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Aldosterone-producing adrenocortical carcinoma with myxoid differentiation in a cat

Short title: Feline myxoid adrenocortical carcinoma

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Abstract

A 10-year-old male neutered Persian cat was presented with an abdominal mass and history of weakness. Blood smear examination found marked elliptocytosis, and serum biochemical analysis revealed hypokalemia, hypochloremia, increased activity of creatine kinase and high aldosterone concentration. Cytologic examination of the mass revealed neoplastic endocrine cells with moderate criteria of malignancy, favoring adrenocortical neoplasia. The adrenal mass was surgically excised and histologically characterized by lobules of mildly pleomorphic, polygonal neoplastic cells with moderate to abundant, occasionally granular, eosinophilic cytoplasm. Lobules were separated by fine fibrovascular trabeculae, and numerous cystic cavities containing amorphous eosinophilic material that stained positive with Alcian blue and periodic acid–Schiff were seen. Neoplastic cells were multifocally positive for cytochrome P450 aldosterone synthase. Based on clinicopathologic and immunohistochemical findings the present case was diagnosed as aldosterone-producing adrenocortical carcinoma with myxoid differentiation. While this entity has not been reported in cats, myxoid differentiation of adrenocortical carcinomas has been found in other species and can pose a major diagnostic challenge on microscopic examination.

Keywords: adrenocortical carcinoma, feline, cytochrome P450 aldosterone synthase, elliptocytes, hyperaldosteronism, myxoid differentiation, intranuclear cytoplasmic invaginations

Case Presentation

A 10-year-old male neutered Persian cat was presented to the Queen Mother Hospital for Animals (QMHA) at the Royal Veterinary College, for a one-year duration of polyuria and polydipsia and a six-month history of intermittent inappetence and weakness. One week prior to presentation, the cat was diagnosed with severe hypertension by the referring veterinarian. On physical examination at the QMHA, a large, firm, and irregular intraabdominal mass was palpated. Blood smears, stained with a modified Wright's stain (Hematek, Siemens, Munich, Germany), revealed marked elliptocytosis (Figure 1) while the CBC (ADVIA 2120, Siemens, Munich, Germany) had no clinically significant abnormalities. Serum biochemistry analysis (ILab 600, Instrumentation Laboratory, San Diego, USA) found hypokalemia [3.20 mmol/L; reference interval (RI) 3.80 to 5.50 mmol/L], hypochloremia (105.0 mmol/L; RI 111.0 to 123.0 mmol/L), a sodium concentration within reference limits (157.0 mmol/L; RI 148.0 to 160.0 mmol/L), increased CK activity (1221 U/L; RI 52 to 506 U/L), and a mildly increased urea concentration (15.2 mmol/L; RI 6.1 to 12.0 mmol/L). Urine specific gravity was 1.014. Contrast-enhanced abdominal computed tomography (CT) scan revealed a large right adrenal mass, approximately 6.5 cm in diameter. The mass was compressing the vena cava along its length, but there was no evidence of vascular invasion. The left adrenal gland could not be identified on the CT scan.

Smears of an ultrasound-guided fine-needle biopsy of the adrenal mass were stained with modified Wright's stain (Hematek, Siemens, Munich, Germany). The smears had moderate to high nucleated cellularity, low numbers of erythrocytes and lysed cells on a light pink background with abundant pink, streaming, extracellular matrix, occasionally found in lakes (Figure 2). The predominant nucleated cell population consisted of round to polygonal cells, arranged in loose sheets and clusters, rarely in rows or acinar-like patterns, and with a predominance of free nuclei within a background of continuous cytoplasmic material on

certain areas of the slides. Cells had round nuclei with finely stippled chromatin, one to two small, variably prominent nucleoli and occasional cytoplasmic invaginations into the nucleus (Figure 2, inset). Cells had moderate amounts of pale basophilic to amphophilic, finely granular cytoplasm, which often contained variable numbers of small cytoplasmic vacuoles. Cytologic criteria of malignancy included moderate to rarely marked anisocytosis and anisokaryosis (10 to 25 μm), occasional binucleation (occasionally with nuclear molding and intracellular anisokaryosis), and nucleoli of varying number, shape, and size, including rare macronucleoli. Occasional mitotic figures were observed. Cytologic findings were consistent with an epithelial neoplasm with moderate criteria of malignancy; adrenocortical neoplasia was favored, but medullary origin could not be excluded. Additional analysis revealed a serum aldosterone concentration (SAC) greater than 5000 pmol/L (RI: 87 to 224 pmol/L), basal cortisol below 27.6 nmol/L (RI: 30.0 to 170.0 nmol/L), and a tT_4 concentration of 52.5 nmol/L (RI: 19.0 to 65.0 nmol/L), consistent with a diagnosis of Feline Primary Hyperaldosteronism (Conn's syndrome), an adrenal disorder characterized by excessive and independent aldosterone secretion.

