Review Article

NEOPLASTIC DISEASE

Short Title: Standards for Reporting Canine Prostatic Epithelial Lesions

Histopathological Terminology Standards for the Reporting of Prostatic Epithelial Lesions in Dogs

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Summary

The terminology applied to canine prostatic epithelial lesions, especially carcinomas, is currently not standardized and this hampers the ability of pathologists to study the biological and clinical significance of these lesions. The aim of this review is to present the essential histomorphological diagnostic attributes of a wide spectrum of prostatic epithelial lesions in dogs. In addition to the traditionally recognized prostatic hyperplasia, hormonal atrophy, prostatitis, squamous metaplasia, adenocarcinoma and transitional cell (urothelial) carcinoma, new entities are described and discussed in order to provide veterinary pathologists with a basic atlas of common histological lesions of the canine prostate that is comprehensive and easy to use.

Keywords: dog; prostate; histopathology; diagnostic standards

Introduction

Five veterinary pathologists (CP, RF, VG, GAW and RLA) and one oncologist (CEFA) with a common interest and expertise in canine prostatic disorders convened at the School of Veterinary Medicine and Animal Science, São Paulo State University, Botucatu, Brazil, in May 2018 in conjunction with the International Symposium on Animal Models for Translational Research–Canine Prostate Cancer. This group meeting was held in order to develop a set of histopathological standards for the surgical pathology reporting of prostatic epithelial lesions in dogs. One member of the subgroup (CP) assembled an archive of 87 scanned slides retrieved retrospectively from the collections of the University of Queensland

and the São Paulo State University. All slides were reviewed by the members of the group with the contribution of a human urological pathologist (AMDM) and a molecular biologist (HME). The current recommendations present the outcome of this meeting and are intended as an informative and educational resource rather than a mandate. The intention is to provide veterinary pathologists with a minimum dataset and guidelines that are comprehensive, easy to use and in keeping with local capacities and practice.

Prostatic Hyperplasia

Prostatic hyperplasia (PH) is a spontaneously arising disease of entire male dogs that begins as glandular hyperplasia as early as 2–3 years of age (Brendler *et al.*, 1983). With time, almost all entire dogs will develop PH, with >95% affected by 9 years of age (Gobello and Corrada, 2002). Most will not show any clinical signs until the hyperplastic gland grows large enough, generally late in life (Sun *et al.*, 2017).

As it is not considered to be a precursor to prostate cancer and therefore lacks malignant potential (Foster, 2016), the term PH is preferred, rather than the traditionally applied term of benign PH (BPH), with further classification into glandular and complex hyperplasia (Berry *et al.*, 1986).

In glandular PH, there is normal architecture with an increase in the amount of secretory epithelium. The alveoli are larger and contain more elaborate papillary projections than in the normal gland. The glandular proliferation usually occurs in all portions of the gland and the amount of stroma is relatively less than in the normal gland (Fig. 1A). Complex PH contains areas of nodular glandular hyperplasia and often is admixed with foci in which the secretory epithelium is atrophic and attenuated. In the atrophic areas, there is a mild to moderate increase in the stroma. Some of the alveoli are dilated and cystic, lined by

columnar to small cuboidal cells without obvious morphological evidence of secretory activity. Chronic inflammation is common and occasional squamous metaplasia may be present (Fig. 1B).

Prostatic Inflammation

A pathologically significant prostatitis occurs when large numbers of neutrophils with variable numbers of macrophages, lymphocytes and/or plasma cells are seen within the acini (intra-acinar) (Fig. 2A) and/or in the interstitium (interstitial) (Figs. 2B, 2C), with or without fibrosis. However, prostatitis may be observed even in dogs with no clinical signs (Smith, 2008; Palmieri *et al.*, 2014), eventually in association with glandular or complex PH, as focal or multifocal interstitial aggregates of small numbers of lymphocytes and plasma cells and should be reported as an incidental finding.

