*Renal carcinoma with metastatic spread in a tiger (*Panthera tigris*): morphological and immunohistochemical study*

Lorella Maniscalco[1*], Pier Luigi Acutis[1], Cristina Biolatti[1], Daniele R. Laguardia[2], Alessia Di Blasio[1], Alessandro Dondo[1], Elena Bozzetta[1], Katia Varello[1]

[1]*Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta.*
[2]*Giardino Zoologico Safari Ravenna.*

[*]*Corresponding author at: Istituto Zooprofilattico del Piemonte, Liguria e Valle d'Aosta.*
E-mail: lorella.maniscalco@i-vet.it.

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Tiger,
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Summary
A 12-year-old intact male *Panthera tigris* presented with pain and weight loss was euthanatized. Necroscopical examination revealed a neoplastic mass expanding to the left renal pelvis with metastatic dissemination to local lymph node, adrenal gland, and lung. Immunohistochemical characterization was performed revealing co-expression of both cytokeratin and vimentin and negativity for both PAX8 and c-KIT. Considering histochemical and immunohistochemical results the tumour was classified as renal cell carcinoma with metastatic spread. This report provides insights into the morphological and immunohistochemical features of renal cell carcinoma in *Panthera tigris*.

Primary renal tumours are uncommon in domestic feline representing less than 1% of all neoplasms (Newkirk, Newman et al. 2011, Bonsembiante, Benali et al. 2016). Most tumours diagnosed in domestic feline kidney are lymphomas, representing 78% of the lesions and metastatic lesions (10%) (Meuten and Meuten 2017, Ramos-Vara, Edmondson et al. 2017). Among primary renal tumours in cats, renal cell carcinoma (RCC) is the most common in domestic animals, including cats (Matsumoto, Chambers et al. 2018). To the Authors' knowledge, a single case regarding a primary renal tumour in *Panthera tigris*, diagnosed as renal papilloma (Kloft, Ramsay et al. 2019), was reported, but no reports describing primary renal malignancies are present to date.

A 12-year-old male tiger (*Panthera tigris*) held in captivity at the Safari Park, Ravenna (RA), Italy, developed lethargy, vomiting, constipation and anorexia. The animal was sedated for a complete physical examination and diagnostic work-up. Complete blood cell count and chemistry panel were performed: moderate anaemia, increased urea and creatinine concentration were observed. Abdominal ultrasound revealed hydronephrosis of the left kidney. Due to the poor condition, the animal was euthanized, and a complete necroscopy was immediately performed. Opening the abdominal cavity, a moderate amount (about 150 ml) of sero-hematic effusion was observed with moderate and diffuse presence of chronic peritonitis characterized by the presence of chronic fibrinous adherence between intestinal tract and abdominal wall. The gastrointestinal tract was empty. Left kidney was discoloured and enlarged by a 4 cm in diameter irregular brown to whitish solid multinodular mass expanding the renal pelvis, involving cortex and medulla causing severe hydronephrosis (Fig.1).

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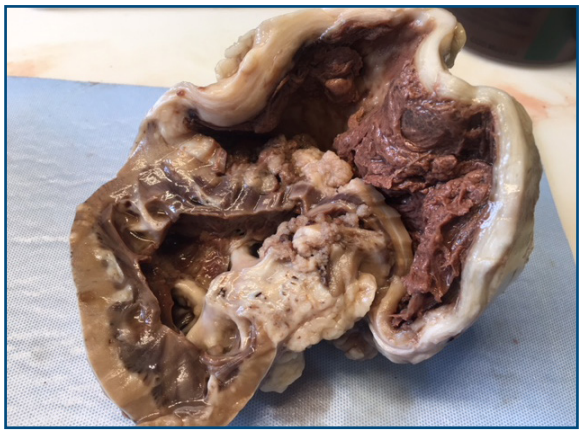

Figure 1. *Kidney with an irregular brown to whitish solid multinodular mass expanding in the renal pelvis and involving cortex and medulla; a severe hydronephrosis.is present.*