The cat underwent exploratory celiotomy, and a 6.5 cm in diameter mass was found, extending from the left to the right kidney. A right adrenalectomy was performed. The mass was closely adhered to the vena cava and both renal veins, but no caval vascular invasion was evident. The left adrenal gland was not identifiable during surgery. The mass was fixed in 10% neutral buffered formalin, processed routinely and embedded in paraffin wax. Sections (4 μm) were stained routinely with H&E. On histopathologic examination the mass was well demarcated, partially encapsulated and multilobular, arising from and replacing the adrenal cortex. The neoplasm extended to the adrenal capsule and multifocally penetrated and seeded along the external capsular surface. There was multifocal intravascular invasion. Neoplastic cells were arranged in lobules, fine trabeculae, packets, and rarely in a cribriform pattern, and

surrounded by a variable fine fibrovascular stroma. Neoplastic cells were moderate in size, polygonal, with moderate to abundant, occasionally granular, eosinophilic cytoplasm that contained a single round to oval nucleus, with finely clumped chromatin, and one to two dense nucleoli. Intranuclear cytoplasmic invaginations were occasionally seen. Mitoses were observed at a rate of four per ten high power (x400) fields, and moderate anisocytosis and anisokaryosis were noted (Figure 3). Multifocally the neoplasm was expanded by cystic cavities containing amorphous eosinophilic material. The extracellular material stained positively for both Alcian blue (pH 2.5) and periodic acid–Schiff (PAS) on cytological smears and histology sections (Figure 4), indicating a myxoid matrix. The neoplastic cells immunolabeled multifocally for cytochrome P450 aldosterone synthase (Figure 5). Based on clinicopathologic and immunohistochemical findings, the cat was diagnosed with an aldosterone-producing adrenocortical carcinoma with myxoid differentiation leading to the clinical presentation of Feline Primary Hyperaldosteronism (Conn's syndrome).

A postoperative ACTH stimulation test indicated the lack of endogenous cortisol production (cortisol concentration pre- and post ACTH stimulation: <27.6 nmol/L); thus, hydrocortisone was supplemented. Additionally, postoperatively marked azotemia developed with mild hyperkalemia, and an ongoing mild, nonregenerative anemia. The cat was discharged nine days postoperatively with tapering hydrocortisone supplementation and recovered well according to the owner. An ACTH stimulation test one month postoperatively documented evidence of endogenous cortisol production, and the elliptocytosis had resolved. Eight months after surgery, the cat was clinically well although still polydipsic, and there was an ongoing mild non-regenerative anemia (hematocrit 21.0%; RI 24.0 to 45.0 %). Azotemia had improved, electrolytes were within reference intervals, and a normal response was found on an ACTH stimulation test. One year after surgery the excessive drinking worsened to become extreme (approximately 800mls per day). A repeat SAC was greater than 5000

pmol/L (RI: 87 to 224 pmol/L) consistent with re-growth of the adrenal tumor and/or a distant metastasis; unfortunately, further investigations and treatment were declined by the owner.

Discussion

Primary adrenal neoplasms are rare in cats and most frequently include cortical adenomas and carcinomas, with pheochromocytomas being far less common.[1] Adrenocortical carcinoma with myxoid differentiation is a variant of adrenocortical carcinoma rarely found in humans, ferrets, and cattle, and to our knowledge has not been reported in cats.[2-4] Here we describe the clinical, cytologic, histopathologic and immunohistochemical findings of an aldosterone-producing adrenocortical carcinoma with myxoid differentiation in a cat.

This cat presented with clinical signs that were compatible with, but not specific for hyperaldosteronism, including weakness, hypertension, polyuria, and polydipsia.[5] Serum biochemistry results were consistent with hyperaldosteronism, and included moderate hypokalemia and increased CK, likely secondary to hypokalemic polymyopathy. Chloride was disproportionately decreased (corrected chloride was approximately 103 mmol/L), indicating a metabolic alkalosis due to the increased loss of H^+ through the kidneys via the stimulation of the hydrogen ATPase pump by aldosterone. Cats with hyperaldosteronism can also have concurrent renal disease, as suggested in this case, by the low USG in light of mild azotemia. Renal disease may be due to prolonged hypokalemia causing renal vasoconstriction and vasopressin-resistance via decreased aquaporin-2 water channel expression, which incites renal injury and is referred to as hypokalemic nephropathy.[6, 7] The most prominent finding from the hematologic examination was the marked elliptocytosis, which has been reported previously in cats with hepatic and bone marrow diseases.[8] The absence of elliptocytes one month postoperatively suggests that the elliptocytosis was probably associated with the

adrenocortical carcinoma and/or the associated electrolyte changes, but the underlying mechanism remains unclear.