Non-invasive Proliferative Epithelial Lesions

Prostatic intraepithelial neoplasia (PIN) is considered to be the precursor of most cases of human prostate carcinoma (Montironi *et al.*, 2011; Trabzonlu *et al.*, 2019). This focal lesion is, histologically, a proliferation of epithelial cells with cytological atypia such that secretory or ductal cells are enlarged with an increased nuclear:cytoplasmic (N:C) ratio and prominent nucleoli. Nuclear crowding and stratification are observed commonly (Bostwick and Qian, 2004). These non-invasive proliferative lesions are described in dog prostates with carcinoma and may be observed rarely in histological specimens (Waters and Bostwick, 1997a, b; Waters *et al.*, 1997; Aquilina *et al.*, 1998; Waters, 1999; Bostwick *et al.*, 2000; Madewell *et al.*, 2004; Matsuzaki *et al.*, 2010). The authors are unaware of any publications that confirm these lesions as true precursor lesions, rather than reactive nuclear atypia with

prominent nucleoli that is secondary to acute or chronic inflammation of the prostatic gland. In cases where the lesions are in close proximity to invasive carcinoma, it can be impossible to distinguish them from retrograde colonization of benign ducts/acini by invasive adenocarcinoma (Haffner *et al.*, 2016). Further immunohistochemical and molecular characterization of such canine lesions is required, as is reported for man. Therefore, according to presence or absence of a concurrent inflammatory reaction, these lesions should be classified as: (1) reactive nuclear atypia (with inflammation) (Fig. 3A), (2) epithelial dysplasia/atypical hyperplasia (without inflammation) (Fig. 3B).

Prostatic Atrophy

Two different atrophic lesions may be recognized in the prostate gland of entire and neutered dogs: (1) atrophy post-neutering or secondary to dysfunctional testes (e.g. hormone dysfunction, testicular degeneration or atrophy), called hormonal atrophy (Lai *et al.*, 2008); (2) atrophy with chronic inflammation similar to the proliferative inflammatory atrophy (PIA) described in man (De Marzo *et al.*, 1999; van Leenders *et al.*, 2003; Palmieri *et al.*, 2014, 2018;).

Hormonal 'atrophy' is a lack of development of prostatic acini in dogs neutered before puberty, or true atrophy in dogs neutered or given antiandrogenic therapy after puberty. In advanced atrophy, only tubular structures with the appearance of a single lining of epithelial cells remain in the prostate, so it is hard to differentiate ducts from atrophic acini (Fig. 4A).

Atrophy with lymphocyte- and plasma cell-rich inflammation occurs in entire dogs and those neutered after puberty as focal or multifocal interstitial aggregates between normal or hyperplastic glands. These atrophic lesions may be found occasionally in biopsy samples

of prostates with prostatic carcinoma. The key identifying feature of focal/multifocal prostatic atrophy is recognized at low power and consists of an overall hyperchromatic appearance of the involved glands. The atrophic acini contain two layers of epithelial cells. The luminal cells appear as attenuated cuboidal secretory-type cells with reduced amount of cytoplasm compared with the normal epithelium, and many of them are considered as 'intermediate cells', which also occur in human focal atrophy (Palmieri *et al.*, 2018). The basal cells are similar to those observed in the normal epithelium. Some atrophic acini may be so attenuated as to appear to have only one layer of markedly flattened epithelial cells (Fig. 4B).

In both cases, the histological diagnosis should take into account the history, including neutering status and time of neutering, as well as the assessment of testes or hormonal profiles and the concurrent presence of complex hyperplasia.

Prostatic Carcinoma

Until further definition of a standardized immunohistochemical diagnostic panel, three general types of carcinoma of the prostate are identified at the histological level: (1) prostatic urothelial carcinoma (UC); (2) prostatic adenocarcinoma (AC); and (3) prostatic carcinoma with mixed urothelial and glandular phenotypes (i.e. when prostatic carcinoma shows mixed morphological features within the same tissue section).

UC arises from the prostatic urethra or the prostatic ducts near the urethra. This location is helpful in differentiating this type from adenocarcinoma. It consists of neoplastic cells arranged in solid, papillary or cribriform patterns. Most UCs are composed of a heterogeneous cell population. The cell types range from a small polyhedral cell with a high N:C ratio, a round, hyperchromatic nucleus, and scant eosinophilic cytoplasm to large cells with low N:C ratio, abundant eosinophilic cytoplasm and round to oval vesicular nuclei.

Mitoses may be numerous. Characteristic cytoplasmic vacuoles (Melamed–Wolinska bodies), that may be empty or contain homogeneous or stippled eosinophilic material, may be observed (Figs. 5A, 5B).

