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# Metastatic pancreatic carcinoma with neuroendocrine differentiation in a cat

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## Abstract

**Case summary** This report describes the case of a 7-year-old male neutered domestic mixed-breed cat that was initially referred to the Veterinary Hospital of the State University of Londrina for evaluation of a 2-week history of abdominal distension and a 2-day history of anorexia, infected with feline immunodeficiency virus (FIV). Abdominal ultrasound revealed an expansive mass located around the pancreas and right hepatic lobe. In the transoperative macroscopic observation, multiple white nodules were visualized in the liver, pancreas, mesentery, intestine, stomach and peritoneal wall. Immunohistochemical examination revealed that neoplastic cells demonstrated a strong positivity for AE1/AE3 and CK20. A sparse immunoreactivity to chromogranin A was observed, which demonstrates neuroendocrine cell labeling. The histopathologic changes associated with the immunohistochemical profile confirmed the diagnosis of metastatic carcinoma with neuroendocrine differentiation, originating from the pancreas.

**Relevance and novel information** Neuroendocrine tumors of the pancreas are rare and are associated with a poor prognosis in humans. In humans, approximately 7% of neuroendocrine tumors develop in the pancreas, and the 5-year survival rate for a pancreatic neuroendocrine tumor is 53%, according to the American Cancer Society. To our knowledge, only one case has been described in the feline species so far. Due to the rarity of this type of tumor in cats, there is little information about predisposition related to age, sex or breed, as well as the main clinical signs presented, survival time and treatment options.

**Keywords:** Pancreatic carcinoma; neuroendocrine tumor; non-functional pancreatic neuroendocrine tumor; metastasis

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## Introduction

Neuroendocrine carcinomas are rare tumors that occur in humans and animals, originated by dispersed cells of the neuroendocrine system known to produce hormones and peptides.<sup>1,2</sup>

In humans, neuroendocrine carcinomas are more often found in the gastrointestinal tract and bronchopulmonary system.<sup>3</sup> In cats, neuroendocrine carcinomas have been reported in several organs, including the gallbladder,<sup>4</sup> skin,<sup>5</sup> colon,<sup>6</sup> trachea,<sup>7</sup> esophagus<sup>8</sup> and liver.<sup>9,10</sup> To our knowledge, only one case of feline pancreatic neuroendocrine carcinoma has been described so far.<sup>11</sup>

The aim of the present paper is to report the case of a 7-year-old male neutered domestic mixed-breed cat,

infected with feline immunodeficiency virus (FIV), treated at the Veterinary Hospital of State University of

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Londrina, which was diagnosed with pancreatic carcinoma with neuroendocrine differentiation.

### Case description

A 7-year-old male neutered domestic mixed-breed cat, infected with FIV, was originally referred to the Veterinary Hospital of the State University of Londrina for an evaluation of a 2-week history of abdominal distension and a 2-day history of anorexia. Physical examination revealed pale mucous membranes, dehydration (8%), hypothermia (36.2°C), cachexia and ascites.