The cytologic examination of the adrenal mass revealed a population of neoplastic cells with characteristics of adrenocortical origin, including amphophilic, granular cytoplasm containing clear vacuoles. However, these cells also exhibited morphologic features more typical of adrenal medullary neoplasms, such as the focally high proportions of free nuclei and rare formations of rows or acinar-like patterns, making a determination of the cellular origin of this neoplasm challenging. While cytological classification of canine adrenal neoplasms has been well described, data on cytological features of these neoplasms in cats is limited. A recent publication on this topic was based mainly on canine samples and included only 3 feline neoplasms,[1] and thereby provided limited aid for the diagnosis of the case presented here. Nonetheless, cytologic features of feline adrenocortical neoplasms as reported for two individual cases were similar to our findings to support a diagnosis of an adrenocortical carcinoma.[9] While SAC is typically >1000 pmol/L in cats with aldosterone secreting tumors, SAC alone cannot always distinguish primary from secondary hyperaldosteronism. Thus, simultaneous measurement of renin activity would ideally be needed to rule out increased aldosterone production in response to chronic kidney disease or heart failure, but no appropriately preserved samples were obtained to measure this labile hormone in this case. However, markedly increased SAC together with an adrenocortical neoplasm and resolution of clinical signs following tumor removal are most consistent with a diagnosis of primary hyperaldosteronism. Production of other hormones by the tumor was not further explored, and thus could not be ruled out entirely.

The bright eosinophilic material (Figure 4) and the nuclear invaginations (Figures 2 and 3) are unique findings that have not been described previously with adrenocortical carcinomas in cats. On cytology alone, it is difficult to determine the exact nature of the

extracellular eosinophilic material, with possible differentials including mucins, amyloid, and extracellular matrices such as collagen, osteoid, and chondroid. In this case, the positive Alcian blue and PAS staining suggest that the extracellular matrix seen on impression smears corresponds to the content of the cystic spaces in the tissue sections, and is consistent with epithelial mucins.[10] In ferrets, a high degree of malignancy and increased risk of metastasis is reported with this myxoid variant of adrenocortical carcinoma.[4] Similarly, the myxoid differentiation was described only in metastatic adrenocortical carcinoma in cattle and this feline case, the reappearance of hyperaldosteronism also suggests a more aggressive biological behavior.[2] Additionally, in ferrets, the transcription factor GATA-4 has been identified as a marker of anaplasia in adrenocortical neoplasms, and cytochrome b_5 has been found to be upregulated during gonadectomy-induced adrenocortical neoplasia in this species.[11, 12] Thus, besides the myxoid differentiation, these two markers could potentially be used to evaluate if an adrenocortical tumor is highly malignant.

The second unique morphological feature was the intranuclear cytoplasmic invaginations observed in neoplastic cells both on cytological and histopathological examination of the tumor. On cytology, these invaginations may be mistaken for large nucleoli or large cytoplasmic vacuoles overlaying the nucleus, while on histology they could be erroneously interpreted as intranuclear inclusion bodies or viral inclusions. In humans, intranuclear cytoplasmic invaginations have proven to be rich in monomeric actin and are more often encountered in highly de-differentiated or cancerous cell lines, such as human breast cancer cell lines, compared with cells that are well-differentiated (eg, human mammary epithelial cell line). Even though the exact function of these actin-filled invaginations is not clear, it is speculated that the folding increases the surface area of the nucleus and facilitates increased levels of nuclear activity. [13] In veterinary medicine, intranuclear cytoplasmic invaginations have been sporadically described, besides in meningiomas where they are a

well-established cytological feature.[14] In one study transmission electron microscopy was used to report seven interstitial cell tumors with intranuclear cytoplasmic invaginations from a group of 32 canine testicular tumors.[15] Additionally, this feature is commonly found in both neoplastic hepatocytes and normal aging hepatocytes of mice and rats.[16]

Herein was described an aldosterone-producing adrenocortical carcinoma with myxoid differentiation and intranuclear cytoplasmic invaginations in a cat, two features which, to the best of our knowledge, have not been previously described in feline adrenal tumors. Veterinary clinical and anatomic pathologists should be aware of this uncommon morphological presentation, especially in relation to the difficulties that myxoid differentiation poses for diagnoses and should consider it as a differential for adrenal masses in cats.

Conflict of Interest Statement

The authors have indicated that they have no affiliations or financial involvement with any organization or entity with a financial interest in, or in financial competition with, the subject matter or materials discussed in this article.