AC arises from the glandular epithelium and consists of neoplastic cells with small to moderate amounts of eosinophilic cytoplasm, moderate cellular pleomorphism and moderately vesicular to hyperchromatic nuclei. In the World Health Organization (WHO) classification, prostatic carcinomas are classified as adenocarcinoma (intra-alveolar and acinar) and poorly differentiated carcinoma (Kennedy *et al.*, 1998). However, canine prostate cancer displays a high degree of morphological heterogeneity in terms of number and combinations of growth patterns and different histological types have been described (Bell *et al.*, 1991; Waters *et al.*, 1998; Cornell *et al.*, 2000; Lai *et al.*, 2008; Palmieri *et al.*, 2014). Therefore, ACs should be further classified as: (1) simple tubular (replacing the small acinar/ductal type), with neoplastic cells forming small tubules with a focal/multifocal distribution or infiltrating and replacing the stroma (Fig. 5C); (2) papillary, with neoplastic cells forming delicate papillary projections within an extended duct (Fig. 5D); (3) Cribriform, with tumour cells completely filling the lumen with the formation of regular fenestrae, often accompanied with central necrosis (comedonecrosis) (Fig. 5E); or (4) Solid (poorly differentiated), with pleomorphic tumour cells arranged as solid nests or sometimes individual cells within the stroma; tumour cells may be occasionally spindle-shaped (Fig. 5F).

Interestingly, all of these patterns can be found in human prostatic adenocarcinomas: tubular is most similar to the acinar adenocarcinoma with a Gleason pattern 3+3 (discrete, well-formed variably sized glands), papillary is similar to ductal adenocarcinoma and cribriform carcinoma is similar to human cribriform carcinoma and intraductal carcinoma with comedonecrosis (Humphrey, 2003; Humphrey *et al.*, 2016).

Additional histological findings that may be observed in prostatic carcinomas are: (1) perineural invasion, which is a hallmark of human prostatic carcinoma (Humphrey, 2003) and a recognized mechanism whereby cancer cells spread beyond the prostate by using the rich innervation of the posterior aspect of the prostate (Villers *et al.*, 1989). Histologically, different patterns may be observed, including: 'crescentic applications' (Fig. 6A), intraneural invasion, 'tracking' along a longitudinally sectioned nerve or circumferential extension around a nerve (Fig. 6B); (2) squamous differentiation (Fig. 6C); or (3) lymphatic/vascular invasion (Fig. 6D).

Recommendations on Collection of Representative Samples from Post-mortem and Prostatectomy Cases

In the case of prostate carcinoma, tissue samples collected during post-mortem examination and after prostatectomy should include the mass (sample 1) and the urethra/periurethral region (sample 2) in order to have representative material for evaluating the morphological features of the tumour and its relationship to the prostatic urethra. In case of prostatic hyperplasia or prostates without grossly detectable lesions, sectioning should include at least three samples (middle, cranial and caudal ends). This will allow the evaluation of microscopical lesions, such as tubular adenocarcinoma, atrophy and epithelial changes as described in the previous sections.

Conclusions

In summary, this overview and recommendation on nomenclature presents a practical histopathological approach to the diagnosis of prostatic epithelial lesions, especially carcinoma, in dogs. It can be used by diagnostic pathologists, pathology residents and

oncologists and researchers interested in diseases of the prostate (Table 1). The spectrum of prostatic disorders in dogs is wider than originally thought and encompasses histological lesions with different pathogenesis and behaviour with potential prognostic significance. The existing WHO classification system for canine prostate cancer was published in 1998. Twenty years later, there has been little progress in refining this basic classification, with scattered sporadic attempts, and the exact cell-of-origin (urothelium versus duct versus gland) is still debated. Our recommendation is an admission that there is much to learn about the histogenesis and behaviour of these tumours, starting from a reliable classification system, preferably one based simply upon morphology using haematoxylin and eosin stained sections. A more detailed system will depend on future immunohistochemical and molecular studies. This system should facilitate future studies designed to address the lack of a structured pathology report with standardized definition for each component that may enhance the completeness and quality of data provided to the clinicians, as well as facilitate the comparison of studies from different groups of researchers. We suggest that the histological lesions outlined in this paper be used for such studies, as well as in diagnostic settings. Because there is no known prognostic difference between the neoplasms described, we do not suggest that pathologists routinely comment on and/or quantify them. For those pathologists that wish to do so, however, we would recommend using these guidelines.