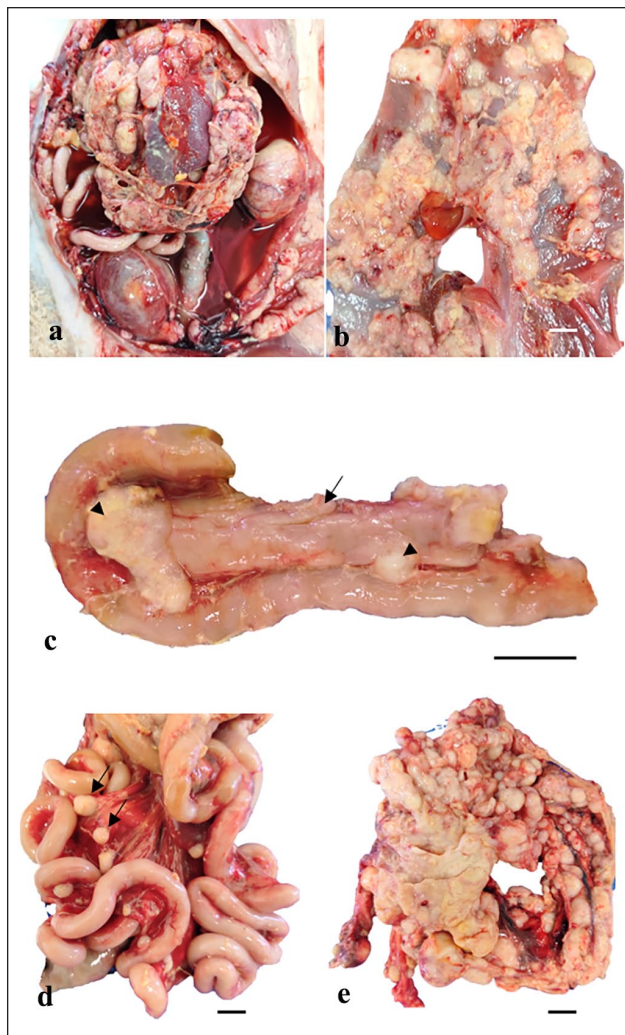
A full blood count revealed mild normochromic normocytic anemia (red blood cells: 5.89 [10.6/mm<sup>3</sup>] [reference interval (RI): 5.0–10.0 (10.6/mm<sup>3</sup>)]); hemoglobin: 7.6g/dl [RI: 8.0–15.0g/dl]; hematocrit: 24.9% [RI: 24.0–45.0%]); platelets (270,000 [RI: 230,000–680,000]) and leukocytes (17,700 [RI: 5500–19,500]) were within the normal range. The biochemical analysis revealed an increase in urea (100mg/dl [RI: 42–64mg/dl]), and the other biochemical tests were within the normal range: creatinine 1.4mg/dl (RI: 0.8–1.8mg/dl), alanine aminotransferase 16 U/l (RI: 6–83 U/l), gamma-glutamyl transferase 10 U/l (RI: 0–10 U/l), albumin 2.9g/dl (RI: 2.1–3.3g/l) and blood glucose 122mg/dl (RI: 73–134 mg/dl).

The peritoneal fluid collected was compatible with modified transudate. It had a hemorrhagic aspect. The results were as follows: density: 1028; albumin: 1.6g/l; proteins: 4.1g/dl, albumin/globulin ratio: 0.73; glucose: 112mg/dl; bacteria absent; red blood cells: 540,000/mm<sup>3</sup>; nucleated cells: 5600/mm<sup>3</sup>; and 78% neutrophils, 19% lymphocytes and 3% monocytes.

Abdominal ultrasound revealed an expansive mass in the epigastric and mesogastric regions, of immeasurable dimensions, multinodular, with mixed echogenicity and irregular contours, located around the pancreas and right hepatic lobe. The perihepatic lymph node was enlarged (1.53 × 2.22 cm) and there was a large amount of free fluid.

