Clinical and Pathological Features of Canine Metastatic Adrenocortical Carcinoma

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ABSTRACT

Background: Adrenocortical carcinomas (ACC) are uncommon in dogs. ACC often invade the posterior vena cava wall and the advential layer of the abdominal aorta; however, metastases to distant organs are rare. Most dogs with ACC show clinical signs of Cushing`s syndrome in a similar way with signs reported in humans. The aim of this study is to report three cases of metastatic ACC in dogs and their clinical and pathological features.

Cases: This report describes three cases of metastatic adrenocortical carcinoma (ACC) in dogs diagnosed *post mortem* through histopathological examination. The animals presented no signs of adrenal hormones overproduction. *Case 1*. A 13-year-old intact, mixed breed female dog was presented with a history of progressive hepatomegaly during the last four months. The animal was submitted to an exploratory laparotomy, but due to anesthetic complications no tissue specimen was sampled and after one week, the animal died. The necropsy examination showed an increased left adrenal (3 cm in diameter), multiple yellow to whitish nodules measuring 1 to 2 cm of diameter in the lung and severe hepatomegaly. The histopathological diagnosis was established as ACC with pulmonary metastasis. A severe and diffuse accumulation of glycogen in hepatocytes was also observed in Periodic acid-Schiff (PAS) staining. *Case 2*. A 9-year-old female intact, mixed breed dog was presented showing ataxia, left head-tilt and dyspnoea. The clinical signs progressed and the dog died after four days. The gross examination showed a diffuse increase of the left adrenal gland (2 cm of diameter) accompanied by a yellowish colour at cut surface; diffuse bronchopneumonia and cerebral and renal discrete congestion. The microscopical diagnosis was ACC with kidney and CNS metastasis. *Case 3*. A 16-year-old male, intact, Poodle dog was presented with apathy, anorexia, vomiting and diarrhoea in the last two days. The animal was submitted to treatment, but the clinical signs progressed and the dog died. In the necropsy exam an increased right adrenal gland was observed (5 cm of diameter) showing multiple yellowish nodules when sectioned. The lung showed multiple whitish nodules mainly in pleural region. In the histopathological examination, the definitive diagnosis was ACC with pulmonary metastasis.

Discussion: Most of adrenocortical tumours in dogs are functional and secrete excessive amounts of cortisol; therefore commonly animals show clinical signs of Cushing`s syndrome. In the present cases, the animals showed no clinical evidence of hormonal changes. Well-differentiated neoplastic cells were observed in cases 1 and 2, whereas in case 3 tumorous cells showed marked features of malignancy as cellular pleomorphism, binucleation, high mitotic index and atypical mitosis. Despite mild anaplastic features observed on cases 1 and 2, animals showed focus of metastases in lung, kidney and CNS. Apparently features of malignancy in ACC were not associated to the risk of metastasis development, indicating that even well-differentiated ACC may metastasize to distant organs. Metastases of ACC to distant sites are rare in dogs and there was no previous report of metastasis to CNS as observed in case 2. Canine ACC are considered uncommon tumours, however, they should be included in the differential diagnosis of mass in the abdominal cavity since the animals may show no clinical signs of adrenocortical hormones overproduction.

Keywords: adrenal tumours, adrenocortical hormones, metastases, dog.
Descritores: tumores adrenais, hormônios adrenocorticais, metástases, cão.

INTRODUCTION

Primary adrenal tumours affect approximately 0.17% to 0.76% of the dogs and represent about 1% to 2% of all canine tumours [17]. The primary adrenocortical tumours can be divided into benign adenomas and malignant carcinomas that can be either endocrinologically inactive or hormonally functional [3,6]. ACC are uncommon in dogs and most are functional tumours [4]. The majority of dogs with functional carcinomas present typical clinical signs of glucocorticoid excess, with polyuria and polyphagia as the dominating features. Other signs of canine Cushing`s syndrome include abdominal obesity, weight gain, fatigue, muscle atrophy, skin changes [3] and are similar to those reported in humans [7,8].

In dogs, ACC often invade the posterior vena cava wall and the advential layer of the abdominal aorta [9,15,16]. Metastases to distant sites are rare and were described in liver, lung, kidney and mesenteric lymph nodes [4,16,19]. In humans, the most common sites of metastasis are the regional lymph nodes, peritoneal ad pleural surfaces, liver, lung and bone [14,18].

