NEOPLASTIC DISEASE

Short Title: Fibromyxoid Stroma in Canine Urothelial Carcinoma

A Fibromyxoid Stromal Response is Associated with Muscle Invasion in Canine Urothelial Carcinoma

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Summary

Canine urothelial carcinoma (UC) is the most common type of cancer of the lower urinary tract and tends to affect elderly neutered female dogs, with a high predisposition for Scottish terriers. Tumour stroma, inflammation and necrosis are poorly characterized in canine UC and their role as prognostic factors is unknown. The aims of this study were to (1) assess histologically 381 canine UCs, with emphasis on myxoid tumour stroma, inflammation and necrosis, and (2) assess possible associations between these features and the available epidemiological data as well as bladder wall muscle invasion. In 103 of 381 (27%) of cases, the stroma was mixed collagenous and myxoid (fibromyxoid), which was strongly associated with invasive growth of muscle (P <0.0001). Peritumoral and intratumoural inflammation was present in 308 of 345 (89%) and 287 of 381 (75%) of cases, respectively, and was mostly mild and lymphoplasmacytic. One hundred and fifteen of the 381 (30%) cases showed a variable eosinophilic inflammation, and 58 of 381 (15%) presented with formations of one or several lymphoid follicles. Twenty-four percent (91 of 381) of cases had tumour necrosis, which was typically mild. In 83 of 91 (91%) of cases, the necrosis was comedo-like. Moderate to severe tumour necrosis was associated with the presence of moderate to predominant fibromyxoid tumour stroma (P <0.02). The results of this study indicate that fibromyxoid stroma is common in canine UC and is a strong indicator for invasive growth of muscle, which is consistent with a poor prognosis. Based on histomorphology, tumour necrosis in canine UC is best described as comedonecrosis.

Keywords: dog; urothelial carcinoma; fibromyxoid stroma; muscle invasion

Introduction

Urothelial (transitional cell) carcinoma (UC) is the most common cancer of the lower urinary tract in man and dogs (Norris *et al.*, 1992; Mutsaers *et al.*, 2003; Knapp *et al.*, 2014; Dy *et al.*, 2017). Canine UC most commonly affects elderly neutered female dogs, with a strong predisposition for Scottish terriers (Norris *et al.*, 1992; Knapp *et al.*, 2014; de Brot *et al.*, 2018). At the time of diagnosis, affected dogs typically present with advanced disease and options for curative therapy are often limited (Boria *et al.*, 2005; Abbo *et al.*, 2010; Henry *et al.*, 2018). In order to improve therapy, and therefore the prognosis of UC affected dogs, a genetic and morphological tumour characterization, ideally on an individual basis, is crucial. When assessing the morphology of urothelial, or any other type of, tumours, features such as tumour stroma, inflammation and necrosis need to be included, due to their potential importance in tumour prognosis and response to therapy (Samaratunga *et al.*, 2005; Wang *et al.*, 2015; Inoue *et al.*, 2017; Hodgson *et al.*, 2018). In veterinary medicine, the stroma present in UC is characterized poorly, and is typically described as variably dense, fine fibrovascular to desmoplastic (Valli *et al.*, 1995). In man, conventional UC is characterized by a desmoplastic tumour stroma (Samaratunga *et al.*, 2005). Other, much less common stromal responses, reported only in people, include a myxoid and pseudosarcomatous reaction (Ro *et al.*, 1993; Harik *et al.*, 2006; Cox *et al.*, 2009; Tavora and Epstein, 2009). Significant myxoid stroma can be seen in sarcomatoid UC, in UC with a pseudosarcomatous stromal response, and in rare cases of UC with abundant myxoid stroma (Mahadevia *et al.*, 1989; Ikegami *et al.*, 2003; Cox *et al.*, 2009; Tavora and Epstein, 2009; Sanfrancesco *et al.*, 2016). A pseudosarcomatous stromal reaction is uncommon in the lower urinary tract and is characterized by the presence of plump, activated fibroblasts, associated with myxoid changes and increased cellularity (Young *et al.*, 1988; Mahadevia *et al.*, 1989). This type of stromal response has been reported in benign and malignant human urinary tract lesions, and occurs

spontaneously, in response to trauma or tumour treatment, or in association with neoplasia (Pearson *et al.*, 1989; Hughes *et al.*, 1991; Harik et al., 2006; Ambrosini-Spaltro and Melissari, 2011; Vasilakaki *et al.*, 2014).

Tumour inflammation is a well-known phenomenon, which has been studied extensively in various human tumours (Mantovani *et al.*, 2008). In human UC, inflammation can play a prognostic role (Offersen *et al.*, 2002; Samaratunga *et al.*, 2005; Inoue *et al.*, 2017; Hodgson *et al.*, 2018). A mild, predominantly lymphoplasmacytic, intratumoral or peritumoral inflammation is a common finding in human and canine UC (Mihatsch *et al.*, 1979; Offersen *et al.*, 2002; Samaratunga *et al.*, 2005; Inoue *et al.*, 2017). The prognostic significance of tumour inflammation in UC is controversial, but studies suggest that prominent inflammation is associated with better prognosis in people (Offersen *et al.*, 2002; Hodgson *et al.*, 2018). In this context, cancer-associated inflammation may be interpreted as evidence for antitumor immunity.

Tumour necrosis is widely accepted as a poor prognostic factor in various cancers. In general, tumour necrosis is understood to represent increased intratumoral hypoxia due to rapid cell turnover (Caruso *et al.*, 2014; Hodgson *et al.*, 2018). In human UC, the presence and extent of tumour necrosis has been shown to be associated with worse prognosis (Langner *et al.*, 2006; Lee *et al.*, 2007; Ord *et al.*, 2007; Zigeuner *et al.*, 2010; Soave *et al.*, 2015). Where available, histological description of the necrosis in UC was consistent with coagulative necrosis (Langner *et al.*, 2006; Ord *et al.*, 2007; Zigeuner *et al.*, 2010). One of these studies included a morphological variant of coagulative necrosis (i.e. comedo necrosis), in their histological assessment (Ord *et al.*, 2007). By definition, comedo-type necrosis is characterized by the presence of well-circumscribed epithelial nests containing central necrotic material (Caruso *et al.*, 2014).