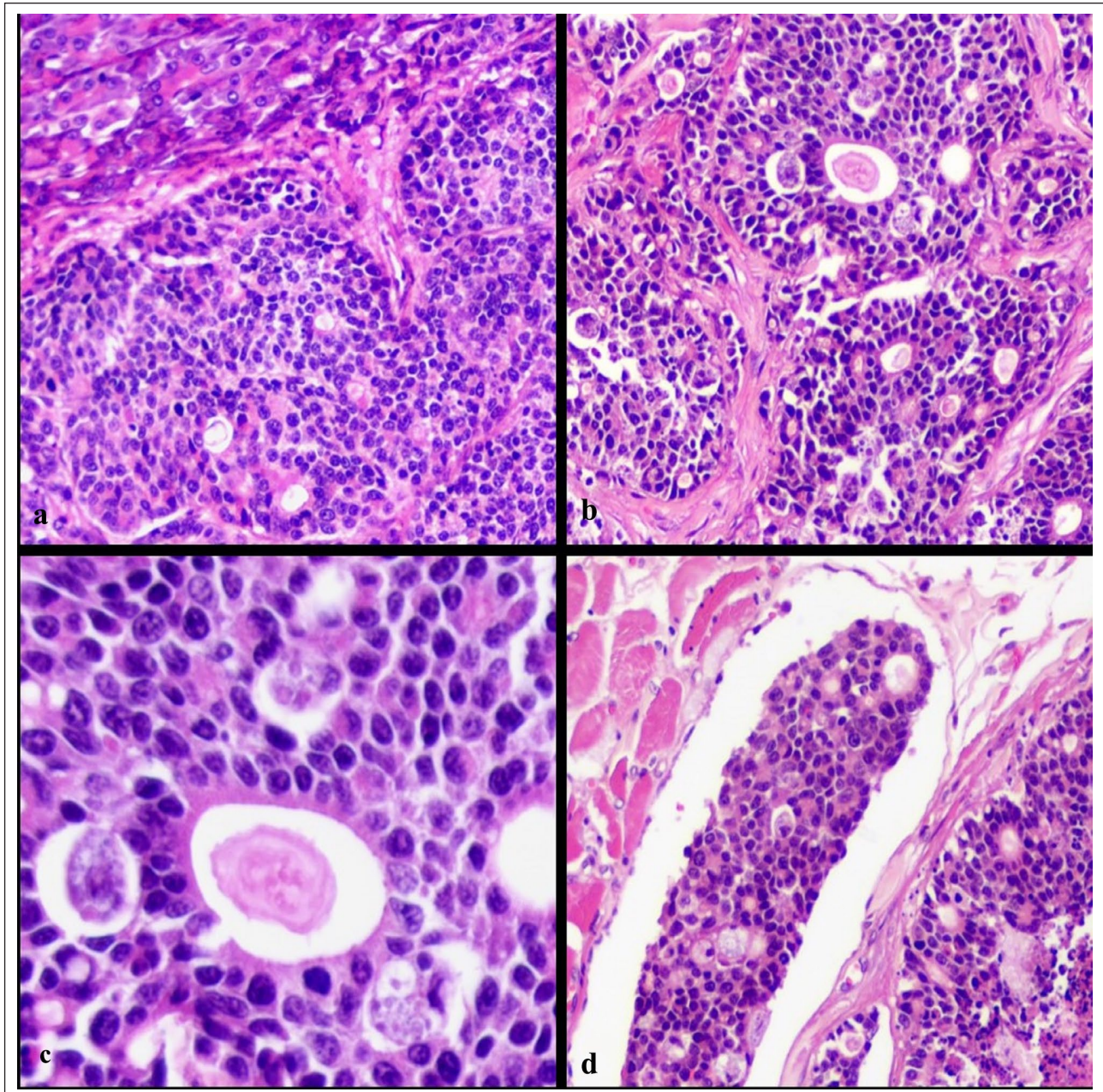
An exploratory laparotomy was performed, where multiple white nodules were visualized in the liver, pancreas, mesentery, intestine, stomach and peritoneal wall. Due to the impossibility of surgical excision and the poor prognosis, euthanasia was indicated.

The necropsy examination showed mild hydroperitoneum and accentuated fibrinous peritonitis, and in the abdominal cavity there were numerous multifocal to coalescent firm nodules spread throughout serosa (Figure 1a and b). The pancreas was severely atrophied and had multiple white, well-delimited plaques neofor- mation, 0.2–0.5cm in size, located in the pancreatic head and tail (Figure 1c). Metastases were observed in the mesenteric lymph nodes, omentum, diaphragm, mesentery and peritoneal serosa (Figure 1d). An in situ evaluation of the abdominal cavity evidenced a solid irregular mass, topographically arising from the mesenteric lymph nodes and replacing the epiploon, and adhered to