References

1. Bertazzolo W, Didier M, Gelain ME, et al. Accuracy of cytology in distinguishing adrenocortical tumors from pheochromocytoma in companion animals. *Vet Clin Pathol*. 2014; 43: 453-9.
2. Grossi AB, Leifsson PS, Jensen HE, Vainer B and Iburg T. Histologic and immunohistochemical classification of 41 bovine adrenal gland neoplasms. *Vet Pathol*. 2013; 50: 534-42.
3. Mondal S, Dasgupta S, Mandal P and Sinha M. Cytodiagnosis of myxoid adrenocortical carcinoma and role of immunocytochemistry to differentiate it from renal cell carcinoma. *Journal of Cytology*. 2014; 31: 111-3.
4. R. A. Peterson I, Kiupel M and Capen CC. Adrenal Cortical Carcinomas with Myxoid Differentiation in the Domestic Ferret (Mustela putorius furo). *Veterinary Pathology*. 2003; 40: 136-42.
5. Ash RA, Harvey AM and Tasker S. Primary hyperaldosteronism in the cat: a series of 13 cases. *J Feline Med Surg*. 2005; 7: 173-82.

6. Suga S-I, Phillips MI, Ray PE, et al. Hypokalemia induces renal injury and alterations in vasoactive mediators that favor salt sensitivity. *American Journal of Physiology-Renal Physiology*. 2001; 281: F620-F9.
7. Marples D, Frøkiaer J, Dørup J, Knepper MA and Nielsen S. Hypokalemia-induced downregulation of aquaporin-2 water channel expression in rat kidney medulla and cortex. *Journal of Clinical Investigation*. 1996; 97: 1960-8.
8. Harvey JW. *Veterinary Hematology: A Diagnostic Guide and Color Atlas*. 2nd ed. St. Louis Missouri, USA: Elsevier Saunders, 2012.
9. Renschler JS and Dean GA. What is your diagnosis? Abdominal mass aspirate in a cat with an increased Na:K ratio. *Vet Clin Pathol*. 2009; 38: 69-72.
10. Astudillo VG, Schaffer-White A, Allavena R and Palmieri C. Multiple intra-abdominal serosal myxosarcomas in two koalas (Phascolarctos cinereus). *J Comp Pathol*. 2015; 152: 283-6.
11. R. A. Peterson I, Kiupel M, Bielinska M, et al. Transcription Factor GATA-4 Is a Marker of Anaplasia in Adrenocortical Neoplasms of the Domestic Ferret (Mustela putorius furo). *Veterinary Pathology*. 2004; 41: 446-9.
12. Wagner S, Kiupel M, R. A. Peterson I, Heikinheimo M and Wilson DB. Cytochrome b5 Expression in Gonadectomy-induced Adrenocortical Neoplasms of the Domestic Ferret (Mustela putorius furo). *Veterinary Pathology*. 2008; 45: 439-42.
13. Johnson N, Krebs M, Boudreau R, Giorgi G, LeGros M and Larabell C. Actin-filled nuclear invaginations indicate degree of cell de-differentiation. *Differentiation*. 2003; 71: 414-24.
14. Rose E. Raskin and Meyer DJ. *Canine and Feline Cytology*. 3rd ed. St. Louis, MO: Saunders Elsevier, 2015, p.438-40.
15. Sanford SE, Miller RB and Hoover DM. A light and electron microscopical study of intranuclear cytoplasmic invaginations in interstitial cell tumours of dogs. *Journal of Comparative Pathology*. 1987; 97: 629-35.
16. Thoolen B, Maronpot RR, Harada T, et al. Proliferative and nonproliferative lesions of the rat and mouse hepatobiliary system. *Toxicol Pathol*. 2010; 38: 5S-81S.

