, alkaline phosphatase, albumin, and total protein, as well as urinalysis and imaging techniques (thoracic radiography in three views and abdominal ultrasound). To rule out any involvement of anal glands, a cytology specimen was collected from both anal sacs. Results of blood tests and urinalysis were within the normal range for the species and imaging results showed no alterations; cytology showed a scattered population of normal epithelial cells.

A second pathologist confirmed the presence of undifferentiated epithelial cells infiltrating the bone tissue, and a complete IHC profile was recommended. IHC was positive for AE1/AE3, 34BE12, CK7, and vimentin (Table 1). According to IHC, a squamous cell carcinoma (SCC) was diagnosed. Then, we performed thoracic CT and biopsy of bilateral tonsils to rule out SCC of these organs. No evidence of masses in the thorax or any neoplastic cells in the tonsils were found. Thus, CUP was diagnosed based on these results.

After diagnosis, a doxorubicin-based chemotherapy protocol was proposed and accepted by the owner. The patient was administered 5 doses of doxorubicin (30 mg/m^2 intravenous, Fauldoxo, Libbs, São Paulo, Brazil) every 21 days and piroxicam (0.3 mg/kg orally, once daily, Feldene, Medley, São Paulo, Brazil) was concomitantly administered. Thorax radiography and abdominal ultrasound were performed every month during chemotherapy protocol and for three months after finishing chemotherapy, then every three months thereafter.

After 737 days of diagnosis, the patient was presented to the emergency department of our hospital because of mixed dyspnea, lethargy and reluctance to move noted by the owner 7 days ago. During consultation patient experienced a respiratory failure followed by cardiac arrest. Cardiorespiratory resuscitation procedure was unsuccessful and the patient died. Necropsy was authorized by the owner and performed by the Pathology department at our institution. Multiple infiltrating and coalescent white areas were observed throughout both lungs and kidneys (Fig. 1). Samples of these tissues were collected and sent for analysis. Histopathological results showed undifferentiated neoplastic cells infiltrating pulmonary and renal parenchyma. IHC evaluation showed positive expression for AE1/AE3, 34BE12, Vimentine and CK7 (Fig. 2). Based on the histopathologic characteristics and IHC results, we confirmed the diagnosis of CUP.

CUP represents a group of metastatic carcinomas in which a primary tumor cannot be found. It seems to be rare in veterinary medicine, therefore information regarding tumor biology, prognosis, and treatment decision is still lacking in current literature [12]. In contrast, it represents approximately 3–5% of all tumors in humans [1], and some theories about CUP development have been discussed. One theory proposes that after a metastatic nodule arises, the primary tumor is eliminated by delayed recognition of the immune system [3]. Other authors explain that different genetic profiles were found in primary and metastatic nodules, so metastatic nodules have different characteristics and behavior induced at aberrant locations throughout the body [5].

Another theory postulates that the primary nodule enters dormancy after releasing metastatic cells into the blood stream, thus remaining microscopic and unidentified by imaging techniques [7]. Furthermore, some other authors propose that metastatic cells are released into circulation because of insufficient vascularization in the primary tumor, which is later eliminated, and that escaped malignant cells remain in dormancy in any other tissue until complete vascular supply and appropriate conditions are present to start growing and form a tumor [9].

We report a case of CUP without any evidence of a primary tumor in a patient with metastatic carcinoma of the bone that later affected the lungs and kidneys. Metastatic tissue is defined as malignant epithelial tissue carcinoma,

Table 1. Immunohistochemistry results of bone tissue removed after amputation in a dog

Antibody	Result
AE1/AE3	+
34BE12	+
Vimentin	+
CK7	+
Cerb-B2	−
RE	−
RP	−
35BH11	−
CK20	−
Chromogranin	−
S100	−

AE1/AE3: Pan-cytokeratin, 34BE12: Cytokeratin 34 beta E 12, CK7: Cytokeratin 7, Cer-B2: Her2/neu, RE: Estrogen receptor, RP: Progesterone receptor, 35BH11: Cytokeratin 8, CK20: Cytokeratin 20.

