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Mini Review

Cytology and Other Diagnostic Tools in Urothelial Carcinoma

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Abstract

Urothelial carcinoma also known as transitional cell carcinoma, is the most frequent neoplasm affecting urinary bladder. It can also arise in urethra, prostate or vagina. Clinical signs are usually nonspecific and are similar to other processes affecting the lower urinary tract, like cystitis, urolithiasis or lower urinary tract infection. Diagnostic approach includes a minimum database (complete blood count, serum chemistry and urinalysis) and more specific test, like urinary culture and sensitivity, imaging of the lower urinary tract, cytology, histopathology and/or molecular tests (i.e. detection of BRAF mutation).

On cytology, we can detect epithelial cells, with basophilic cytoplasm, typically showing abundant criteria of malignancy, such as anisokaryosis, anisonucleoliosis, and increase in nuclear-to-cytoplasmic ratio. The presence of eosinophilic cytoplasmic inclusions also called Melamed-Wolinska bodies may be a diagnostic clue and give some more support on urothelial carcinoma diagnosis.

Keywords: *Urothelial Carcinoma; Transitional Cell Carcinoma; Cytology; Melamed-Wolinska Bodies*

Abbreviations

UC: Urothelial Carcinoma; TTC: Transitional Cell Carcinoma; MWB: Melamed-Wolinska Bodies; BRAF: B-Raf (Proto-Oncogene); FNA: Fine Needle Aspiration; BTA: Bladder Tumour-Associated Antigen

Introduction

Neoplasms of urinary bladder are relatively infrequent, accounting about 2% of total malignancies reported in dogs [1]. Urinary bladder is the most common site of total urinary tract neoplasia in dogs, and the second one in cats, followed by renal lymphoma [2]. Urothelial carcinoma (UC) also known as transitional cell carcinoma (TCC) is the most frequent cancer in urinary bladder in dogs and cats. Other neoplasms in bladder, although reported less frequently, include squamous cell carcinoma, adenocarcinoma, undifferentiated carcinoma, rhabdomyosarcoma, lymphoma, hemangiosarcoma, fibroma and other mesenchymal tumours [1].

Most studies and published bibliography regarding this neoplasm refer to the canine counterpart, and cats have significantly less available information. Trigone region of the bladder is a predilected location for UC, which can also localize in urethra, ureter, prostate or vagina [3].

Usual clinical signs are: stranguria, pollakiuria, haematuria, dysuria, urinary incontinence or a combination of them. They are typical from lower urinary tract problems, mimicking bacterial cystitis, urolithiasis or lower urinary tract infection [4]. Less frequently, lameness

may be present due to either bone metastases in extremities, spinal cord metastases or paraneoplastic hypertrophic osteopathy in pulmonary metastases of UC [1,5].

Diagnosis

Diagnostic approach should ideally include: physical examination and history, complete blood count, serum chemistry profile and urinalysis, including culture and sensitivity tests. Abdominal ultrasonography is recommended to investigate the bladder and urethra. After the demonstration of an urethral or vesical mass, the nature of the mass and a complete staging (using tumour, lymph node and metastasis grading system) should be performed. For staging, it is recommended: thoracic radiographs, abdominal imaging (either ultrasonography or computerized tomography) and urinary tract imaging [1,4].

Histopathologic exam usually gives a definitive diagnosis of UC. In difficult cases, confirmation of urothelial origin could be made with immunohistochemistry for uroplakin III and GATA-3. Biopsied tissue may be obtained by cystotomy, cystoscopy and traumatic catheterization [1]. Cytology may provide a UC diagnosis with different degree of certainty, depending on findings and representativity of the sample. In a study with 118 feline TCC, a definitive diagnosis was obtained by cytology without histopathology in 56 of 78 cases were cytology was performed [2]. Other molecular tests, like BRAF detection (proto-oncogene B-Raf), allow a diagnosis of UC [6,7].

Urinary sediment

Sediment exam should be exhaustive, since occasionally, tumoral cells can be seen on it. However, cytological specimens obtained from urine samples hardly ever allow a final diagnosis of neoplasia [8]. When epithelial cells are seen in high quantity, a dry-mount urine sediment preparation is recommended. Besides, cells usually degenerate when are storage in fluids, specially in urine, due to its toxic characteristics [9]. In order to prepare dry-mount samples, we can use line concentration technique, or preferably, cytocentrifugation or extensions of urine sediment avoiding supernatant [8,9].

Acquiring cytological specimens

Cytological smears could be sampled directly from a mass by traumatic urethral catheterization, ultrasound-guided fine needle aspiration (FNA) and touch imprint of surgical [1,8,9] or cystoscopic [1] biopsy. Independently the location of a mass, when FNA is used to sampling, is recommended to recover tissue from central and peripherical areas. If fluid was recovered, it could be smeared and the exceeding fraction kept in an EDTA tube to avoid coagulation [8].