Figures

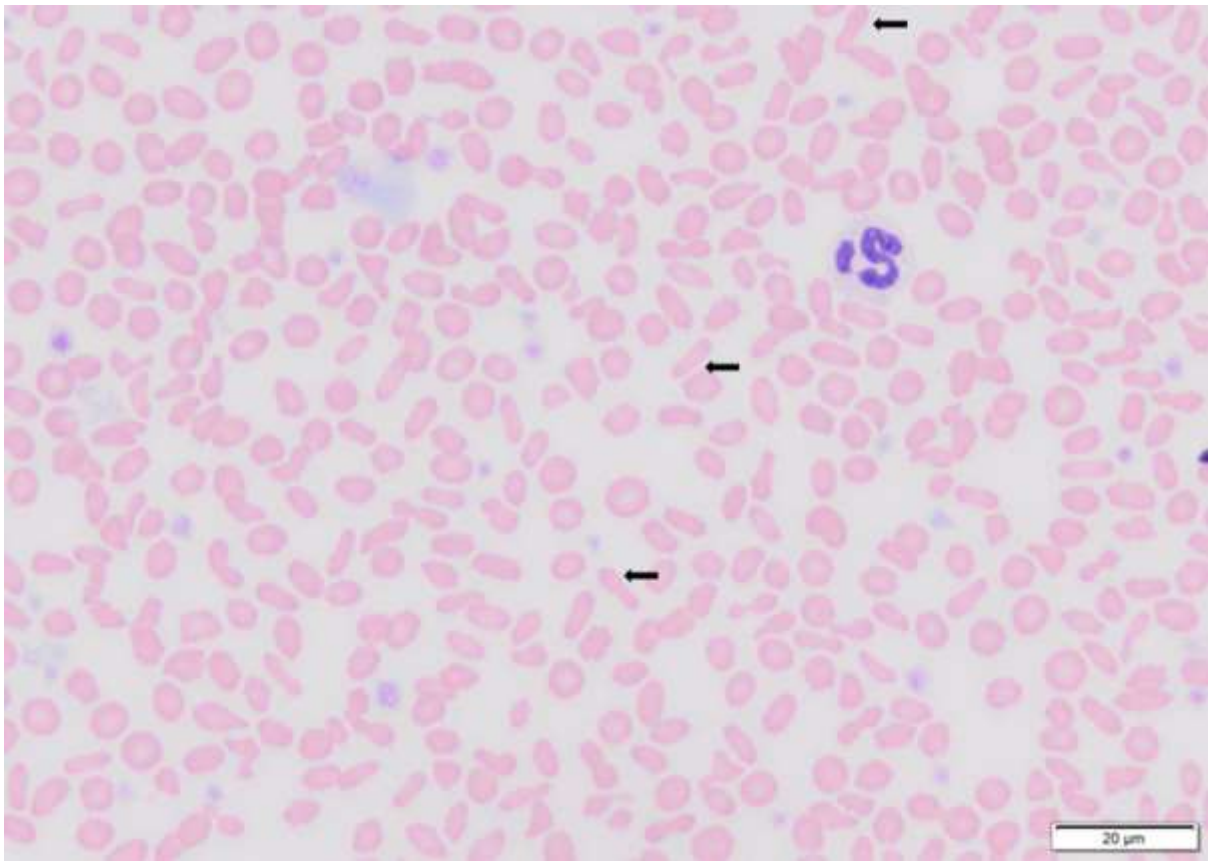

Figure 1. A photomicrograph of a peripheral blood smear showing marked elliptocytosis (arrows) from a cat with adrenal neoplasia. Modified Wright's stain. Bar = 20 µm.