The aim of this report was to present three cases of metastatic ACC in dogs and their clinical and pathological features.

CASES

Three dogs presenting metastatic ACC were attended at a Veterinary Teaching Hospital (Universidade Estadual de Londrina, Paraná, Brazil - HV/UEL) for clinical examination. Diagnoses of ACC were performed after the necropsy exam and histopathological evaluation.

Case 1. A 13-year-old intact, mixed breed female dog was presented with a history of progressive hepatomegaly during the last four months. No other changes were detected on clinical examination. The animal was submitted to an exploratory laparotomy, but due to anaesthetics complications no tissue specimen was sampled and after one week, the animal died. The necropsy examination showed an increased left adrenal (3 cm in diameter). At cut surface, an irregular, necrotic, yellowish surface was observed (Figure 1). In addition, multiple yellow to whitish nodules measuring 1 to 2 cm of diameter in the lung and severe hepatomegaly with evident lobular pattern were observed. Histopathological evaluation revealed a proliferation of neoplastic cells with large eosinophilic cytoplasm, with

occasional vacuolation, an oval and hyperchromatic nucleus, occasional conspicuous nucleolus, moderate anisokaryosis and low mitotic index (Figure 2A) The neoplastic cells were disposed in solid arrangements interspersed by delicate collagenous stroma, located in the cortical region (fasciculate layer) with infiltrative areas in the medullar region; the lung present areas of neoplastic cellular proliferation with similar features to the observed in the adrenal (Figure 2B). Hepatocytes presented a severe and diffuse glycogen accumulation observed by Periodic acid-Schiff (PAS) staining. The definitive diagnosis was ACC with pulmonary metastasis.

Case 2. A 9-year-old female intact, mixed breed dog was presented showing ataxia, left head-tilt and dyspnoea. Clinical examination reveals no others clinical alterations. The clinical signs progressed and the dog died after four days. The gross examination showed a diffuse increase of the left adrenal gland (2 cm of diameter) accompanied by a yellowish colour at cut surface; diffuse bronchopneumonia, cerebral and renal discrete congestion. Microscopically, the adrenal presented a proliferation of neoplastic cells with large eosinophilic cytoplasm with occasional vacuolation, hyperchromatic nucleus, occasional binucleation and conspicuous nucleolus, discrete anisokaryosis and moderate mitotic index (Figure 2C). The neoplastic cells were disposed in trabecular arrangements interspersed by delicate collagenous stroma, located in the cortical region with infiltrative areas into subjacent tissue. The left kidney, brain, cerebellum and brainstem showed multifocal areas of tumorous emboli. The definitive diagnosis was ACC with kidney and CNS metastasis.

Case 3. A 16-year-old male, intact, Poodle dog was presented with apathy, anorexia, vomiting and diarrhoea in the last two days. The animal was submitted to treatment, but the clinical sigs progressed and the dog died. In the necropsy exam an increased right adrenal gland was observed (5 cm of diameter) showing multiple yellowish nodules when sectioned. The lung showed multiple whitish nodules mainly in pleural region, measuring 0.2 cm to 0.5 cm of diameter. In the histopathological examination, the adrenal gland presented a proliferation of polyhedral cells with moderate to large vacuolated amphophilic cytoplasm, vesicular nucleus, conspicuous nucleolus, elevated anisokaryosis and high mitotic index with presence of atypical mito-

sis (Figure 2D). The neoplastic cells showed a diffuse pattern of growth with a delicate collagenous stroma into the cortical region that compressed the medullar area. There was multifocal haemorrhage and vascular invasion subjacent to neoplasm. The lungs presented multiple focus of neoplastic proliferation similar to adrenal gland. Moreover, there was presence of tumoral emboli into blood vessels. Others changes observed were chronic interstitial nephritis and enteritis. The definitive diagnosis was ACC with pulmonary metastasis.
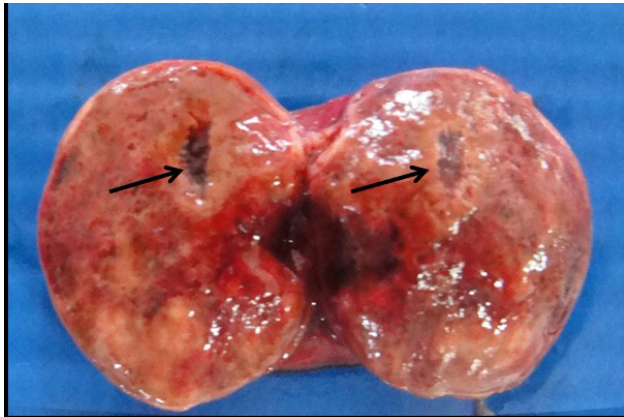