**Figure 1** Macroscopic appearance of a metastatic pancreatic carcinoma with neuroendocrine differentiation in an adult cat: (a) in situ evaluation of the abdominal cavity evidenced carcinomatosis, mild hydroperitoneum, accentuated fibrinous peritonitis and a solid irregular mass, topographically arising from mesenteric lymph nodes and replacing the epiploon, and adhered to the spleen; (b) numerous white solid plaques, multifocal to coalescing, 0.5–2.0 cm in size, spread throughout the serosa of the diaphragm; (c) the pancreas was marked by severe atrophy (arrow) and had multiple white, well-delimited plaques neofor- mation, 0.2–0.5 cm in size, located in the pancreatic head and tail (arrowhead); (d) multiple white solid nodules, 0.5–1.0 cm in size, spread throughout the mesentery (arrow); and (e) dissected irregular mass of panel (a), topographically arising from the mesenteric lymph nodes and replacing the epiploon. Bar = 1.0 cm

the spleen (Figure 1e). Numerous white solid plaques, multifocal to coalescing, 0.5–2.0 cm in size, were spread throughout the serosa of the diaphragm. Tissue samples 5µm thick from all visceral organs were fixed in 10% formalin for 48 h, dehydrated in a graded series of ethanol, embedded in paraffin, stained with hematoxylin and eosin (H&E), and observed under a light microscope.



**Figure 2** Pancreatic carcinoma with neuroendocrine differentiation in a domestic cat. (a) Expansive proliferation, unencapsulated, compresses the remainder of the non-neoplastic pancreatic parenchyma. Hematoxylin and eosin (H&E),  $\times 20$ . (b) Proliferation is composed of polygonal cells arranged predominantly in a glandular pattern, with acinar structures in various sizes, separated by thin fibrous stroma. H&E,  $\times 20$ . (c) Higher magnification demonstrates an epithelial neoplastic cell population with well-defined, abundant eosinophilic and finely granular cytoplasm. The nucleus is round with coarse chromatin and conspicuous nucleoli. Filling the central gap of the acini is an amount of homogeneous, eosinophilic material. H&E,  $\times 40$ . (d) Section of the abdominal wall demonstrates a lymphatic vessel with a neoplastic embolus. H&E,  $\times 20$

Microscopically, the pancreatic nodule consisted of an expansive proliferation, unencapsulated, composed of polygonal cells arranged predominantly in glandular pattern, with large acinar structures that usually present variable amounts of homogeneous eosinophilic material filling the central gap of these structures (Figure 2a and b). Neoplastic cells were supported by a fine fibrovascular

stroma, which compressed the remainder of the non-neoplastic pancreatic parenchyma. The neoplastic cells were polygonal, well-defined and had abundant eosinophilic cytoplasm (Figure 2c). The nucleus was composed of coarse chromatin and conspicuous nucleoli. Marked cell pleomorphism, anisocytosis and anisokaryosis, as well as several areas of intratumoral and peripheral lymphatic

invasion (Figure 2d), intratumoral necrosis and 41 mitotic figures 2.37 mm<sup>2</sup> in size were observed, including bizarre ones. Although there was neuroendocrine differentiation confirmed by immunohistochemistry, no histomorphological features that could promote the distinction of the two cell populations were observed. The metastatic lesions spread in the abdominal cavity showed the same architectural and cellular features as described above. In addition, in the section of the abdominal wall and peritoneum, there were multiple foci of invasion lymphatic vessels by neoplastic cells.

To confirm the tumor cell origin, a paraffin block was sent to the VetMol laboratory, which performed standardized techniques at the laboratory of the Service of Veterinary Pathology of FMVZ – UNESP – Botucatu, with the following panel of antibodies: cytokeratin (CK); anti-pan-cytokeratin (AE1/AE3); CK20; chromogranin A (CgA); vimentin; CK7; synaptophysin; Wilms tumor (WT-1); calretinin; Caudal Type Homeobox 2 (CDX2); and thyroid transcription factor (TTF-1).