In a previous study, we assessed epidemiologically and histologically 260 canine UCs (de Brot *et al.*, 2018). In that population, elderly neutered female Scottish terriers were at highest risk for developing UC. The histological assessment included subgross tumour growth (papillary versus non-papillary), muscle-invasive growth and histological tumour classification. Tumour stroma, inflammation and necrosis were not specifically assessed. Given the human literature and the lack of studies which characterize canine UC histologically, the aims of the present study were: (1) to histologically assess and characterize 381 canine UCs, with emphasis on myxoid tumour stroma, inflammation and necrosis, and (2) to assess possible associations between these features and the available epidemiological data as well as muscle invasion.

Materials and Methods

Samples

The present retrospective study includes archived formalin-fixed and paraffin wax-embedded tissues from 381 dogs with primary urinary bladder (*n* = 302), urethral (*n* = 77) or renal pelvic (*n* = 2) carcinomas, which were submitted between 1989 and 2016, to four different institutions located in the UK (*n* = 260; Bridge Pathology Ltd., Bristol, UK) and Switzerland (*n* = 121; University of Zurich; University of Bern; Zyto-Histo Diagnostics, Freienstein). The following case information was available: age at the time of tumour diagnosis, sex, neutering status and dog breed. No follow-up data were available.

Histology

Haematoxylin and eosin (HE)-stained tissue sections from all 381 cases were available as both glass and digital (scanner 3DHISTECH Panoramic 250 Flash III; UK) or only glass (Switzerland) slides. The samples were assessed histologically by a board-certified veterinary

pathologist (SdB), with support of a certified human uropathologist (BR) and five certified veterinary pathologists (LGR, MD, FG, DM and TS). All cases were classified for tumour subtype according to the World Health Organization (WHO) human tumour classification system (Humphrey *et al.*, 2016). On all cases, semiquantitative assessment of peritumoural and intratumoural inflammation and tumour necrosis was performed as follows: 0, absent; 1, focal mild; 2, multifocal mild; 3, multifocal moderate; 4, multifocal severe or diffuse. The degree of severity was defined as follows (percentage of affected tissue per tissue section): for inflammation as mild, <10%; moderate, 10–30%; or severe, >30%; and for necrosis as mild, <5%; moderate, 5–20%; or severe, >20%.

Cases with peri- and/or intratumoural inflammation were assessed for the presence of lymphoid follicles (0, none; 1, 1 follicle; 2, 2–3 follicles; 3, >3 follicles), and number of eosinophils per 1 and 10 high power fields (HPF). For this purpose, HPFs with the highest number of eosinophils were selected. A HPF was defined as 0.237mm^2 (×40 objective and ×10 ocular with field number 22) following the recommendations by Meuten *et al.* (2016). Cases with tumour necrosis were estimated for the presence of comedo-like necrosis, and calculated as % of total necrosis (area percentage) (0, absent; 1, <10%; 2, 10–50%; 3, >50%). For all cases, a semiquantitative assessment of fibromyxoid tumour stroma was performed as follows: 0, absent; 1, focal mild; 2, multifocal mild; 3, multifocal moderate; 4, predominant. The degree of severity was defined as follows (percentage of fibromyxoid stroma within the total tumour stroma per tissue section): mild, <10%; moderate, 10–50%; predominant, >50%. Fibromyxoid stroma was defined as a variably dense mixed collagenous and myxoid stroma, irrespective of the extent and distribution of the two components. The fibromyxoid stroma was assessed on HE-stained tissue sections. In order to confirm both the myxoid and collagenous stromal component, serial sections from seven representative cases were stained with Alcian blue and Masson's trichrome stains. Cases with partial or full-thickness tunica

muscularis (137 of 381) were assessed for muscle-invasive tumour growth. Muscle invasion was defined as tumour invasion into any level of the muscularis propria.

Statistics

Breed predisposition and the risk of the other available epidemiological factors were assessed statistically following previously reported criteria (de Brot *et al.*, 2018). The Chi-square test was used to test for associations between tumour muscle invasion, tumour necrosis, fibromyxoid tumour stroma, peri- and intratumoural inflammation, and squamous and/or glandular differentiation of the tumour. $P < 0.05$ was accepted as significant. Statistical analyses were performed using SPSS v.24.0 (IBM Corp., Armonk, New York, USA).

Results

In a previous study (de Brot *et al.*, 2018), we reported the epidemiological data from 260 out of the current 381 cases. The additional cases ($n = 121$) included in the present study confirmed the previously reported predisposition for elderly neutered female Scottish terriers,. Assessing all 381 cases, Scottish terriers were significantly younger (mean 9.04 $\pm$ 1.7 years) at the time of tumour diagnosis, when compared with all other dog breeds grouped together (mean 10.27 $\pm$ 2.2 years) ($P < 0.01$). The same effect was seen in male dogs (mean 9.78 $\pm$ 2.4 years) when compared with female dogs (mean 10.32 $\pm$ 2.1 years). A more detailed summary of the epidemiological data from the dogs included in the study is presented in Table 1.

Table 2 details the primary tumour location, tumour type and subtype, and muscle invasion of the studied cases. Urinary bladder was the primary tumour location in the majority (79%) of cases and UC was (88%) the most common type of tumour. Squamous differentiation, either partially in cases with UC ($n = 22$), or diffusely in cases with squamous

cell carcinoma (SCC; $n = 7$), was present in 29 of 381 (8%) cases. Glandular differentiation was seen in 13 of 381 (3%) UCs and was diffuse in one case, the latter being consistent with primary bladder adenocarcinoma. Muscle invasion, defined as tumour invasion of any level of the tunica muscularis of the bladder or urethra, was common, and was present in two thirds of all assessable tumours.