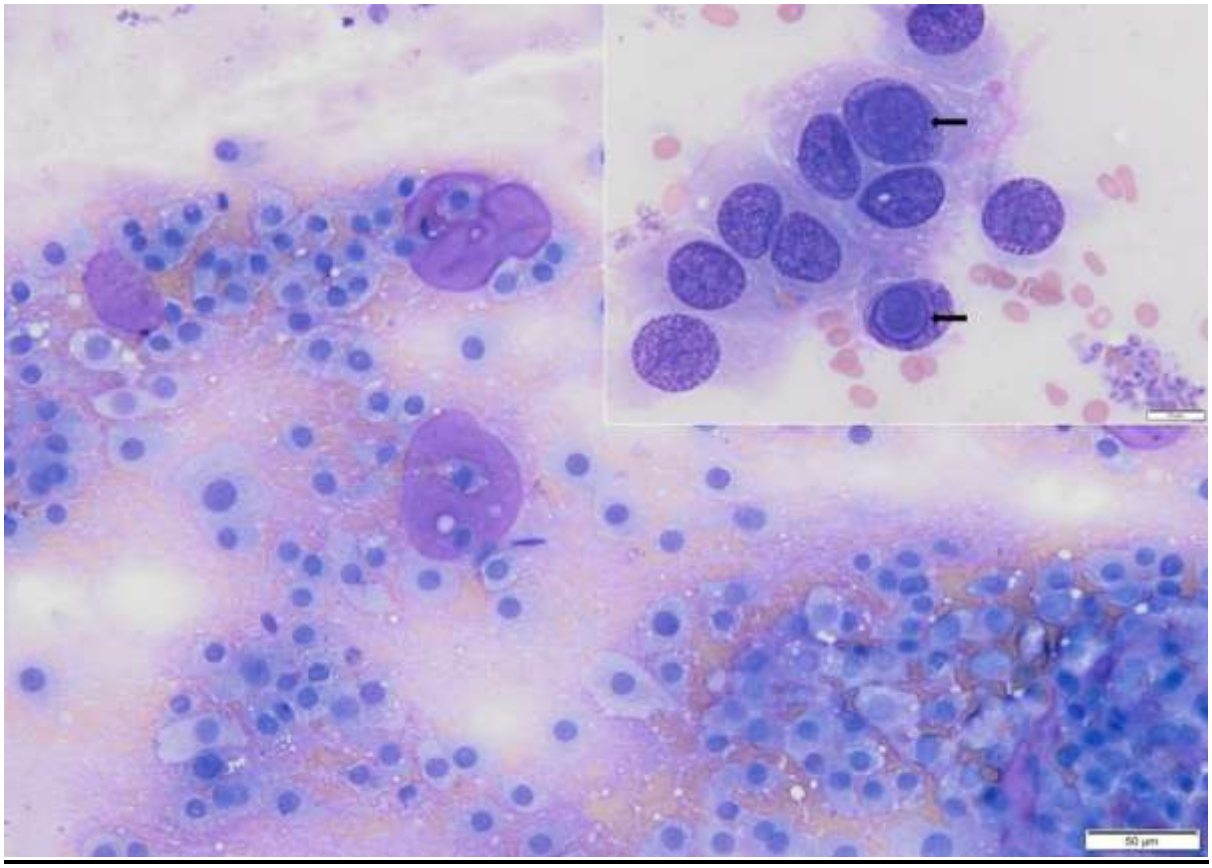

Figure 2. A photomicrograph of a fine-needle aspirate from the adrenal mass of a cat. Neoplastic cells are arranged in loose sheets often within large lakes of pink matrix. Modified Wright's stain. Bar = 50 µm. Inset: Neoplastic cells containing intranuclear cytoplasmic invaginations (arrows). Modified Wright's stain. Bar = 10 µm.