Aspiration of a lower urinary tract mass through the abdominal wall is a highly controversial procedure. Needle tract implantation of tumoral cells after FNA of TCC have been reported in dogs as a rare but serious complication [10,11]. The use of traumatic catheterization when possible is recommended, but percutaneous FNA should be done if catheterization of urethra is not possible [10]. Dogs with TCC located in abdominal wall have a poor prognosis, with a median survival time of only 57 days. Although this is a rare event, Higuchi., *et al*. report this phenomenon in 24 of 544 (4.4%) of their TCC cases, and they recommend to avoid percutaneous aspiration of these masses [12]. Important internal medicine and oncological textbooks recommend avoid this procedure [1,4] and catheterization of bladder and prostatic washes are preferred [3]. At the same time some cytological textbooks consider that FNA biopsy can continue being the best method for acquiring tissue-associated cells, maximizing the cytologic exam. Traumatic urethral catheterization usually extracts superficial cells and leads to a false negative diagnosis [8].

Cytological diagnostic features

Cytologic features in UC include exfoliation in different patterns: laminar sheets, small clusters or isolated large cells. The cellular shape varies from roundish, cubic, polygonal or even fusiform. In general, transitional cells have a relatively low nuclear-to-cytoplasmic ratio, which sometimes can be increased as malignancy indicator. UC are typically pleomorphic on cytology (Figure 1), with evident malig-

nant criteria, including moderated-to-marked anisocytosis and anisokaryosis, pleomorphic nuclei and prominent nucleoli with variability in their size, shape and number [3,8]. Nuclei usually contain most of the features that allow to make a malignant interpretation. They have been described to be up to 5-10-fold larger than in normal urothelium [13]. Marked basophilia, coarse chromatin pattern, multinucleation and eosinophilic cytoplasmic inclusions, called Melamed-Wolinska bodies, may also be present [3,8].

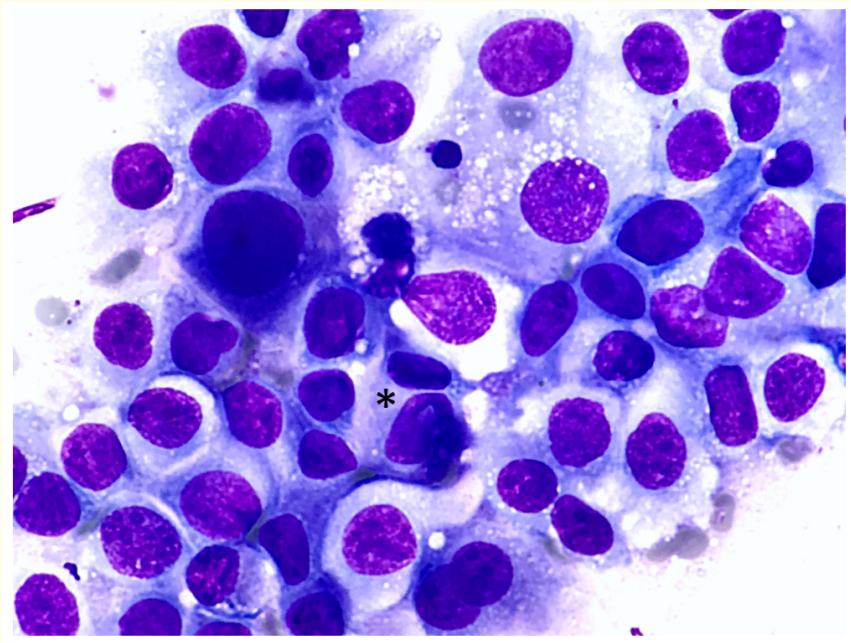

Figure 1: *Micrograph of cytological smear recovered by traumatic catheterization. Moderate-to-marked anisokaryosis, variable cell pleomorphism and mild nuclear moulding (asterisk). (Diff-Quick stain, x100 objective, original magnification).*

Melamed-Wolinska bodies (Figure 2-4) are variable size, oval-to-round, homogeneous or granular, eosinophilic structures. They are seen in urothelial cells, either benign or malignant. Usually one but sporadically more Melamed-Wolinska bodies are seen by cell. Their exact meaning is not well understood, but Arya., *et al*. suggested that may reflect a degenerative change of urothelial cells [14]. Their presence in specimens out of the urinary tract, should increase the possibilities of being a metastatic UC. In 1961, Melamed and Wolinska were two first describers after their study with 500 urinary sediment smears. But they did not find any association between the inclusions and any disease [15].

