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CASE REPORT

Immunohistochemical Analysis of Renal Solid and Papillary-Ductal Carcinoma in Dogs

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ABSTRACT

The paper includes description of neoplastic lesions in canine kidneys, sampled during surgery or post mortem. The tumours were subjected to morphological and immunohistochemical analysis using antibodies specific for c-kit, cytokeratin AE1/AE3, CD10, vimentin, Carcinoembryonic Antigen (CAE), Ki-67. The obtained results permitted to specify an accurate histopathological diagnosis.

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INTRODUCTION

Primary renal tumours in domestic animals comprise around 1% of all detected neoplasias (Nowak *et al.*, 2010). On the basis of analysis of 82 renal tumours in dogs renal carcinoma was demonstrated to be the most frequent pathology, followed by kidney sarcoma and nephroblastoma (Newman, 2012). In dogs renal carcinoma accounts for 0.3-1.5% of all tumours (Meuten, 2002; Gil da Costa *et al.*, 2011). Renal carcinoma develops mainly in average age dogs and it used to be diagnosed twice as frequently in males than in females (Nowak *et al.*, 2010). In the area of Lower Silesia (Poland) in the years of 2000-2004 tumours of urinary system accounted for 0.8% and in the years of 2005-2008 already for 1.4% of all diagnosed tumours (Nowak *et al.*, 2006; Nowak *et al.*, 2010).

Within kidneys, several subtypes of tumours can be distinguished which develop from renal tubule epithelium. The lesions differ in morphology but differences in their biological behaviour remain to be accurately recognised. About 90% of the cases are diagnosed as a carcinoma. Cross-section of the kidney with the diagnosed tumour used to demonstrate a delimited lesions of yellow, brownish or a cream colour. Large tumours contain in addition foci of necrosis, extravasation or calcifications (Meuten, 2002). In animals histological outlook allows to distinguish solid, papillary, papillary-ductal carcinomas and carcinosarcomas (Gil da Costa *et al.*, 2011).

Clinical examination and post-mortem findings: One of the kidneys originated from 3 years old male German sheep-dog, in which haematuria persisted for a month and a tumour could be palpated in abdominal cavity. Ultrasonography demonstrate a solid hyperplastic lesion at the cranial pole of the left kidney, with no alterations in the right kidney. The dog was subjected to unilateral nephrectomy.

Another kidney originated from an autopsy of a male dog of mixed breed, aged 10 years and subjected to euthanasia due to a pronounced renal insufficiency and rapidly progressing uraemia. Macroscopically, the two lesions resembled each other: the hyperplastic neoplastic mass disfigured renal surface, altering shape of the entire organ (Fig. 1A). Cross-section of the organ demonstrated a well delimited tumour. Cream-yellowish to brown in colour, with foci of haemorrhages, necrosis and calcifications (Fig. 1B).

Diagnostic procedure: Fragments of kidneys were fixed for 24 hours in 7% buffered formalin, paraffin sections were stained with hematoxylin and eosin (H&E). Immunohistochemical tests were conducted in 4 μm thick paraffin sections. The paraffin was washed off in xylene and the preparations were passed through a row of alcohols. Antigens were retrieved using EnVision™ FLEX Target Retrieval Solution, High pH Dako®. Antigens of preparations devoted for staining of c-kit and Ki-67 were retrieved in EnVision™ FLEX Target Retrieval Solution, Low pH DAKO®. Endogenous peroxidase was blocked in EnVision™ FLEX Peroxidase-Blocking Reagent. Subsequently the sections were overlayed with primary antibodies from Dako®; Polyclonal Rabbit Anti-Human CD117, c-kit and

Monoclonal Mouse Anti-Human: Cytokeratin Clones AE1/AE3, CD10, Anti-Vimentin, Carcinoembryonic Antigen, Ki-67.

All the preparations were incubated and rinsed with EnVision™ FLEX Wash Buffer. Following washing, the sections were overlayed with the EnVision™ FLEX/HR SM802 and incubated. The immunohistochemical reaction was developed using a solution of DAB, EnVision™ FLEX DAB+ Chromogen DAKO® and counterstained with hematoxylin.

Diagnosis: The histopathological pattern and guidelines of WHO allowed to diagnose solid carcinoma in the first case and a papillary-ductal carcinoma in the other. The solid carcinoma was found to contain cells of an abundant cytoplasm. Their cell nuclei were round or oval, with uniformly distributed chromatin, with numerous mitotic figures pointing to a high proliferative potential of the tumour. The tumour was characterized by a scanty fibrovascular stroma and at margins of the solid lesions vesicles of cellular hyperplasia were present (Fig. 2A and 2B).

