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PROPOSAL OF GLEASON-LIKE GRADING SYSTEM OF CANINE PROSTATE CARCINOMA IN VETERINARY PATHOLOGY PRACTICE

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Abstract
Gleason grading - the most useful predictor of prognosis for prostate cancer in men - was updated at a 2005 consensus conference by the International Society of Urological Pathology. Since Gleason-like growth patterns have been recognised in dogs, this study aimed to apply the modified Gleason grading to 45 canine prostate carcinomas.

A single primary growth pattern was observed in 28 cases, a secondary pattern in 11 cases and a tertiary pattern in 6 cases. Cribriform, solid and small acinar/ductal were the most common primary, secondary and tertiary morphological patterns, respectively.

The highest Gleason score (GS10) was obtained in 46.7% of cases. Nine of 14 metastasizing cases were classified as GS10. Gleason pattern 5 was present in 33 of cases.

This study suggests that the modified Gleason grading, based on specific histological growth patterns existing in canine prostate carcinomas, may be accepted as a grading system for histopathology in the practice settings in order to complete the clinical assessment for the best management of the patient.

Keywords: dog, prostate, carcinoma, Gleason grading
Introduction

Human prostatic carcinomas (PCs) are graded by pathologists using the Gleason system (Gleason, 1966), which remains one of the most powerful prognostic indicators in PC (Humphrey, 2004; Young et al., 2000). An important feature of the Gleason grading is that it does not rely on detailed assessment of nuclear morphology, but it assigns numerical grades (1-5) based upon the architectural patterns of the tumour that are best evaluated at low power magnification. Patterns 1, 2 and 3 represent tumours that most closely resemble normal prostate gland, and patterns 4 and 5 are tumours showing increasingly abnormal glandular architecture (Gleason, 1966). A primary grade is then assigned to the most prevalent pattern, while the second most prevalent pattern is the secondary grade and the sum of these grades provides the overall Gleason score (GS). If there is only one pattern, its grade is simply doubled to reach the score (Gleason, 1966).

PC has changed dramatically since the late 1960s from the clinical, diagnostic and therapeutic point of view. In the 1960s, serum prostate-specific antigen (PSA) had not yet been discovered and there was no screening for PC other than by digital rectal examination. The method of obtaining prostate tissue was also different and radical prostatectomy relatively uncommon. New variants and patterns of PC have also been described since the original grading system. Finally, clinical outcomes have also changed over the past several decades (Shah and Zhuo, 2012). Therefore in 2005, the International Society of Urological Pathology (ISUP) proposed a modified Gleason system in response to evolving clinical practice and understanding of prostate cancer pathology (Epstein et al., 2005). In this modified system, certain patterns originally considered as Gleason pattern 3 are now graded as pattern 4 (e.g. ill-defined glands or cribriform glands with irregular borders). Prostatic cancers with a Gleason score of 2-4 (i.e. patterns 1 and 2 occurring either alone or in combination) should rarely, if ever, be diagnosed on needle biopsies. In practice, Gleason score starts from 3+3 = 6. In addition, the ISUP consensus also recommended that the cancer in the needle biopsy be graded with the most common Gleason pattern as the primary pattern and the highest scoring Gleason pattern as the secondary pattern,
accounting for the potential presence of a tertiary most prevalent pattern, not included in the original Gleason grading system (Epstein et al., 2005; Shah and Zhuo, 2012).

Recently, in dog prostate cancers, we have recognized patterns of growth corresponding to those described by Gleason in human prostate cancer (Palmieri et al., 2014). The mixture of growth pattern we have seen in canine PC closely resembles the mixture of growth pattern seen in human androgen refractory prostate cancer, whose majority of cases showed a mixture of Gleason grades 4 and 5 (Shah et al., 2004). Since canine PC shares several similarities with human PC and may serve as a valuable model for human prostate cancer, this study aims to apply the modified Gleason grading system to score canine prostate carcinomas.

Materials and methods
Histologic evaluation, classification and grading
This study cohort included 45 formalin-fixed, paraffin-embedded samples of canine prostatic carcinoma retrieved from the archives of the School of Veterinary Science – Diagnostic Pathology Service of the University of Queensland and the Department of Veterinary Science and Public Health, the University of Milan. Specimens were represented by tissue samples collected during necropsy (n = 20), prostatectomy (n= 4) or biopsy (by ultrasound or exploratory laparotomy; n = 20). In one case, the information about the type of sample was not available. Clinical data were obtained from the histological reports. Prostatic samples were collected from dogs with the following clinical signs: haematuria (3/20 biopsies; 3/20 necropsies; 1/4 prostatectomy), tenesmus (1/20 biopsies; 2/20 necropsies), hind limb weakness (1/20 biopsy; 4/20 necropsies), enlarged prostate (14/20 biopsies; 6/20 necropsies), dysuria (7/20 biopsies; 1/20 necropsy), anuria (2/20 biopsies), abdominal pain (1/20 necropsy), and stranguria (1/20 biopsy; 5/20 necropsies; 1/4 prostatectomy). No history was available in 12 cases.

Five-micron-thick sections were stained with haematoxylin and eosin (H&E) for the histopathological examination.
The classification of prostate cancer was based on the human WHO classification of Tumors of the Urinary System and Male Genital Organs (Eble et al., 2004) and growth patterns recently described in dogs (Palmieri et al., 2014).