They should be differentiated from other eosinophilic inclusions (either nuclear or cytoplasmic), that occur in urothelial cells under viral infections or metal intoxications, like lead. Nowadays, their nature is still controversial. In first studies, they were suggested to contain mucopolysaccharides, while other works support that they are enlarged lysosomes due to cellular degeneration [14]. Since their morphology could sometimes resemble erythrophagocytosis, some studies tried to show immunoreactivity to erythroid membrane antigens (GLUT-1 and Glycoprotein-C). It could not be demonstrated and erythroid origin has been excluded [16].

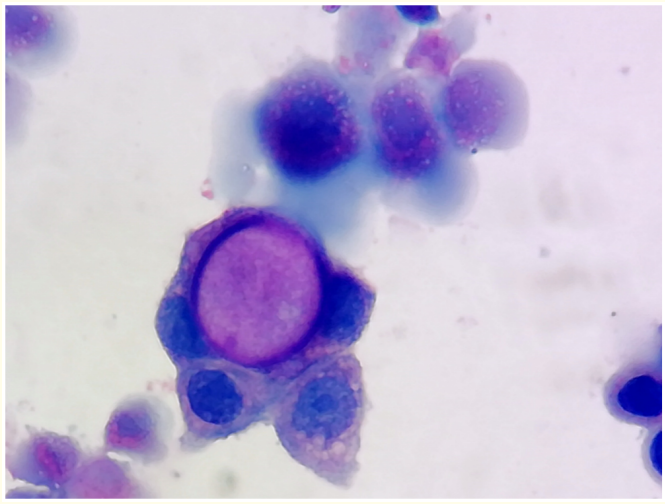

Figure 2: *Micrograph of cytological smear obtained by traumatic catheterization, showing a Melamed-Wolinska body, prominent and large nucleoli (cells on top of the image), and moderate-to-marked anisonucleoliosis, comparing with smaller nucleoli of cells on bottom of the image. (Modified Romanowsky stain, x100 objective, original magnification).*

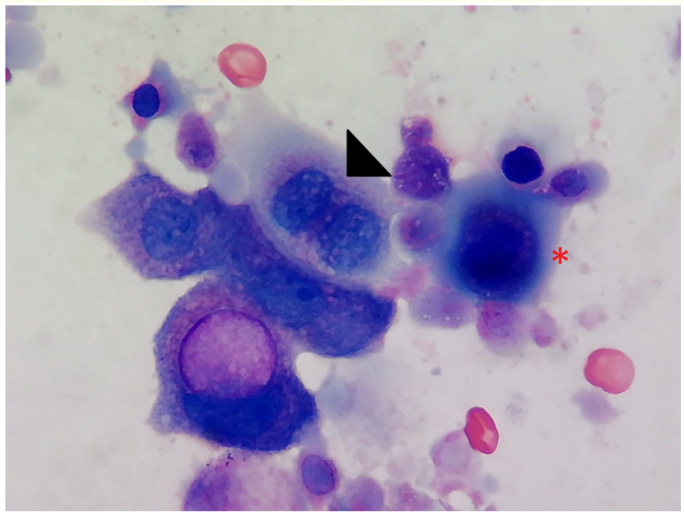

Figure 3: *Micrograph of cytological smear obtained by traumatic catheterization, showing a Melamed-Wolinska body, prominent, large nucleoli (red asterisk), apparently larger than a RBC diameter, anisokaryosis, anisonucleoliosis and binucleated cell (black arrowhead). (Modified Romanowsky stain, x100 objective, original magnification).*

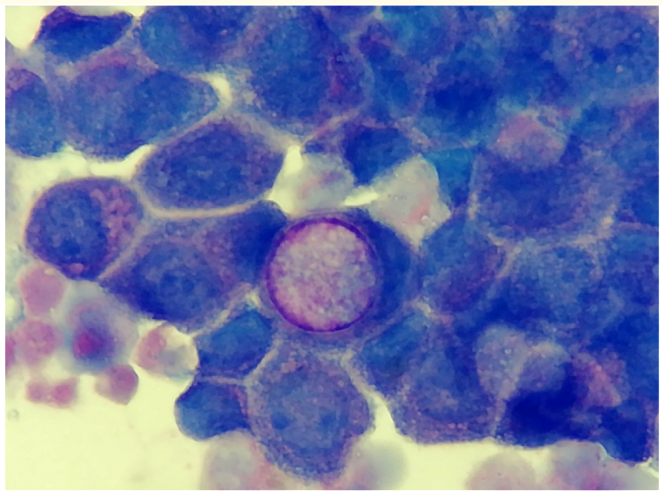

Figure 4: *Micrograph of cytological smear obtained by traumatic catheterization, showing a Melamed-Wolinska body, intense degree of cytoplasmic basophilia and anisokaryosis. (Modified Romanowsky stain, x100 objective, original magnification).*

There is a diagnostic challenge when atypical urothelial cells are seen together with inflammatory cells. Hyperplastic transitional epithelium with dysplastic changes secondary to inflammation in the bladder, and UC or TTC accompanied by inflammation, are the two possible explanations [3]. Other tests and the response to antibiotherapy, could help on the interpretation of each specific clinical case.