Immunohistochemical examination revealed that neoplastic cells demonstrated a strong positivity for AE1/AE3 and CK20 (Figure 3a and b). Immunoreactivity of approximately one-third (30%) of the cells to CgA was observed, which demonstrates neuroendocrine cell labeling (Figure 3c and d). In contrast, the tumor cells were negative for vimentin, CK7, synaptophysin, WT-1, calretinin, CDX2 and TTF-1. The histopathologic findings associated with the immunohistochemical profile confirmed the diagnosis of metastatic pancreatic carcinoma with neuroendocrine differentiation. Immunohistochemical features of the metastatic tumor cells were similar to those of the primary pancreatic tumor cells.

## Discussion

Neuroendocrine neoplasia is produced by tumor cells that express neuroendocrine differentiation markers, including CgA and synaptophysin, as well as hormones, and transcription factors, which are tissue-specific and assimilate the tumor cell to its normal counterpart neuroendocrine cell.<sup>12</sup>

In humans, neuroendocrine tumors that develop in the pancreas have predominant neuroendocrine differentiation and are classified into histologic subtypes according to the current human World Health Organization (WHO) classification, including well-differentiated neuroendocrine tumors, poorly differentiated neuroendocrine carcinomas and mixed neuroendocrine-non-neuroendocrine neoplasms (MiNENs), which include mixed acinar-neuroendocrine carcinoma, mixed ductal-neuroendocrine carcinoma and mixed acinar-neuroendocrine-ductal carcinoma.<sup>13</sup>

The immunohistochemical profile is fundamental to establishing the morphologic type of the pancreatic tumor. The positivity for cytokeratin (AE1/AE3) – a marker of all epithelial cell types and carcinomas – and

the absence of staining for vimentin – a marker of cells of mesenchymal origin and sarcomas – confirmed the carcinoma.<sup>14</sup>

CK19 is a marker used in the immunohistochemical staining of neoplastic processes to demonstrate a higher ductal immunophenotype when endocrine cells become positive for it.<sup>15</sup> In the present case, it was not possible to test for CK19 and confirm the morphologic classification of the pancreatic carcinoma, due to the unavailability of testing for CK-19 in our location.

Tumors with the histologic features of both adenocarcinoma and neuroendocrine carcinoma (NEC), where each component has to exceed 30% of the neoplastic cells, are classified as MiNEN.<sup>13</sup> In the histologic results of this case report, over 30% of the tumor had a neuroendocrine composition, so it was categorized as an MiNEN.

The only case of feline pancreatic neuroendocrine carcinoma that has been described occurred in a 2-year-old mixed-breed female cat that had presented similar clinical signs, such as hyporexia, ascites, diarrhea and weight loss, and also had multiple metastatic liver nodules.<sup>11</sup>

Pancreatic tumors are generally rare in cats, dogs and humans. Among them, carcinoma is the most common type of tumor in dogs and cats.<sup>16</sup> Previous studies indicate that exocrine pancreatic carcinomas normally occur in elderly cats, with no breed predisposition.<sup>17,18</sup> In a retrospective study of 34 cats with exocrine pancreatic carcinoma, the average age was 11 years, with a higher predisposition for male cats.<sup>19</sup>