In the second case large cells prevailed, of hexagonal to cylindrical shape, scanty acidophilic cytoplasm, arranged into papillary and papillary-ductal structures. The cells contained centrally or peribasally located cell nuclei and few mitotic figures. A delicate fibrovascular stroma formed small septa, containing cells tightly arranged into usually single rows (Fig. 2C and 2D).

The results obtained in immunohistochemical reactions are presented in Table 1. While intensity of the reactions varied for individual antibodies, the results were similar in both cases of cancer. The highest reactivity, evaluated in the scale of Remmele, was demonstrated for vimentin, AE1/AE3 and Ki-67. A less intense reaction was noted in the case of antibodies specific for c-kit. No reactions were obtained in cases of CD10 and CAE.

DISCUSSION

To a certain extent results obtained by us seem to resemble those in accessible publications (Bonsib *et al.*, 2010; Shen *et al.*, 2012; Park *et al.*, 2012). Expression of vimentin, the cytoskeleton protein in cells of mesenchymal origin has confirmed pronounced proliferation of renal epithelium in the neoplastically altered organ. Investigations proved that renal epithelium develops from mesenchymal embryo. In the course of the organ development expression of vimentin vanishes and, in parallel, cytokeratin becomes expressed (Grieco *et al.*, 2006; Gil da Costa *et al.*, 2011). Positive reaction for vimentin has indicated that renal carcinoma represents a particularly primitive tumour, manifesting low differentiation of its cells (Fig. 3A and 3B). An antibody specific for cytokeratin is very important in diagnosis of neoplastic lesions manifesting an epithelial origin. In our study we have employed the AE1/AE3 antibody, which has demonstrated a pronounced expression in solid carcinoma and papillary-ductal carcinoma (Figs. 3C and 3D). The accessible references related to use of the antibody in diagnosis of renal tumours in animals demonstrated a negative reaction in cases of solid carcinoma while in cases of papillary-ductal carcinoma

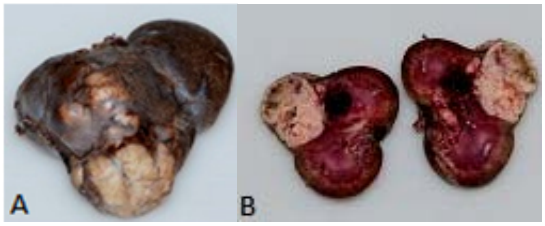

Fig. 1: A. Hypertrophy of tumour mass in kidney; B. Kidney cross-section.

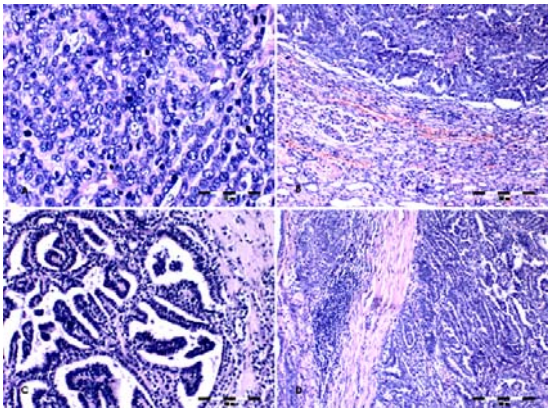

Fig. 2: A and B. Kidney. Solid carcinoma. H&E. A. 400x, B. 100x. C and D Kidney. Papillary-ductal carcinoma. H&E. C. 200x, D. 100x.

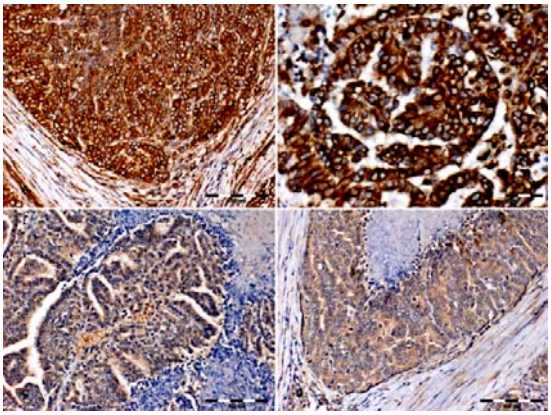

Fig. 3: Cytoplasmic expression of vimentin: A. solid carcinoma, B. papillary-ductal carcinoma. A. 200x, B. 400x. Cytoplasmic expression of AE1/AE3: C. solid carcinoma, D. papillary-ductal carcinoma. C. 200x, D. 200x.