Both left renal lymph node and left adrenal gland appeared irregular and moderately enlarged by a brown to whitish solid mass. Splenomegaly and hepatomegaly were also present. In thoracic cavity a moderate amount (about 100 ml) of sero-hematic pleural effusion was present. Multifocal chronic pleuritis was present and examining pulmonary parenchyma, multiple white to brownish 0.5-8 cm in diameter nodular lesions were observed. Representative tissue samples from lungs, liver, kidney, spleen, alimentary tract and renal lymph nodes were fixed in 10% buffered formalin, routinely processed, and paraffin-embedded. Sections of 4 mm were obtained and stained with haematoxylin and eosin and then observed by three pathologists (L.M, K.V, E.B). Histopathological examination revealed a renal neoplastic multinodular mass expanding to renal pelvis and medulla end cortex. The neoplastic lesion was composed by polygonal cells arranged in tubules, papillae and solid areas intermingled with abundant desmoplastic stroma. Cells were about 20 microns in diameter, polygonal, with well distinct cell borders, moderate amount of cytoplasm and round nucleus with stippled chromatin and one round magenta nucleus sometimes evident. Neoplastic cells showed moderate anisocytosis and anisocaryosis, moderate N/C ratio and 0/2 mitoses per high-power field were present (Fig. 2a).