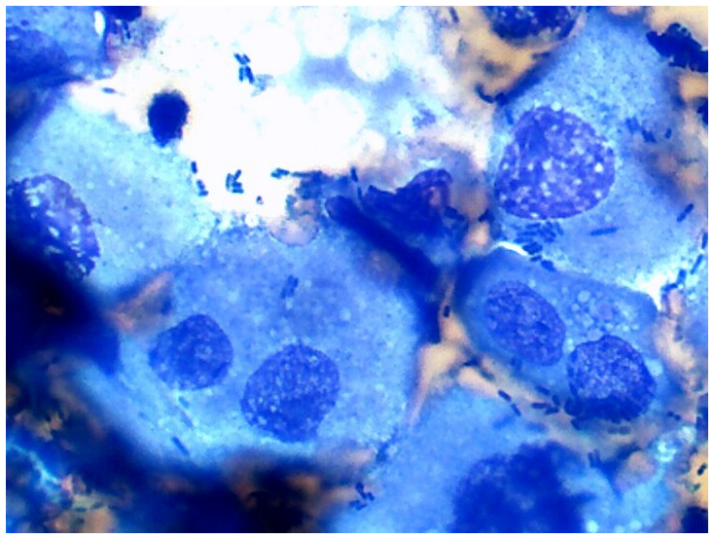

Figure 5: *Micrograph of cytological smear of a dry-mount urinary cytocentrifugated sediment from a dog. Epithelial cells show moderate atypia. Many rod-shaped bacteria are present. (Modified Romanowsky stain, x100 objective, original magnification).*

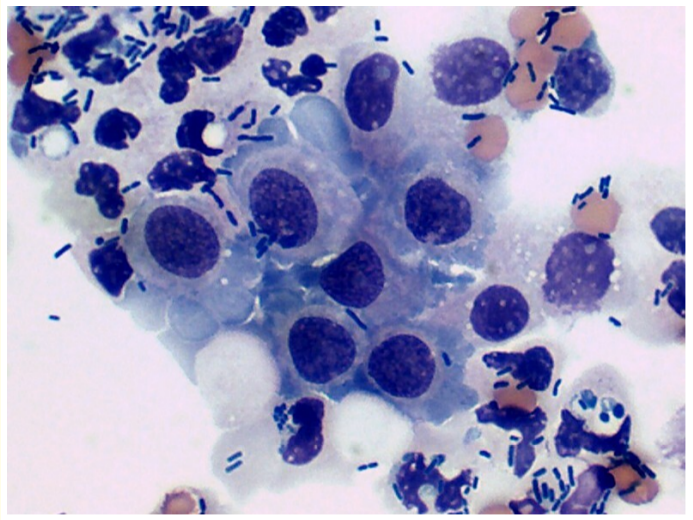

Figure 6: *Micrograph of cytological smear of a dry-mount urinary cytocentrifugated sediment. Same case that figure 5. Marked septic neutrophilic inflammation and mild epithelial dysplasia. Rod-shaped bacteria are present on the background and inside degenerated neutrophils. (Modified Romanowsky stain, x100 objective, original magnification).*

Molecular tools

Bard® BTA (bladder tumour-associated antigen) test has been tested for screening purposes in canine UC, although was developed for human TCC. It showed 90% and 78% sensitivity and specificity respectively. This relatively high sensitivity allows to rule out UC in old patients or patients with clinical signs of lower urinary tract. False positives were seen with haematuria, proteinuria and glucosuria [17].

A veterinary version of previous test (V-BTA) was created with similar results. It discriminates lower tract affected cases from non-affected dogs. Again, the high sensitivity makes this test useful as screening test, but not recommended as definitive diagnosis for UC [18,19].

Proto-oncogene BRAF is present in about 85% of TCC. It was determined after studied the prevalence of the BRAF variant V595E. It has 70% and almost 100% of sensitivity and specificity respectively. This test could be performed on samples of different nature, including: urine, urinary sediment, fine needle aspirates, or biopsy specimens. The only requirement is to have enough cells of the neoplasm. This test helps to confirm uncertain UC cases but the absence of the mutation cannot rule out UC. It constitutes a recent, early detecting, minimally invasive, diagnostic tool for UC [6,7].

Conclusion

Cytology may continue being an important tool on diagnosis of UC. It is a simple, widely used method, with potentially many different sampling techniques. However, a proper knowledge of its limitations is required, and the use of other diagnostic tools is sometimes recommended, depending on the specific necessities and limitations of each particular case.

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