Table 3 gives results for the presence of fibromyxoid stroma, inflammation and necrosis of all studied tumours. The tumour stroma was fibromyxoid in 27% of cases, being moderate to severe (i.e. affecting ≥10% of the total tumour stroma per tissue section) in two thirds of the cases. The fibromyxoid stroma was characterized typically by a dense fibrous stroma with variable multifocal to coalescing accumulations of intercellular, myxomatous substance (Figs. 1, 2). The presence of fibromyxoid stroma was strongly associated with tumour muscle invasive growth (P <0.0001). Tumours with evident (5–100% of the tumour tissue section) squamous or glandular differentiation were associated with the presence of moderate to predominant fibromyxoid stroma. This association was stronger in tumours with glandular (P <0.0001) compared with squamous differentiation (P = 0.005). Inflammation, peritumoural (including tumour front) as well as intratumoural, was common and seen in 89% and 75% of cases, respectively. The inflammation was predominantly lymphoplasmacytic, multifocal to coalescing, and typically mild. Almost one third of all cases (115 of 381; 30%) showed a variable, ranging from mild to severe, peri- and/or intratumoural infiltration with eosinophils (Fig. 3). Fewer cases (58 of 381; 15%) presented with formations of one or several lymphoid follicles, typically located in peritumoural tissue (Fig. 4). Moderate to severe intratumoural and peritumoural inflammation was associated with squamous (P <0.04) and glandular (P = 0.001) differentiation, respectively. Moderate to severe peritumoural inflammation was associated with moderate to severe intratumoural inflammation (P <0.0001). Twenty-four percent of all cases had variable tumour necrosis, which was typically

mild and multifocal to coalescing (Table 3). In the majority (91%) of cases, the necrosis was comedo-like (Fig. 5). Moderate to severe tumour necrosis was associated with the presence of moderate to predominant fibromyxoid tumour stroma (P <0.02). No other significant associations were observed between the different studied parameters.

Discussion

UC is known to have a variably dense fibrous tumour stroma (Amin 2009; Humphrey *et al.*, 2016). UC with myxoid stroma is rare in people (Tavora and Epstein, 2009) and has not been described in dogs or other animals. In people, myxoid stroma is reported in both invasive and non-invasive urothelial carcinomas (Amin 2009; Humphrey *et al.*, 2016). The proposed terminology for UC with prominent myxoid stroma includes 'myxoid urothelial carcinoma with abundant myxoid stroma' (Behzatoğlu *et al.*, 2012). In cases with chordoid morphology, characterized by prominent cellular cording and associated myxoid stromal matrix, the UCs are proposed to be referred to as 'urothelial carcinoma with abundant myxoid stroma showing chordoid-like features' (Cox *et al.*, 2009). In man, the myxoid stroma is typically loose with no significant collagenous stromal component. This contrasts with the canine cases reported here, which were characterized by a dense, mixed collagenous and myxoid (hence the term 'fibromyxoid') tumour stroma. The primarily dense fibromyxoid stroma in the canine cases studied here appears to closely resemble the fibromyxoid stroma described in human vulvar squamous cell carcinoma (Jeffus *et al.*, 2015). In accordance with previous studies (Ambros *et al.*, 1996; Pinto *et al.*, 1999), Jeffus *et al.* (2015) defined a fibromyxoid stromal response as extracellular matrix composed of immature collagen and fibroblasts with a myxoid component which had a blue hue on HE-stained tissue sections compared with the fibrous stroma. This histomorphological description defines well the appearance of the fibromyxoid stroma observed in the canine cases in the present study. The blue hue of the myxoid stromal

component was observed in HE-stained tissue sections and was even more evident when the tissue was stained with Alcian blue. In people, UC may be associated with an exuberant stroma with variable myxoid matrix and extensive mesenchymal changes, which may stimulate a sarcoma or sarcomatoid differentiation, referred to as 'pseudosarcomatous stromal reaction' (Ro *et al.*, 1993; Ambrosini-Spaltro and Melissari, 2011). In the present canine cases, such a highly proliferative stromal spindle cell population, characterized by a high cellularity of plump fibroblasts, was absent. Canine cases in the present study lacked any chordoid-like features (i.e. cellular cording in a loose myxoid stroma).

The fact that the presence of fibromyxoid stroma was strongly associated with muscle invasive tumour growth in these dogs with UC is significant. Muscle invasion *per se* is an indicator for higher tumour stage and poor prognosis in patients with UC (Humphrey *et al.*, 2016). In a study of human UC, all cases with abundant myxoid stroma were invasive, with 69% and 31% of cases invading the lamina propria and tunica muscularis, respectively (Tavora and Epstein, 2009). The presence of fibromyxoid stroma is also known to be a poor prognostic factor in vulvar squamous cell carcinoma (Ambros *et al.*, 1996; Jeffus *et al.*, 2015) and uterine endometrioid endometrial carcinoma (Murray *et al.*, 2003). In vulvar squamous cell carcinoma, the presence of a fibromyxoid stroma was associated with older age group, poorer survival rate and more extensive lymph node metastases (Ambros *et al.*, 1996) as well as infiltrative tumour morphology, perineural invasion and lymph node metastasis (Jeffus *et al.*, 2015). In endometrial carcinoma, a fibromyxoid stromal reaction is known to be associated with a higher frequency of death or recurrence and is frequently accompanied by lymphatic or blood vessel invasion (Murray *et al.*, 2003). The role of fibromyxoid tumour stroma in the pathomechanism of aggressive tumour growth remains unknown. Infiltrative tumours with a fibromyxoid stromal response have been proposed to behave more aggressively through epithelial-to-mesenchymal transition (Holthoff *et al.*, 2016).

Tumour necrosis was a common finding in the present study, and was observed in ~25% of cases. This is in agreement with human upper and lower urinary tract UC where tumour necrosis is a frequent and a well-described feature (Lee *et al.*, 2007; Zigeuner *et al.*, 2010; Soave *et al.*, 2015; Hodgson *et al.*, 2018). Tumour necrosis is a poor prognostic factor in human UC, and is associated with increased tumour size, decreased recurrence-free survival and decreased disease-specific survival, older age, advanced tumour stage, higher tumour grade, lymph node metastasis, positive surgical margin status and lymphovascular invasion (Lee *et al.*, 2007; Zigeuner *et al.*, 2010; Soave *et al.*, 2015; Hodgson *et al.*, 2018). In these canine cases, no association of tumour necrosis with older age or tumour muscle invasion was observed. It was not possible to determine additional clinicopathological associations, as information with respect to tumour size, survival time, lymph node metastasis and surgical margins was not available, which is a limitation of this study. Interestingly however, moderate (affecting 5–20% of the tissue section) to severe (affecting >50% of the tissue section) tumour necrosis was significantly associated (P <0.02) with the presence of moderate (10–50% of the total tumour stroma) to predominant (>50% of the total tumour stroma) fibromyxoid tumour stroma, which in turn is strongly associated with muscle invasion. Nevertheless, no direct association was observed between tumour necrosis and muscle invasion.