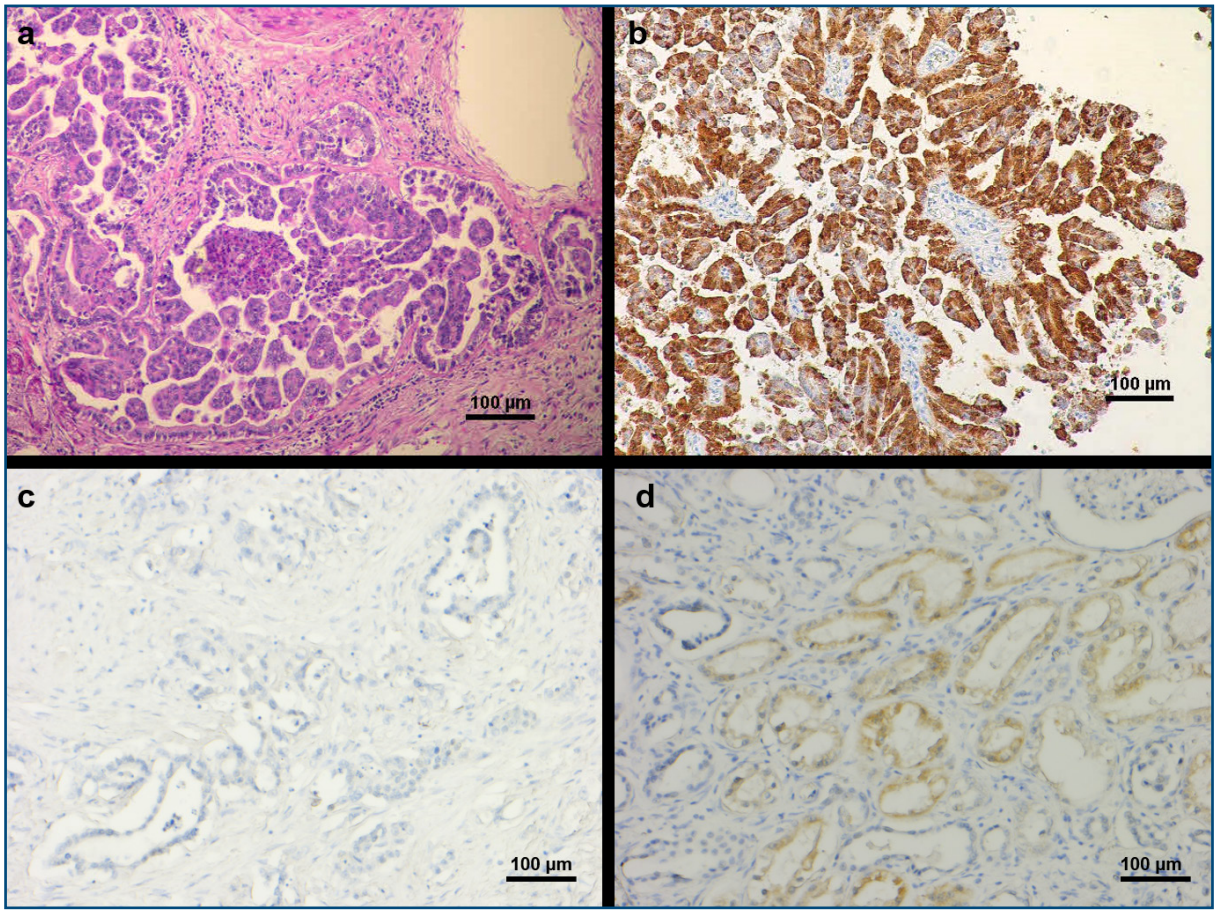

Figure 2. *Kidney. Renal cell carcinoma. Neoplastic cells are arranged in tubules and papillae surrounded by fibrous stroma. Neoplastic cells show moderate anisocytosis and anisocaryosis. Hematoxylin and eosin; b: Renal cell carcinoma. Immunohistochemistry for cytokeratin (CK). Neoplastic cells are characterised by diffuse and strong cytoplasmic immunolabelling for CK. Streptavidin–biotin–peroxidase method. Mayer's haematoxylin counterstain; c: Renal cell carcinoma. Immunohistochemistry for c-KIT. Neoplastic cells are characterised by diffuse lack of immunolabelling for c-KIT. Streptavidin–biotin–peroxidase method. Mayer's haematoxylin counterstain; d: Kidney, Immunohistochemistry for PAX8. Cortical tubules cortical tubules with cytoplasmic immunolabelling for PAX8.*

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Multifocal areas of the adrenal gland cortex, lymph node and pulmonary parenchyma were effaced by a neoplastic lesion whose histological morphology appeared like the one observed for the renal mass. In order to confirm the diagnosis, immunohistochemistry (IHC) was performed on representative sections of the primary renal neoplasm and metastases applying the following commercial antibodies: anti-cytokeratin AE1/AE3 (Dako, monoclonal, heat-induced epitope retrieval high pH, 1:50), anti-vimentin (Dako, monoclonal, heat induced epitope retrieval high pH, 1:100), anti PAX8 (Biocare Medical, monoclonal, heat-induced epitope retrieval high pH, 1:50), anti c-KIT Dako (polyclonal, heat induced epitope retrieval high pH, 1:100)

IHC was performed using the streptavidin–biotin–peroxidase method. From each block, 4-mm-thick sections were cut on polarized slides. All sections were deparaffinized in Bio-Clear (Bio-Clear, Bio-Optica, 20134 Milano, Italy), hydrated with graduated ethanol, and finally immersed in distilled water. In order to block endogenous peroxidase activity, slides were treated with 3% hydrogen peroxide in distilled water for 10 min.

Antigen retrieval was performed with 0.01 mM sodium citrate buffer pH 6.0. The peroxidase reaction was developed using a diaminobenzidine (EnVision®+ Dual Link System-HRP (DAB+), Dakocytomation, Glostrup, Denmark) and the sections were counterstained with Mayer's' haematoxylin.

Normal skin from a *Panthera tigris* from archive cases was used as positive controls for both cytokeratin and vimentin, while feline normal kidney and feline mast cell tumour were used as positive control for PAX8 and c-KIT, respectively.

Negative controls were obtained with the omission of the primary antibodies.

Neoplastic cells from both primary and metastatic lesions showed similar immunohistochemical expression. Neoplastic cells resulted strongly and diffusely positive for both cytokeratin (Fig.2b) and vimentin; interstitial stroma showed no immunoreactivity for cytokeratin while was strongly positive for vimentin. c-KIT was diffusely negative in both neoplastic and stromal compartment (Fig 2c). PAX8 had strong expression in thick and thin loops of Henle in the medulla, renal pelvic urothelium and occasional cortical tubules while neoplastic cells were diffusely negative (Fig 2d).

Negative controls showed no immunostaining to all the antibodies tested.

Based on these results the tumour was classified as tubulo-papillary renal carcinoma. These conclusions were first achieved by macroscopic examination, confirmed by histopathology, and followed by immunohistochemical characterization and are in agreement with the immunohistochemical profile of feline renal carcinomas described in literature (Ramos-Vara, Edmondson et al. 2017). In that paper co-expression of cytokeratin and vimentin were observed in a large amount of renal cell carcinomas (19 out of 20) together with the 95% of cases expressing PAX8.

PAX8 showed a weak diffuse cytoplasmic positivity in renal tubules similar to what is described in domestic cat kidney but was surprisingly negative in neoplastic cells. Since there are no previous reports regarding the expression of this marker in a *Panthera leo,* our results cannot be compared. Similarly, c-KIT was not detected in this neoplasia while it is mostly reported as positive in domestic cat RCC (Matsumoto, Chambers et al. 2018). We can speculate that this neoplasia can represent one of the unusual RCC negative case for these markers or that the immunohistochemical profile of RCC is different in wild felines.

This observation suggests that also in *Panthera tigris*, RCC epithelial to mesenchymal transition (EMT) transcriptional Pathways could be activated, as previously demonstrated for a number of epithelial neoplasms in domestic cat and other animals (Matsumoto, Chambers et al. 2018).

In conclusion, to the best of our knowledge, this is the first report of an RCC with metastatic dissemination in a *Panthera tigris*.

Our findings provide insights into the morphological and immunohistochemical features of this tumour.

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