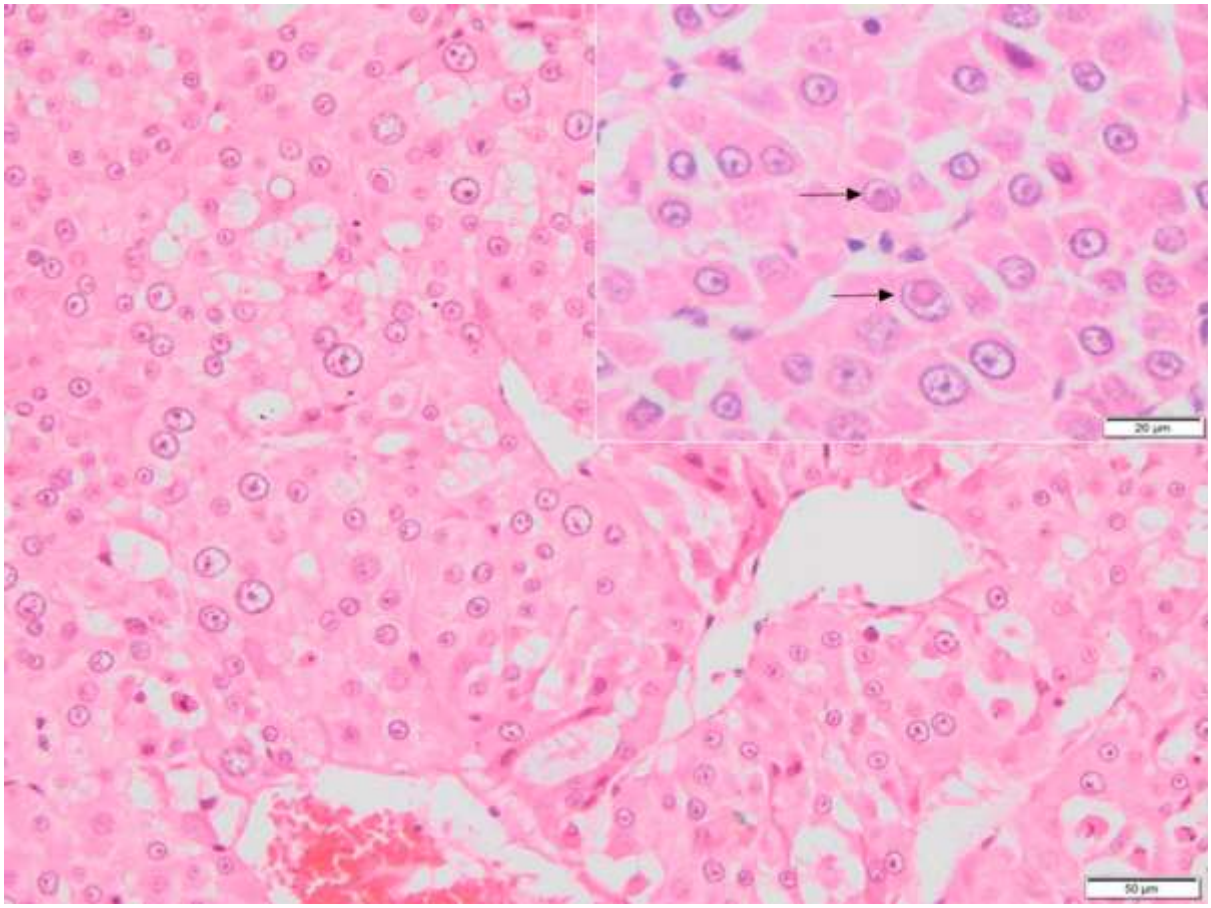

Figure 3. A photomicrograph of a histopathologic section from the adrenal mass of a cat consists of polygonal cells with abundant, eosinophilic cytoplasm, and round-oval nuclei. H&E stain. Bar = 50 μm. Inset: Neoplastic cells containing intranuclear cytoplasmic invaginations (arrows). H&E stain. Bar = 20 μm.