Figure 1. Adrenocortical carcinoma in dogs (case 1). At section, the adrenal showed an irregular surface, yellowish color with necrosis areas (black arrows).
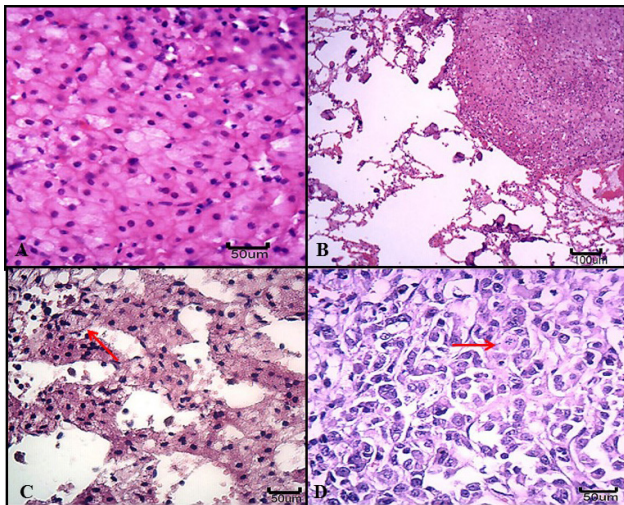

Figure 2. Adrenocortical carcinoma (ACC) in dogs. (A)- ACC case 1: proliferation of neoplastic cells disposed in solid arrangement with eosinophilic cytoplasm, hyperchromatic nucleus and moderate anisokaryosis. [HE, bar = 50 μm]. (B)- Pulmonary metastasis in case 1: proliferation of neoplastic cells with similar aspects observed in adrenal gland. [HE, bar =100 μm]. (C)- ACC case 2: proliferation of neoplastic cells disposed in trabecular arrangement with eosinophilic cytoplasm, hyperchromatic nucleus and presence of mitotic figure (red arrow). [HE, bar = 50 μm]. (D)- ACC case 3: proliferation of neoplastic cells disposed in solid arrangement with amphophilic and vacuolated cytoplasm, vesicular nucleus, conspicuous nuclei, elevated anisokaryosis and presence of atypical mitosis (red arrow). [HE, Bar = 50 μm].

DISCUSSION

The adrenal gland consist of two distinct regions, cortex and medulla, that differ morphologically and functionally due their distinct embryologic origin [5]. The adrenal cortex is subdivided into three layers or zones: the glomerulosa layer (outer zone) is composed of columns of cells that show a sigmoid arrangement and is responsible for the secretion of mineralocorticoid hormones (e.g. aldosterone); the cells of the fasciculate layer (middle zone) are disposed in long anastomosing cords separated by numerous small capillaries and constitute about 80% of the total volume of cortex region and secrete glucocorticoid hormones (e.g. cortisol); the reticularis layer (inner zone) presents cells arranged in small groups surrounded by capillaries and is responsible for the secretion of sex steroids (e.g. progesterone, oestrogens and androgens). The cells of the medullar region secrete catecholamine hormones [5,6].

Adrenocortical carcinomas are uncommon tumours in humans [14] and dogs [4]. In humans, the use of oral contraceptives and smoking may increase the risk of adrenal cancer development [6]. However, there are no data about the predisposition to adrenal tumoral development in dogs. In humans, ACC are aggressive endocrine tumours affecting mainly children younger than five years and adults after 40 years-old [6]. In dogs, ACC are uncommon and can develop in adult to older dogs and present no breed or sex predisposition [4].

In goats, ferrets, hamsters and mice, the frequency of functional adrenocortical neoplasms is higher in gonadectomized animals. These gonadectomy-induced adrenocortical tumours may produce an excessive amount of sex steroids [4]. In dogs, the gonadectomy does not impact in the incidence of adrenocortical tumours [3]. Most of adrenocortical tumours are functional and secrete excessive amounts of cortisol; therefore commonly the animals show clinical signs of Cushing`s syndrome [2,4]. In the present cases, the animals showed no clinical evidence of hormonal changes. Hepatocyte glycogen accumulation was observed in case 1 probably related to overproduction of endogen cortisol induced by neoplastic cells.