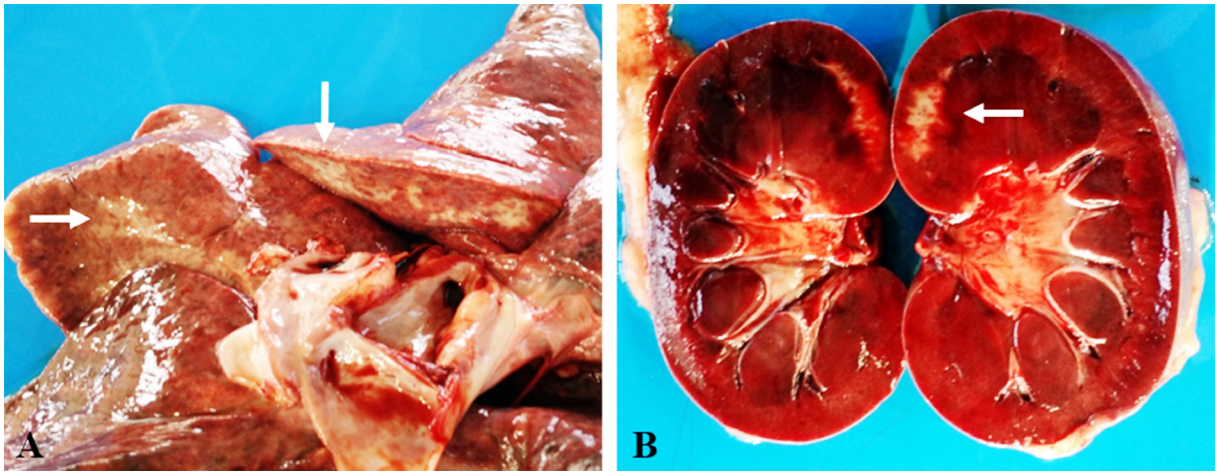

Fig. 1. Post-mortem examination of a dog with carcinoma of unknown primary. (A) Lung: Diffuse and coalescent white areas seem to infiltrate the pulmonary parenchyma (arrows). These areas constituted of neoplastic epithelial malignant cells; (B) Left kidney: Diffuse white areas identified as metastasis are found in the cortex (arrow) of both kidneys.