In the canine UCs reported here, tumour necrosis presented primarily as comedo-type necrosis, closely resembling comedonecrosis in human and canine breast and prostate cancer (Yagata *et al.*, 2003; Akter *et al.*, 2015; Palmieri and Grieco, 2015; Epstein *et al.*, 2017; Al-Mansour *et al.*, 2018; Fine *et al.*, 2018). The histomorphological description of comedonecrosis (i.e. intraluminal necrotic cells and/or karyorrhexis displaying cribriform or solid architecture; Fine *et al.*, 2018), describes well the comedo-type necrosis in the examined canine UCs. However, the term comedonecrosis is not commonly used when referring to

tumour necrosis in human or canine UCs, except in cases with prostatic UC (Humphrey *et al.*, 2016). Reasons for this may be: (1) necrosis in human (non-prostatic) UC is not of comedo-type, and/or (2) necrosis in canine UC has not previously been further characterized. Given that comedonecrosis in human UC appears to be rare, in contrast to canine UC, its presence may indicate a different pathogenesis of tumour necrosis in people and dogs. Given the frequent presence of comedonecrosis in canine UC in the present study, which includes over 380 dogs of different breeds, ages and gender, with origin in two different countries, it seems reasonable to assume that comedonecrosis is a common feature of canine UC.

Regarding the prognostic significance of comedonecrosis in the present study, only an indirect association between the presence of moderate to severe tumour necrosis and tumour muscle invasion was found. No significant association between the presence of comedonecrosis and age, sex, neutering status or dog breed was identified. In human breast carcinoma, disease-free survival was significantly shorter in patients with the comedo-type compared with the non-comedo-type tumour (Yagata *et al.*, 2003). Due to the lack of information about survival times of the dogs studied here, no conclusions could be drawn for canine UC. In prostate cancer, a finding of comedonecrosis has been near universally associated with high-grade disease, and comedonecrosis is a defined feature of Gleason grade 5 tumours (Epstein *et al.*, 2017). Considering that canine UC is typically a high-grade disease *per se*, the frequently occurring comedonecrosis may potentially reflect, and be associated with, high tumour grades.

Tumour inflammation, both peritumoural and intratumoural, was common in the studied canine UCs. Lymphocytes and plasma cells were the predominate inflammatory cell type and most cases had only a mild inflammatory response. This is in agreement with previous studies of human and canine UC (Mihatsch *et al.*, 1979; Offersen *et al.*, 2002; Samaratunga *et al.*, 2005; Inoue *et al.*, 2017). The prognostic significance of peri- and

intratumoural inflammation in UC is controversial in the human literature (Samaratunga *et al.*, 2005). Inflammation has been reported to be associated with longer survival times, particularly in cases with an intense, tumour-front inflammatory response (Offersen *et al.*, 2002; Hodgson *et al.*, 2018). This association could unfortunately not be tested in the present canine cohort due to a lack of information about survival times. Apart from mononuclear inflammatory cells, polymorphonuclear cells, in particular neutrophils and eosinophils, are also described in human UC (Offersen *et al.*, 2002; Samaratunga *et al.*, 2005). Almost one third of all cases in the present series showed a variable, peri- and/or intratumoural infiltration with eosinophils. The occurrence of eosinophils within neoplastic tissue is referred to as tumour-associated tissue eosinophilia (TATE; Popov *et al.*, 2018). Similar to TATE in human UC, the extent of the eosinophilic inflammatory response in canine cases varied greatly, ranging from absent to a high-density inflammation. The prognostic significance of TATE in UC remains controversial (Mihatsch *et al.*, 1979; Lowe and Fletcher, 1984; Hodgson *et al.*, 2018; Popov *et al.*, 2018). In addition to lymphoplasmacytic and eosinophilic inflammation, about 15% of all canine UCs presented with formations of one or several lymphoid follicles, typically located in peritumoural tissue. The presence of tertiary lymphoid structures (TLSs) has been reported to occur in bladder and other types of cancer (Koti *et al.*, 2017). Studies of the prognostic significance of TLSs in bladder cancer are rare. Koti *et al.* (2017) reported a poor prognostic significance of TLSs, which was seen more commonly in high-grade muscle invasive, compared with non-muscle invasive, bladder cancer. In the current series, no association between the presence and extent of TATE or TLSs, and muscle invasive growth, age, sex, neutering status or dog breed was identified. In conclusion, TATE and TLS are regularly occurring features in canine UC, but are of unknown prognostic significance

Divergent, including squamous and glandular, differentiation is frequent in human UC and has diagnostic, prognostic and therapeutic implications (Amin 2009; Shanks and Iczkowski, 2009; Minato *et al.*, 2018). In the present study, squamous differentiation (8%) was found more commonly than glandular differentiation (3%). This has already been reported and discussed in a previous study, which included histological assessment of a subset of 260 out of the currently reported 383 cases (de Brot *et al.*, 2018). As an additional finding, the present study shows an association between divergent (i.e. glandular, and to a lesser extent, squamous differentiation) and the presence of fibromyxoid tumour stroma. Partly based on evidence from the literature, and partly speculative, it could be proposed that this association may be explained as follows: (1) tumour cells with squamous or glandular differentiation promote the development of a tumour stroma with a myxoid component, (2) tumour stroma with a myxoid component promotes the divergent differentiation of neoplastic urothelial cells, or (3) divergent differentiation (Amin 2009; Shanks and Iczkowski, 2009; Minato *et al.*, 2018) and myxoid stroma (Tavora and Epstein, 2009) both represent poor prognostic features and are therefore likely to be seen together in highly malignant subtypes of UC. These explanations are, however, speculative and require further studies for clarification. Another feature which was associated with divergent (i.e. glandular, and to a lesser extent, squamous differentiation) was the presence of moderate to severe inflammation. As for the association with myxoid stroma, it remains unknown whether in these cases inflammation promoted divergent differentiation of neoplastic urothelial cells or vice versa.