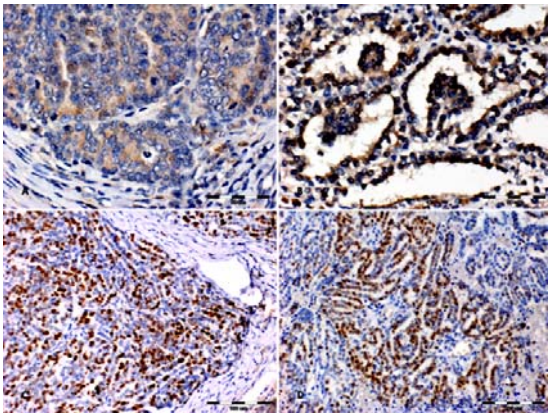

Fig. 4: Cytoplasmic expression of c-kit: A. solid carcinoma, B. papillary-ductal carcinoma. A. 400x, B. 400x. Nuclear expression of Ki-67: A. solid carcinoma, B. papillary-ductal carcinoma. C. 200x, D. 200x.

Table 1: Evaluation of expression manifested by studied antigens

	Renal solid carcinoma	Renal papillary-ductal carcinoma
Vim	+++ (11pts)	+++ (10pts)
AE1/AE3	+++ (10 pts)	+++ (12 pts)
Ki-67	+++ (> 50%)	+++ (>50%)
c-kit	+ (2 pts)	+ (2 pts)
CD10	- (0 pts)	- (0 pts)
CAE	- (0 pts)	- (0 pts)

the expression was positive in 1/3 of the cases, the remaining cases being negative (Gil da Costa *et al.*, 2011).

A less pronounced reaction has been manifested using a polyclonal antibody specific for c-kit, the trans-membrane receptor with activity of tyrosine kinase (Figs. 4A and 4B). The marker is used in diagnosis of, i.a., mesenchymal tumours developing in the abdominal cavity. Other authors obtained positive reactions with the antibody also in almost all renal tumours, but at a variable level (Gil da Costa *et al.*, 2011). Studies related to renal tumours in humans documented a positive reaction for c-kit only in cases of chromophobe carcinoma (Bonsib *et al.*, 2010; Shen *et al.*, 2012; Park *et al.*, 2012). In our diagnoses we have used Ki-67 antigen as a marker of cellular proliferation, demonstrating its intense expression, pointing to a high mitotic potential of the tumours (Figs. 4C and 4D).

In cases of CD10 and CEA we have obtained negative reactions. CD10 represents a surface protein, belonging to transmembrane metalloproteases and it used to be applied in human medicine for diagnosis of lymphomas and leukaemias, termed the common antigen of acute lymphoblastic leukaemia. The marker is useful also on diagnosis of renal clear cell carcinoma. CEA, belonging to the family of carcinoembryonic glycoproteins, is physiologically present in foetal life. In adult humans it is present in low concentrations but quantities of the antigen increase in patients with certain neoplastic diseases, particularly with epithelial tumours. Studies related to renal carcinoma in animals have demonstrated a variable reactivity of CD10 and CEA. A positive reaction for CD10 has been obtained in 1/3 of solid carcinoma cases and in all cases the reaction has been positive for CEA. In papillary-ductal carcinoma expression of CD10 was detected in 1/3 of cases and a similar proportion of cases were positive after application of CEA (Gil da Costa *et al.*, 2011). In diagnosis of renal tumours in humans CD10-specific antibody is useful in detection of all types of tumours (Shen *et al.*, 2012; Park *et al.*, 2012). The results obtained in our investigations resemble those in reports illustrating diagnosis of renal tumours in humans and animals (Bonsib *et al.*, 2010; Gil da Costa *et al.*, 2011).

Conclusion: Both in medicine and in veterinary practice application of the modern diagnostic technique of immunohistochemistry allows for a more precise diagnosis of tumours. Unfortunately, not always owners of the patients express consent for surgical removal of the affected kidney, an alternative involves obtaining the investigated material by a thick needle biopsy. However, the amount and quality of biopsy material and application of the routine staining not always permit to establish the diagnosis. In such cases, application of appropriately selected antibodies provides a helpful tool for a histo-pathologist, opening potential for an accurate examination of the material, establishing a correct diagnosis and implementation of an appropriate, targeted therapy.

Author's Contribution: Rafal Ciaputa, Marcin Nowak, Malgorzata Kandefer-Gola, Bartlomiej Liszka executed the experiment and analyze the results, photographic recording, preparing and elaboration the manuscript while Jakub Nicpon and Bartlomiej Liszka carried out clinical diagnosis and surgical procedures.

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