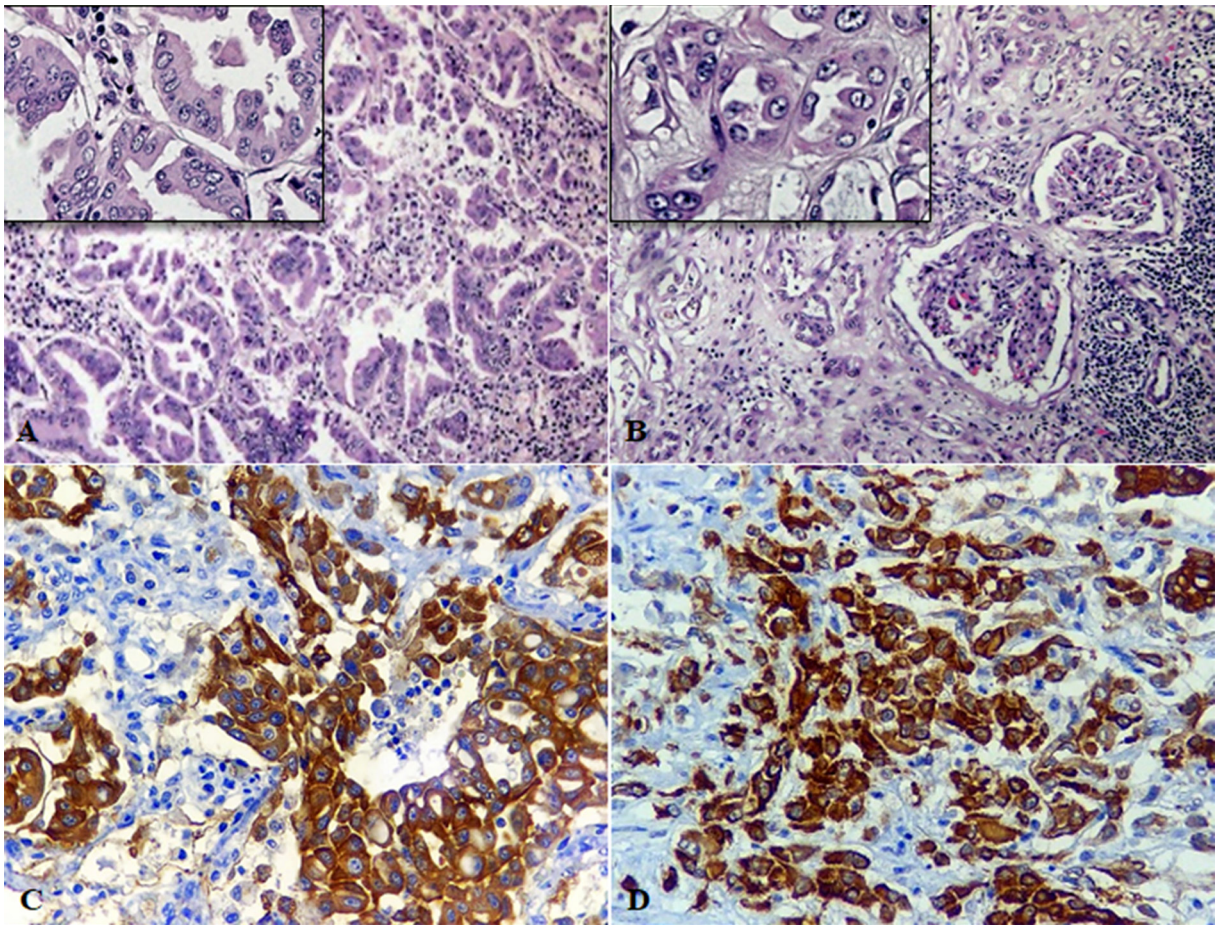

Fig. 2. Histopathologic findings in a dog with carcinoma of unknown primary. (A) Lung: Epithelial neoplastic proliferation shows high cellularity with poorly demarcated and infiltrative growth. These cells are organized in a tubular to papillary pattern, with marked anisokaryosis and discrete kariomegaly (Inset) (Hematoxylin and Eosin [H&E] stain, 100×; Inset, 400×; (B) Kidney: Neoplastic cells of epithelial origin, similar to that observed in the lung are seen. There is a predominant tubular distribution pattern, anisocytosis, anisokaryosis, and kariomegaly (Inset) [H&E stain, 100×; Inset, 400×]; (C and D) Lung: A strong immunohistochemistry expression of both AE1/AE3 (C) and 34BE12 (D) antibodies (brown color) is observed in the cytoplasm of the epithelial neoplastic cells (400×).

because of the classic characteristics found on histopathology including large and rounded nuclei, abundant eosinophilic cytoplasm, and distribution of cells forming blocks called clusters. In accordance with the World Health Organization guidelines of bone tumors [14], primary tumors of bone emerge from a mesenchymal cell or from one of its precursors (cartilage, fibrous or adipose tissue, etc.), but not from epithelial cells. Therefore, finding epithelial cells in bone suggests a metastatic origin and not a primary tumor. Additionally, IHC results showed a positive expression to pan-cytokeratin AE1/AE3, a selective antibody for epithelial cells, which is commonly expressed in undifferentiated carcinomas [4]. Positive expression of vimentin in epithelial cells was associated with phenotypic transformation of epithelial cells into mesenchymal cells. This process is called epithelial-mesenchymal transition and is associated with more aggressiveness and metastatic profile of epithelial cells, as seen in this case [4].