In conclusion, the presence of fibromyxoid tumour stroma is common in canine UC and is strongly associated with muscle-invasive growth, which is an indicator of poor prognosis. Tumour inflammation and necrosis are common in UC, but their prognostic significance remains unknown. Based on histomorphology, tumour necrosis in canine UC is predominantly comedo-like and therefore best referred to as comedonecrosis.

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Figure Legends

Fig. 1. Muscle-invasive urothelial carcinoma in the urinary bladder of a 7-year-old neutered male crossbreed dog. Note the mixed collagenous and myxoid (fibromyxoid) tumour stroma. HE. Bar, 100 μm.

Fig. 2. Muscle-invasive urothelial carcinoma in the urinary bladder of a 7-year-old neutered male crossbreed dog (same case and approximately same location within the tumour as shown in Fig. 1). Note the mixed collagenous and myxoid (fibromyxoid) tumour stroma with positive Alcian blue and green Masson's trichrome (inset) staining. Alcian blue stain with nuclear fast red counterstain. Bar, 100 μm. Inset: Masson's trichrome stain. Bar, 100 μm.

Fig. 3. Urothelial carcinoma in the urethra of a 12-year-old male curly-coated retriever. Note the marked infiltration of the lamina propria with eosinophils (*). HE. Bar, 100 μm.

Fig. 4. Non-muscle invasive urothelial carcinoma in the urinary bladder of a 6-year-old giant schnauzer. Note the formations of lymphoid follicles (*) in the peritumoural lamina propria. HE. Bar, 1000 μm.

Fig. 5. Muscle-invasive urothelial carcinoma in the urinary bladder of a 5-year-old female Labrador retriever. Note the severe comedonecrosis, characterized by well-demarcated, large central areas of necrotic material (*) surrounded by neoplastic epithelial cells. The tumour stroma is fibromyxoid. HE. Bar, 200 μm.

Table 1

Country of residence, sex, neuter status, breed and age of the dogs included in this study

	Previously reported cases[*] *(n = 260)*	*Additional cases (n = 121)*	*All cases combined (present study) (n = 381)*
Country of residence	UK	Switzerland	Switzerland and UK
Sex	Female 195/260 (75%) Male 65/260 (25%)	Female 86/121 (71%) Male 34/121 (28%) Unknown (1%)	Female 281/381 (74%) Male 99/381 (26%) Unknown 1/381 (<1%)
Neuter status	Neutered 190/260 (73%) Entire 7/260 (3%) Unknown 63/260 (24%)	Neutered 71/121 (59%) Entire 47/121 (39%) Unknown 3/121 (2%)	Neutered 260/381 (68%) Entire 54/381 (14%) Unknown 67/381 (18%)
Breed[†]			
Absolute	1) Crossbred 38/260 (15%) 2) Labrador retriever 33/260 (13%) 3) West Highland white terrier 22/260 (8%) 4) Scottish terrier 16/260 (6%) 4) Cocker spaniel 16/260 (6%)	1) Crossbred 25/121 (21%) 2) Scottish terrier 7/121 (6%) 2) German shepherd dog 7/121 (6%) 2) Bernese mountain dog 7/121 (6%)	1) Crossbred 63/381 (17%) 2) Labrador retriever 36/381 (9%) 3) West Highland white terrier 27/381 (7%) 4) Scottish terrier 23/381 (6%)
Relative[‡]	1) Scottish terrier 16/354 (4.52%) 2) West Highland white terrier 22/2526 (0.87%) 3) Crossbred 38/13050 (0.29%)	1) Scottish terrier 7/387 (1.81%) 2) West Highland white terrier 5/6156 (0.08%) 3) Crossbred 25/129806 (0.02%)	1) Scottish terrier 23/741 (3.10%) 2) West Highland white terrier 27/8682 (0.31%) 3) Labrador retriever 36/38669 (0.09%)

	4) Labrador retriever 33/13007 (0.25%)	4) Labrador retriever 3/25662 (0.01%)	4) Crossbred 64/142856 (0.04%)
Age (years)			
Range	4–15	2–16	2–16
Mean	9.95 ± 2.0	10.70 ± 2.4	10.19 ± 2.2
	Females 10.06 ± 1.9; males 9.99 ± 1.7 Neutered 10.06 ± 1.9; entire 10.04 ± 1.9 Scottish terrier 8.74 ± 1.0; all other breeds 10.06 ± 1.9	Females 10.70 ± 2.2; males 10.70 ± 1.9 Neutered 10.81 ± 2.1; entire 10.93 ± 2.1 Scottish terrier 10.15 ± 1.4; all other breeds 10.89 ± 2.2	Females 10.32 ± 2.1; males 9.78 ± 2.4 (P <0.05) Neutered 10.18 ± 2.0; entire 10.57 ± 2.8 (P >0.05) Scottish terrier 9.04 ± 1.7; all other breeds 10.27 ± 2.2 (P <0.01)

*de Brot *et al.*, 2018

†Only the top four breeds are shown

‡Relative to control population, UK; Bridge Pathology Ltd., Bristol, UK (total canine submissions during time period when studied tumour samples were submitted); Switzerland, AMICUS (www.amicus.ch) (total canine population living in Switzerland in 2016)

Table 2

Primary tumour location, tumour diagnosis and muscle-invasive growth of all studied tumours

	Previously reported cases[*] *(*n = *260)*	*Additional cases (*n = *121)*	*All cases combined (present study) (*n = *381)*
Primary tumour location			
Urinary bladder	199/260 (77%)	103/121 (85%)	302/381 (79%)
Urethra	61/260 (23%)	16/121 (13%)	77/381 (20%)
Kidney (pelvis)	0/260 (0%)	2/121 (2%)	2/381 (1%)
Tumour diagnosis			
Urothelial carcinoma (UC)	234/260 (90%)	102/121 (84%)	336/381 (88%)
UC with glandular and/or squamous differentiation	15/260 (6%)	16/121 (13%)	31/381 (8%)
Squamous cell carcinoma	5/260 (2%)	2/121 (2%)	7/381 (2%)
Other	6/260 (2%)	1/121 (1%)	7/381 (2%)[‡]
Muscle invasion			
Absent	24/260 (9%)	22/121 (18%)	46/381 (12%)
Present[†]	52/260 (20%)	39/121 (32%)	91/381 (24%)
Not assessable (lack of muscular layer)	184/260 (71%)	60/121 (50%)	244/381 (64%)