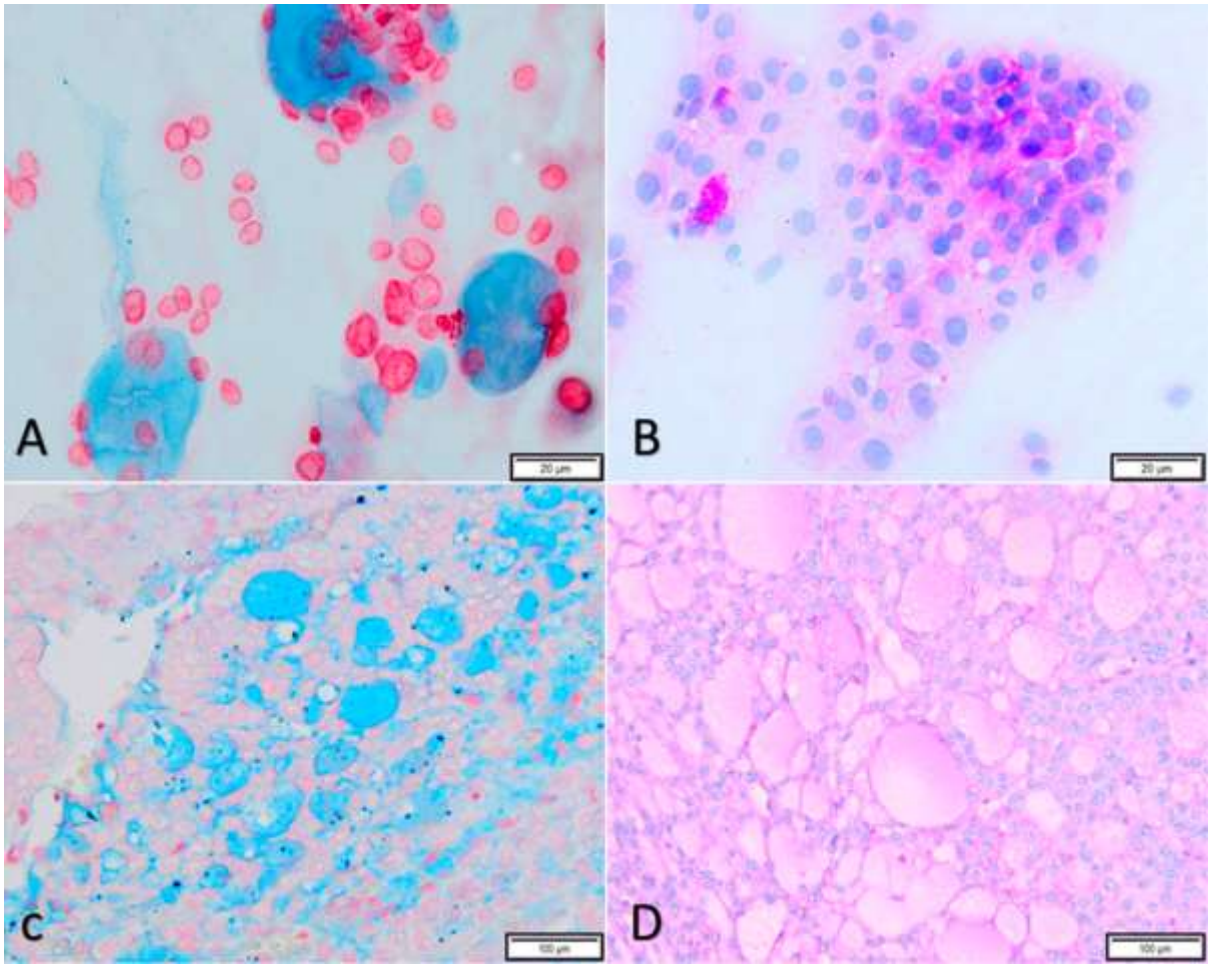

Figure 4. Photomicrographs of special stains from an adrenocortical carcinoma with myxoid differentiation in a cat. Extracellular material stains strongly positive (A) with the Alcian blue stain (pH 2.5), and moderately positive (B) with the Periodic acid–Schiff (PAS) stain; Bars = 20 μm. Extracellular material in the cystic spaces of the tissue section stains strongly positive (C) with the Alcian blue stain (pH 2.5) and moderately positive (D) with the PAS stain; Bars =100 μm.

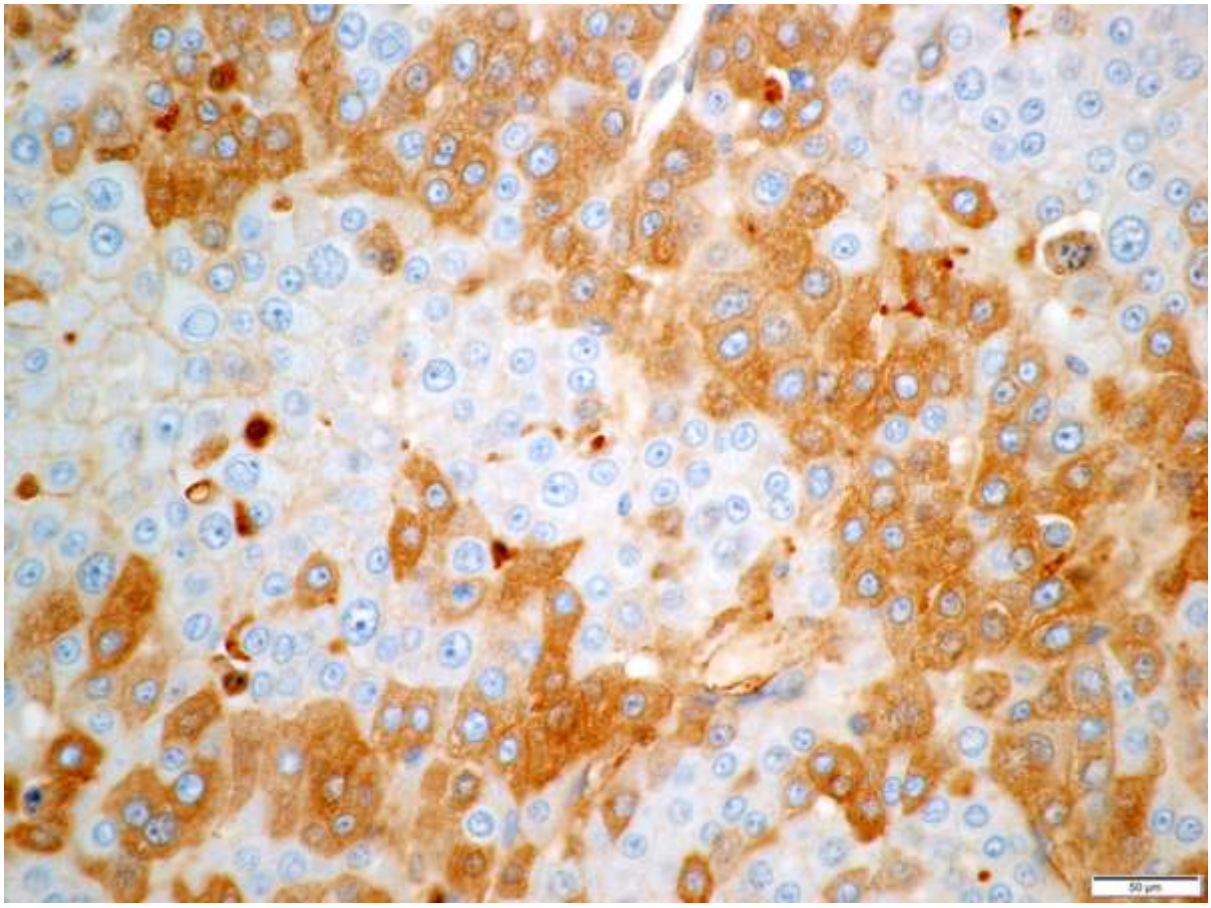

Figure 5. A photomicrograph of P450 aldosterone synthase immunohistochemical staining of an adrenocortical carcinoma with myxoid differentiation in a cat. Multifocal areas of strongly positive neoplastic cells for cytochrome P450 aldosterone synthase. Bar = 50 μm.