Several endocrinological tests have been used to diagnosis hyperadrenocorticism. The most common in veterinary routine are urine cortisol:creatinine ratio, ACTH stimulation test and low-dose dexamethasone suppression test (LDDS) [2,10,12]. Ancillary exams as ultrasonography, magnetic resonance imaging and com-

puted tomography contribute to identify both clinical and subclinical adrenal tumours [2,4]. The definitive diagnosis of ACC is obtained by histopathological examination [4]. Surgical removal of the affected adrenal gland is indicated if the tumour is suspected to be functional, exceeds 4 cm in diameter or presents any evidence of vascular invasion [2]. In our cases, no ancillary tests or surgical excision were performed since the animals showed no clinical signs of hormonal adrenocortical overproduction and the diagnosis of ACC was achieved *post mortem.*

ACC are usually large tumours compared to adenomas and may develop in both glands [2]. Grossly, they present yellow to brownish red colour, can be friable and have multiple areas of mineralization, necrosis, haemorrhage and occasionally cystic degeneration [4,14]. Microscopically, ACC are composed usually by large and polyhedral cells, with densely eosinophilic or vacuolated cytoplasm; the nuclei may appear small and uniform or may exhibit pronounced atypia manifested by pleomorphism, coarse chromatin granularity, multiple nuclei and multiple conspicuous nucleoli [8]. Mitotic activity is variable but often ACC show high mitotic index and atypical mitosis [4,13]. The pattern of neoplastic cells growth include trabecular, lobular, nests or solid arrangements and also admixtures of them [4,8] and the fibrovascular stroma varied in thickness [4,18]. Invasion of the adrenal capsule, blood and lymphatic vessels (forming emboli) by neoplastic cells are often observed in ACC [4]. In cases 1 and 2, ACC showed well-differentiated neoplastic cells, whereas in case 3 tumorous cells presented features of malignancy as cellular pleomorphism, binucleation, high mitotic index and atypical mitosis. Despite the discrete histopathological malignancy features observed on cases 1 and 2, the animals showed focus of metastases in lung, kidney and CNS. Therefore, apparently features of malignancy in ACC were not associated to the risk of metastasis development, indicating that even well-differentiated ACC may metastasize to distant organs as lung and CNS.

The principal differential diagnoses of ACC are adrenocortical adenomas, renal cell carcinomas and adrenomedullary tumours [18]. Adenomas tend to be smaller, homogeneous and lacking haemorrhage and necrosis. Microscopically, they present discrete cellular atypia and low mitotic index [4,14]. However, the distinction between adenoma and well-differentiated ACC can be a challenge, except in cases where metastases are detected as in the present study. Renal cell carcinomas can invade the adrenal gland directly, metastasize ipsilaterally or contralaterally; microscopically, this tumour shows a glandular pattern with abundant glycogen in cytoplasm. In immunohistochemical exam, renal cell carcinomas show strong immunostaining for cytokeratin, EMA (epithelial membrane antigen), CD10, whereas ACC show strong immunostaining for inhibin, A103, Melan-A and synaptophysin [18]. In some cases, the histopathological differential diagnosis between ACC and adrenomedullary tumours can be difficult and the immunohistochemical exam can be an important tool. Immunostaining for chromogranin is negative in ACC but is positive in pheocromocytomas [14,18]. In the present study, immunohistochemistry was not necessary as the cellular features were indicative of ACC in all cases.

In dogs, metastases of ACC to distant sites are rare; normally animals with ACC present invasion of posterior vena cava wall and advential layer of the abdominal aorta [9,15,16]. The few reports of ACC metastases to distant sites describe metastases in liver, lung, kidney and mesenteric lymph nodes [4,16,19]. There was no previous report of metastasis to CNS as observed in case 2. Data about the survival time of animals with ACC are scarce. Anderson *et al.* [1] reported a mean survival time of 778 days for dogs with ACC. The prognosis is reserved to poor [2,11] even in cases of well-differentiated tumours as observed in the present cases.

Canine ACC are considered uncommon tumours, however, they should be included in the differential diagnosis of mass in the abdominal cavity since the animals may show no clinical signs of adrenocortical hormones overproduction. On the other hand, animals with ACC diagnosis must perform regularly image exams to control metastases as even well-differentiated ACC can metastasize to kidney and others distant organs such lung and CNS.

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