In this case, the proximal region of the left scapula was infiltrated by neoplastic cells, and metastatic carcinomas tend to localize in proximal regions of long bones [2]. Some authors [2] have described 19 cases of metastatic carcinomas in bones, where 11 (58%) cases were diagnosed with CUP because of absence of evidence of a primary tumor. In the largest study in veterinary medicine [12], the authors confirmed only one primary tumor in 21 dogs, wherein this dog had metastatic melanoma of the mandibular lymph node and developed melanoma in the ipsilateral thoracic limb after seven months.

We performed several examinations and imaging techniques to try to identify the primary tumor during the course of the disease; however, no evidence of a primary tumor was ever confirmed. Some studies suggested that complete body advanced imaging should be performed to rule out masses in any part of the body [13], but other authors [12] found no advantage with this technique, because no mass was identified after complete body CT of 21 dogs with CUP. We believe that thoracic CT and constant evaluation through abdominal ultrasound and thoracic radiographs are sufficient to confirm the absence of primary tumor in a patient with a metastatic carcinoma of the bone, and this constitutes the key criterion for CUP diagnosis [10].

In human medicine, an algorithm for CUP diagnosis is defined by IHC positive results for CK7 and CK20, which provides origin information and helps to decide the treatment [15]. In this patient, cells tested positive for CK7 and negative for CK20, suggesting a pulmonary, pancreatic, hepatic, or thyroid carcinoma as the primary tumor [15]. Additionally, metastatic cells tested positive for high-molecular weight cytokeratin (34BE12) that identifies basal and squamous cells in the bronchus and prostate tissues in humans, and differentiates squamous cell carcinomas from pulmonary adenocarcinomas [11]. Negative expression of low-molecular weight cytokeratin (35BH11) rules out the possibility of several carcinomas, as this cytokeratin is widely distributed in epithelial cells, but not in squamous cells [11].

The female dog in this report was spayed before her first estrous cycle; therefore, negative expression for antibodies related to the mammary tissue (RE, RP, Her2/neu) was expected, ruling out mammary and reproductive cells as the tissue of origin. Negative expression for S100 and chromogranin ruled out the possibility of neuroendocrine and nerve sheath tumors, as well as melanomas [6].

According to the IHC results, a metastatic squamous cell carcinoma was finally diagnosed. Remarkably, the patient had never presented a cutaneous nodule before diagnosis, nor was there any evidence of cutaneous nodules observed during treatment and follow-up. Moreover, tonsilar biopsy showed no neoplastic cells and thoracic CT did not show any evidence of pulmonary nodules. Thus, we ruled out SCC in the main suspected tissues of origin.

According to some authors [12], an IHC panel is not recommended in veterinary medicine until further studies confirm its clinical applicability. We disagree with this statement, because IHC is one of the few validated techniques for identifying primary tumors in CUP cases in human medicine. Although the antibodies used in this case were not canine validated, IHC results guided diagnosis and helped in treatment decision.

Treatment decision using doxorubicin and piroxicam proved effective in this case. New skin nodules were not observed during the follow-up period of almost two years, until massive pulmonary metastases were evident after 737 days of the diagnosis. This survival time goes beyond the median survival time of 30 days in dogs with CUP as reported by Rossi *et al*., [12]. Humans with CUP have poor prognosis and present a median survival time of approximately 6–12 months, and only 25% of patients are expected to live over a year [10].

In this case, a unique metastatic tumor was identified in the scapula and treated radically with surgery (amputation). The presence of more than two metastatic sites, hepatic involvement, and presence of comorbidities constituted negative prognostic factors in humans [8]. The lack of negative prognostic factors probably favored the longer survival time observed in this case.

We concluded that CUP diagnosis needs several thorough examinations to rule out the presence of a primary tumor. CT and IHC panel were the most important diagnostic techniques used to diagnose CUP. Although veterinary medicine lacks sufficient published data concerning this rare tumor, diagnostic algorithms taken from previous reports on humans helped to diagnose a specific histopathological tissue in this case. With this information, we could decide the most appropriate treatment for the patient. Radical surgery of the unique metastatic site associated with chemotherapy seemed to extend the overall survival time of the patient in this case.

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