[*]de Brot *et al.*, 2018

[†]In cases with renal pelvic UC (*n* = 2), tumour invasion into renal sinus or renal papilla was interpreted as comparable to muscle invasion in bladder and urethra

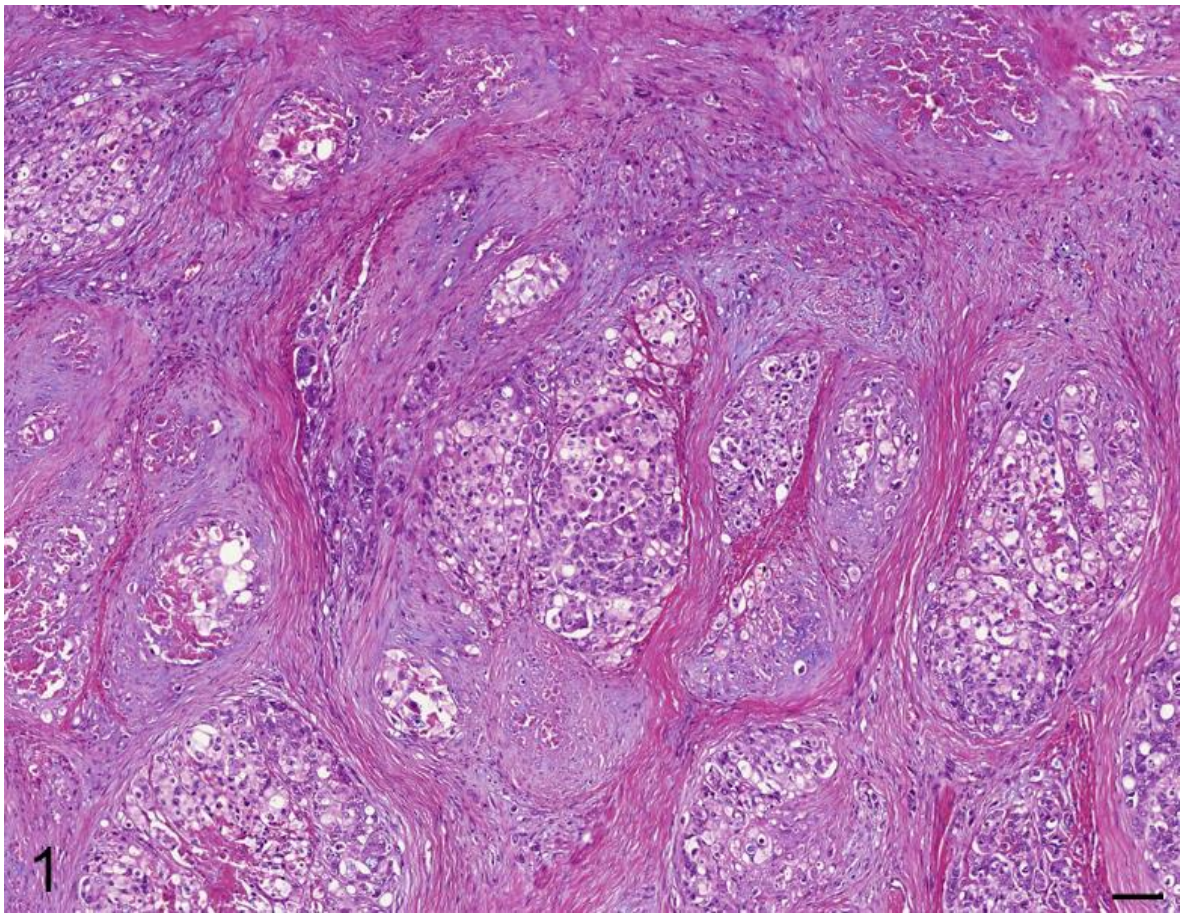
[‡]*n* = 2 neuroendocrine tumour, *n* = 1 plasmacytoid UC, *n* = 1 micropapillary UC, *n* = 1 UC with sarcomatoid differentiation, *n* = 1 UC with microcystic-rich features, *n* = 1 adenocarcinoma

Table 3

Presence of fibromyxoid stroma, peri- and intratumoural inflammation and necrosis of all studied tumours

Fibromyxoid tumour stroma	*Tumour inflammation*	*Eosinophilic and lymphoid follicular inflammation*[†]	*Tumour necrosis*
Present in 103/383 (27%) 0 (absent) 280/383 (73%) 1 (focal mild) 3/383 (0.8%) 2 (multifocal mild) 33/383 (9%) 3 (multifocal moderate) 43/383 (11%) 4 (predominant) 24/383 (6%)	**Peritumoural**[*] Present in 308/345 (89%) 0 (none) 37/345 (10%) 1 (focal mild) 2/345 (0.5%) 2 (multifocal mild) 190/345 (50%) 3 (multifocal moderate) 98/345 (26%) 4 (multifocal severe) 18/345 (5%) Not assessable 38/383 (lack of adjacent non-neoplastic tissue) [*]including tumour front **Intratumoural** Present in 287/383 (75%) 0 (none) 96/383 (25%) 1 (focal mild) 2/383 (0.5%) 2 (multifocal mild) 234/383 (61%) 3 (multifocal moderate) 39/383 (10%) 4 (multifocal severe) 12/383 (3%)	**Eosinophilic inflammation** Present in 115/383 (30%) Number of eosinophils per HPF: mean 22.61 ± 102.4 (range 0–1870) Number of eosinophils per 10 HPFs: mean 92.92 ± 783 (range 0–15,122) **Presence of lymphoid follicles** Present in 58/383 (15%) 0 (none) 325/383 (85%) 1 (1 follicle) 11/383 (3%) 2 (2–3 follicles) 12/383 (3%) 3 (>3 follicles) 35/383 (9%)	Present in 91/383 (24%) 0 (absent) 292/383 (76%) 1 (focal mild) 6/383 (2%) 2 (multifocal mild) 33/383 (9%) 3 (multifocal moderate) 26/383 (7%) 4 (multifocal severe) 26/383 (7%) **Comedonecrosis** Present in 83/91 (91%) cases with tumour necrosis 0 (absent) 8/91 (9%) 1 (<10%)[‡] 8/91 (9%) 2 (10–50%)[‡] 10/91 (11%) 3 (>50%)[‡]65/91 (71%) [‡]Percentages in brackets indicate the amount of comedonecrosis within the total tumour necrosis