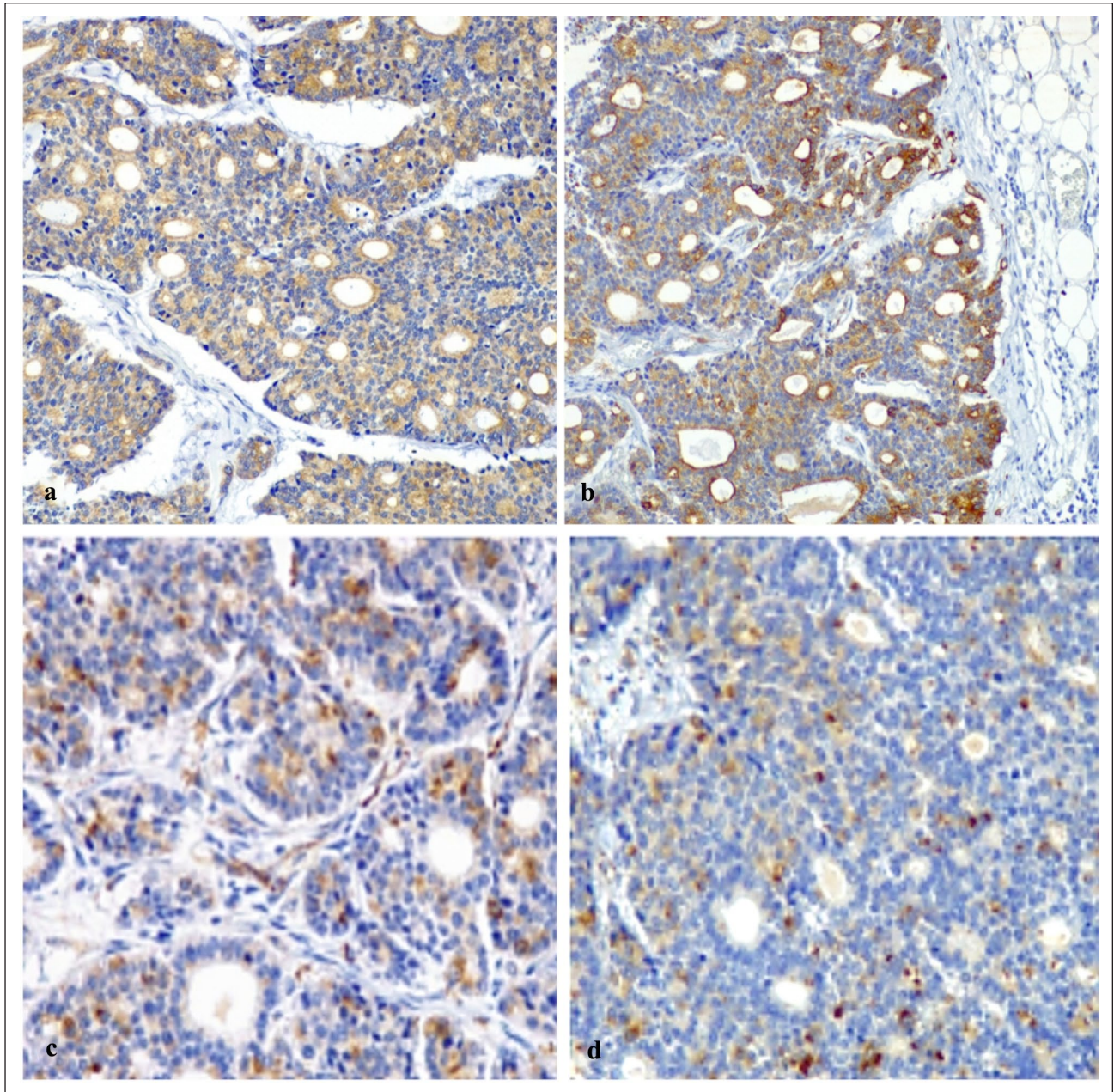
There is still no information about the predisposed age or sex for pancreatic carcinoma with neuroendocrine differentiation in cats; however, the age and sex of the animal reported in this case is similar to those cats diagnosed with exocrine pancreatic neoplasia, both with higher occurrence in elderly and male cats.<sup>18</sup>

Regarding NECs described in other organs, there also seems to be a predisposition towards male cats of advanced age. In a retrospective study of 17 cats with hepatobiliary NEC, the average of the animals was 9 years, mainly affecting male cats.<sup>4</sup>

Pancreatic neoplasms often cause non-specific constitutional and gastrointestinal signs, such as lethargy, anorexia, weight loss, diarrhea, vomiting, abdominal pain or palpable abdominal masses.<sup>16,20</sup>

Pancreatic neuroendocrine tumors can cause clinical signs of mass effect or hormone production<sup>21</sup>; thus, they are classified as functional and non-functional tumors based on the ability to produce bioactive substances.<sup>22</sup> In humans, most (50–75%) of them are non-functional tumors<sup>23</sup> and are at an advanced stage at the time of diagnosis, becoming symptomatic only when they cause the mass effect (compression of structures, abdominal pain, peritoneal effusion).<sup>24</sup>