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Conflict of Interest Statement

The authors declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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Figure Legends

Fig. 1. Prostatic hyperplasia in dogs. (A) Glandular hyperplasia: increased amount of secretory epithelium in an otherwise normal prostate. HE. Bar, 120 μm. (B) Complex hyperplasia: dilated and cystic alveoli admixed with multifocal foci of atrophic epithelium and increased stroma. HE. Bar, 240 μm.

Fig. 2. Prostatic inflammation in dogs. (A) Focal small aggregate of lymphocytes and plasma cells (incidental finding). HE. Bar, 60 μm. (B) Pathologically significant prostatitis with accumulation of high numbers of neutrophils within dilated acini and infiltration of low numbers of mixed inflammatory cells in the interstitium. HE. Bar, 60 μm. (C) Pathologically significant prostatitis with diffuse infiltration of high numbers of neutrophils, macrophages, lymphocytes and plasma cells within the acini and the interstitium. HE. Bar, 60 μm.

Fig. 3. Non-invasive proliferative epithelial lesions of the canine prostate. (A) Reactive nuclear atypia with inflammation. HE. Bar, 60 μm. (B) Epithelial dysplasia/atypical hyperplasia without inflammation. HE. Bar, 30 μm.

Fig. 4. Prostatic atrophy in dogs. (A) Advanced hormonal atrophy: tubular structures lined by a single layer of epithelial cells. HE. Bar, 120 μm. (B) Atrophy with inflammation: focal aggregate of atrophic glands associated with infiltration of lymphocytes and plasma cells into the interstitium. HE. Bar, 60 μm.

Fig. 5 Prostatic carcinoma in dogs. (A) Prostatic urothelial carcinoma arising from the prostatic urethra. HE. Bar, 240 μm. (B) Prostatic urothelial carcinoma with a cribriform pattern and central necrosis. HE. Bar, 120 μm. (C) Simple tubular prostatic adenocarcinoma. HE. Bar, 30 μm. (D) Papillary prostatic adenocarcinoma. HE. Bar, 240 μm. (E) Cribriform prostatic adenocarcinoma with central necrosis. HE. Bar, 120 μm. (F) Solid poorly differentiated prostatic adenocarcinoma. HE. Bar, 60 μm.

Fig. 6. Ancillary histological findings of canine prostatic carcinoma. (A) Perineural invasion with a crescentic application pattern characterized by neoplastic tubules forming small crescents intimately associated with nerves. HE. Bar, 60 μm. (B) Perineural invasion with circumferential growth of neoplastic cells around a nerve. HE. Bar, 60 μm. (C) Squamous differentiation of neoplastic cells. HE. Bar, 60 μm. (D) Neoplastic emboli within lymph and blood vessels (lymphatic/vascular invasion). HE. Bar, 30 μm.

Table 1

Summary of the recommendations for the histological interpretation of canine prostatic lesions

Prostatic hyperplasia:

1. Glandular
2. Complex

Prostatic inflammation:

1. Incidental
2. Predominant: intra-acinar and/or interstitial

Non-invasive proliferative lesions:

1. Without inflammation: epithelial dysplasia/atypical hyperplasia
2. With inflammation: reactive nuclear atypia

Prostatic atrophy:

1. Hormonal atrophy (post-neutering or secondary to dysfunctional testes)
2. Atrophy with chronic inflammation

Prostatic carcinoma:

1. Urothelial carcinoma: solid, papillary, cribriform (with and without necrosis)
2. Adenocarcinoma: intra-alveolar (papillary, cribriform with and without necrosis), simple tubular, solid
3. Carcinoma with mixed urothelial and glandular phenotypes

- Additional histological findings: perineural invasion, squamous metaplasia, lymphatic or vascular invasion

Others:

- Interpretation of prostatic changes in association with testicular changes (if any), history and neutering status
- Collection of representative samples from post-mortem and prostatectomy cases:
    1. In prostate carcinoma cases: two samples (tumour + urethra/periurethral region)
    2. In normal/hyperplastic prostates: three samples (cranial, middle, caudal)