HPF, high-power field
[†]intra- and/or peritumoural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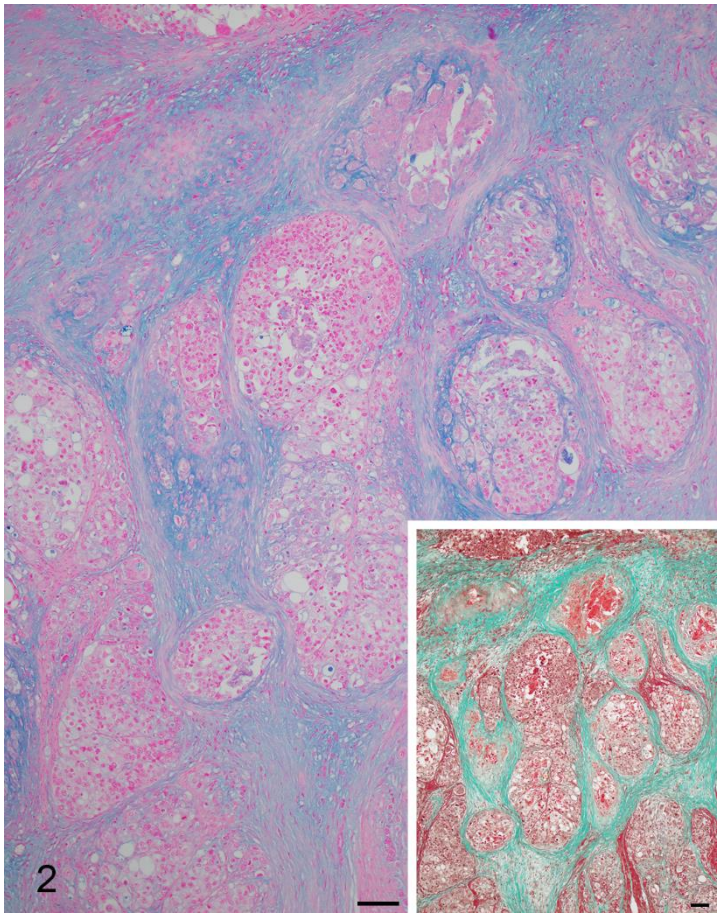
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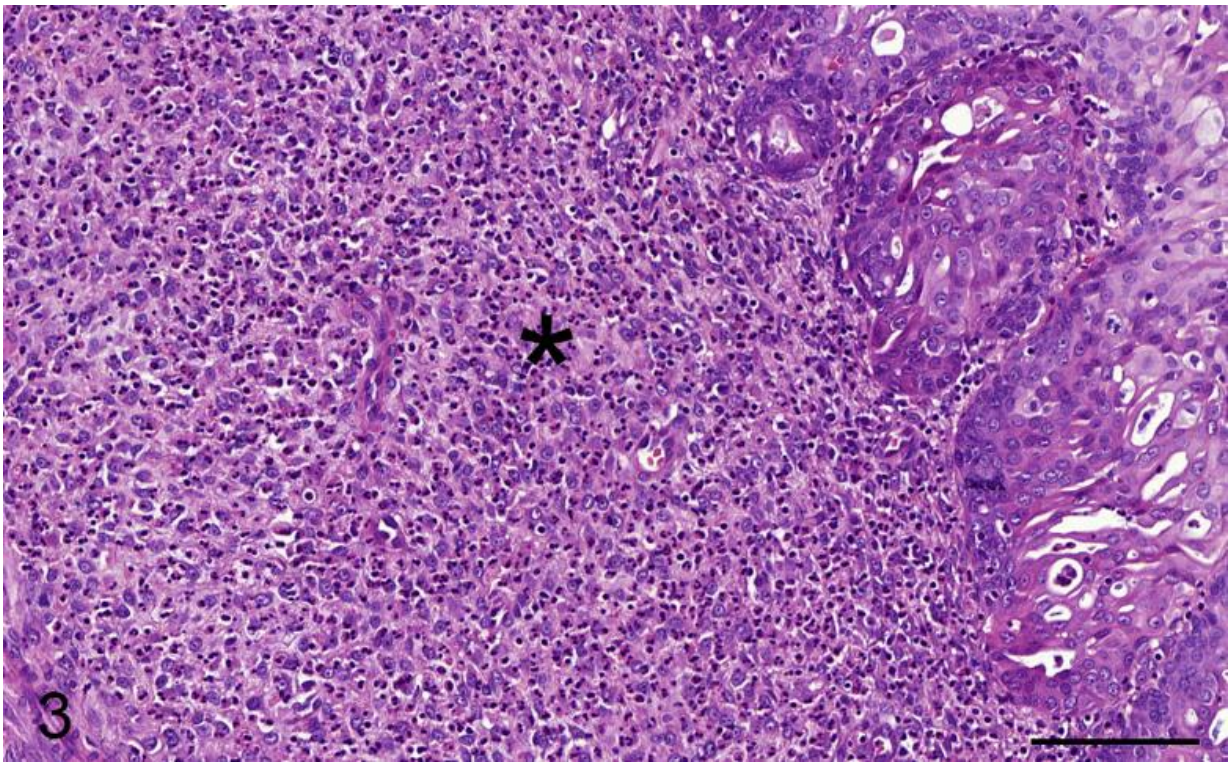