In cats, some functional pancreatic neuroendocrine tumors, including gastrin, glucagon or insulin-producing



**Figure 3** Immunostaining results for pancreatic carcinoma with neuroendocrine differentiation in a domestic cat: (a) neoplastic cells were diffusely positive for cytokeratin(CK)20; (b) neoplastic cells were diffusely positive for anti-pan-cytokeratin(AE)1/AE3; and (c,d) approximately one-third of neoplastic cells were positive for chromogranin A. Immunohistochemistry: 3,3-diaminobenzidine tetrachloride (DAB) for all sections and counterstained with Harris's hematoxylin,  $\times 20$

tumors, have been reported.<sup>9,25,26</sup> Functional pancreatic neuroendocrine tumors cause clinical signs according to the substances they produce, such as necrolytic migratory erythema in glucagon-secreting tumors and hypoglycemia in insulin-producing tumors.<sup>9,26</sup>

In our case report, no skin lesions or seizures were observed, and the concentration of serum glucose remained within the normal range throughout the study period. Considering that the clinical signs did not indicate hypersecretion of these hormones, the neuroendocrine tumor

was probably not functional in this case. For definitive differentiation between functional and non-functional neuroendocrine tumors, serum concentrations of bioactive peptides and immunohistochemistry are required.

The diagnosis of a neuroendocrine tumor is based on a combination of clinical signs, physical examination, histopathologic findings and immunohistochemical staining.<sup>27</sup>

On immunohistochemical examination, pancreatic neuroendocrine tumors can be identified using CgA,

or neuron-specific enolase (NSE) or synaptophysin, along with morphologic features.<sup>11</sup> The tumor described here was immunoreactive for CgA, but not for synaptophysin; NSE has not been performed in this case. In a study that analyzed the immunoreactivity of CgA in pancreatic neuroendocrine tumors of dogs and cats, CgA was expressed in 76% and 100% of cases, respectively, demonstrating that CgA is a useful immunohistochemical marker for pancreatic tumors of neuroendocrine origin.<sup>28</sup>

There is little information about the prognosis, survival time and treatment options of neuroendocrine pancreatic carcinoma. The first-choice treatment is radical surgical excision; therefore, the anatomic location of the tumor has great impact on treatment and prognosis.<sup>22</sup> In the case described, the surgical approach for a complete resection of the tumor was not possible because of the metastases presented in several abdominal organs, so euthanasia was indicated.

For the definitive diagnosis of this tumor, detailed histologic and immunohistochemical examinations are required. Improvements in diagnostic accuracy can help to increase the number of reported cases, making it possible to study the behavior, risk factors, treatment and prognosis of this neoplasm in animals, and compare it with human cases for recognition of potential similarities.

## Conclusions

This case report describes the clinical signs and ultrasonographic, histologic and immunohistochemical findings of a cat diagnosed with pancreatic carcinoma with neuroendocrine differentiation. Although it is an uncommon tumor, neuroendocrine pancreatic carcinoma must be considered to be a differential diagnosis in cats with signs such as ascites, hyporexia and weight loss, with pancreatic masses detected on abdominal ultrasound.


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**Ethical approval** The work described in this manuscript involved the use of non-experimental (owned or unowned) animals. Established internationally recognized high standards ('best practice') of veterinary clinical care for the individual patient were always followed and/or this work involved the use of cadavers. Ethical approval from a committee was therefore not specifically required for publication in *JFMS Open Reports*. Although not required, where ethical approval was still obtained, it is stated in the manuscript.

**Informed consent** Informed consent (verbal or written) was obtained from the owner or legal custodian of all animal(s) described in this work (experimental or non-experimental

animals, including cadavers) for all procedure(s) undertaken (prospective or retrospective studies). No animals or people are identifiable within this publication, and therefore additional informed consent for publication was not required.

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