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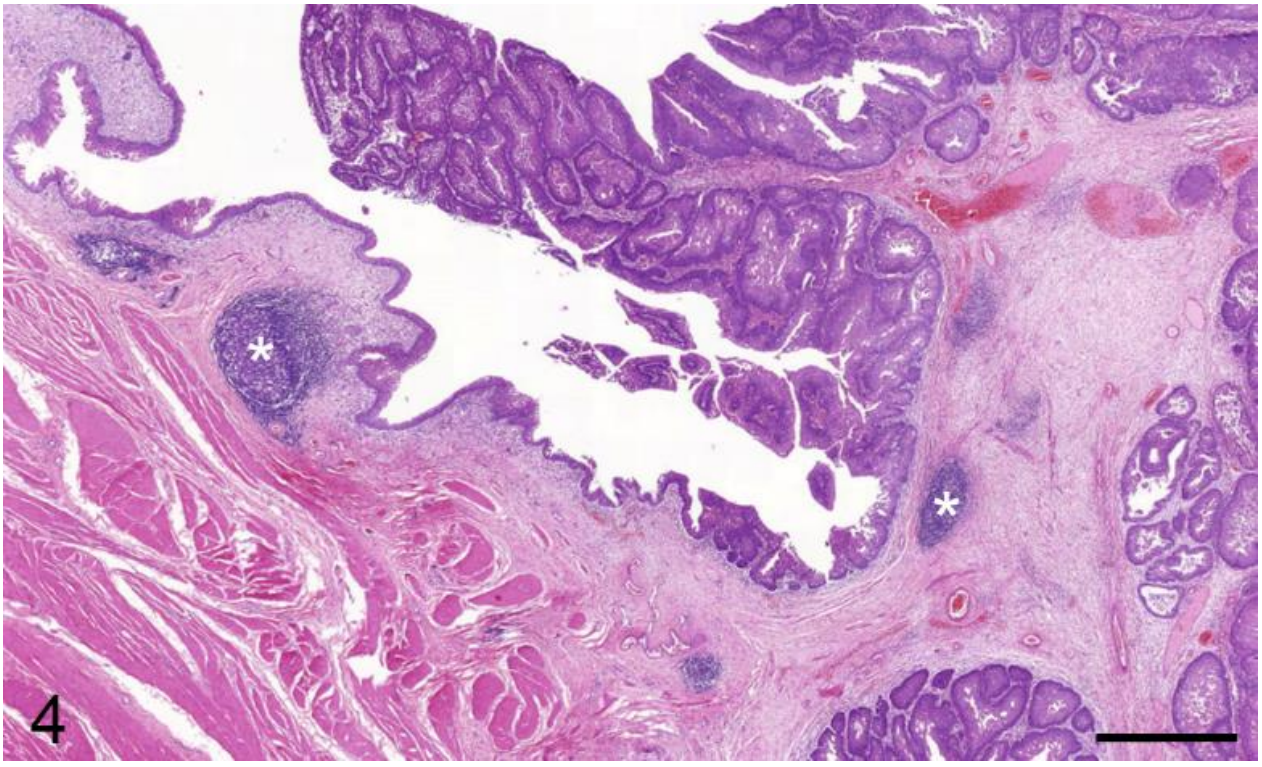
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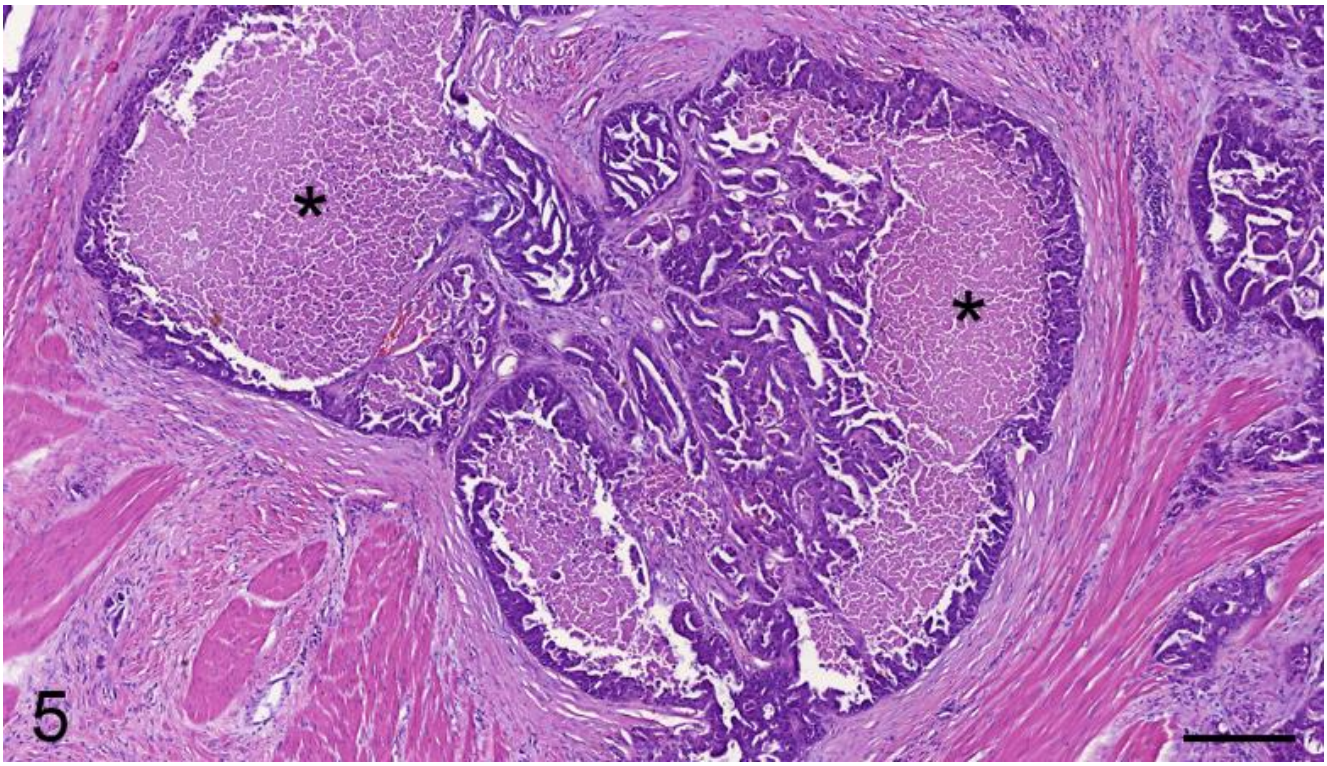
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