Gleason grading was performed according to the 2005 ISUP modified Gleason grading system (Table 1, Fig. 1) (Epstein et al., 2005). The architectural patterns observed in our samples according to the modified Gleason grading system are illustrated in Figures 2, 3 and 4. Gleason score was obtained by adding the primary and secondary grades together. A tertiary pattern higher than the primary and secondary grades has been included in the final Gleason score as the secondary grade. This means that if a biopsy contained multiple patterns with 3, 4 and 5 in various proportions and pattern 5 being the least prevalent (tertiary grade), then the Gleason pattern 5 component was upgraded to a secondary grade before assigning the final Gleason score. The component of intraductal spread (cribriform or papillary) was counted as Gleason pattern 5 if it contained intraluminal comedonecrosis. Any amount of Gleason pattern 5 (even <5% of PC), as long as recognisable at low-power examination, was considered significant and included for analysis.

Prostate carcinomas were initially graded at low magnification using 4x or 10x lens. The grades were verified at 20x lens.

Results

Twenty-eight carcinomas were characterised by a single growth pattern (primary grade) and classified as follows: solid (9 out of 28), small acinar/ductal (8 out of 28), cribriform with (5 out of 28) and without comedonecrosis (4 out of 28), and papillary with (1 out of 28) and without comedonecrosis (1 out of 28).

A primary and secondary grade was observed in 11 out of 45 samples, while a tertiary grade was observed in 6 out of 45 cases. In one case, the tertiary grade(solid, pattern 5) was upgraded to a secondary grade.

The most common primary grades were, in decreasing order of prevalence, cribriform with (11 out of 45) and without (6 out of 45) comedonecrosis, solid (13 out of 45), small
acinar/ductal (10 out of 45), papillary with (1 out of 45) and without comedonecrosis (2 out of 45), mucinous (1 out of 45), and signet ring (1 out of 45).

The most common secondary grades were, in decreasing order of prevalence, solid (5 out of 17), small acinar/ductal (5 out of 17), papillary with (1 of 17) and without (3 out of 17) comedonecrosis, cribriform with (1 out of 17) and without (1 out of 17) comedonecrosis, and signet ring (1 out of 17).

Four types of tertiary grades were observed, specifically small acinar/ductal (3 out of 6), cribriform (1 out of 6), papillary (1 out of 6), and solid (1 out of 6).

Seven (15.6%) dogs were classified as Gleason score 3+3 = 6; 2 (4.4%), 4 + 3 = 7; 7 (15.6%), 4+4 = 8; 2 (4.4%), 5 + 3 = 8; 4 (8.9%), 5 + 4 = 9; 2 (4.4%), 4 + 5 = 9; and 21 (46.7%), 5 + 5 = 10. Fourteen prostatic carcinomas collected during necropsy were associated with metastases in the lumbar skeletal muscle and adipose tissue (1 out of 14), intestinal serosa (1 out of 14), sublumbar andinguinal lymph nodes (6 out of 14), mesentery (2 out of 14), spleen (1 out of 14), lung (6 out of 14), humerus (1 out of 14), peritoneum (1 out of 14), diaphragm (1 out of 14), liver (2 out of 14), mediastinum (2 out of 14), kidney (1 out of 14). Nine metastasising cases were classified as Gleason score 10; 2 as Gleason score 9 and one each as Gleason score 8, 7, and 6.

Gleason score in relation to the type of sample collection is summarised in Table 2. The most common score observed in tissue collected during necropsy (65% of cases) and prostatectomy (50% of cases) was Score 10, while Score 8 in biopsy samples (30% of cases).

Gleason pattern 5 was present in 33 of all prostate cases.

The distribution for Gleason pattern 5 was as follows: primary component (n = 24), secondary component (n = 8), and tertiary component (n = 1).

The following morphologic subpatterns of Gleason pattern 5 were observed: comedocarcinoma (papillary or cribriform), solid sheets, and signet ring.

Gleason pattern 5 subpatterns and its relation to distribution are summarized in Table 3.
Discussion

Prostate biopsy and histopathological assessment are the key steps in PC diagnosis in both humans and dogs and PC grading may be essential for an appropriate treatment decision-making also in the canine species, especially when used at an early stage. In this study, we have demonstrated that canine prostatic carcinoma may show variable morphological features and Gleason-like growth patterns that would aid in the acceptance of the modified Gleason score as a grading system for histopathology obtained from prostate biopsy or radical prostatectomy (RP).

As in humans, in most canine PC more than one histological pattern is present and may be assigned to a primary, secondary or tertiary grade. In men, the presence of a tertiary grade is associated more frequently with Prostate Specific Antigen (PSA) recurrence, extraprostatic extension, surgical margin positivity, seminal vesicle infiltration and lymph node metastases (Delahunt et al., 2012). In our cases, the tertiary grade was observed in 6 cases and associated with the highest GS (GS10) in 4 out of 6. Therefore, the presence of a tertiary grade may be a marker of more aggressive disease, although the limited number of cases coupled with the lack of follow-up information hindered a correct prognostic assessment.

As expected due to the aggressive biological behaviour of canine PC, the most common GS reported in our study is 10 (5+5) and the highest GS was observed in metastasising PCs. In men, GS7 PC is the most commonly diagnosed cancer in both needle biopsy and RP specimen when using the modified Gleason grading system (Huang et al., 2014). This not represents a true discrepancy since canine PC is similar to the late stage, androgen-independent human PC, which is usually associated with a GS 9 to 10. Men with Gleason score 9 to 10 on biopsy have a significantly worse prognosis than men with Gleason score 8 or less in terms of biochemical recurrence (Pierorazio et al., 2013).

Regarding sample collection, the highest GS has been observed in necropsy or prostatectomy specimens, although for the prostatectomy follow-up, only a subset of all needle core cases was studied as only a minority of the patients had undergone radical
prostatectomy. The Gleason score of biopsy, prostatectomy specimens or samples obtained from necropsy may be not the same for several reasons. Borderline grades (tumours displaying features that are intermediate between two gleason score) and pathological errors are possible explanations (Montironi et al., 2005). Most frequently, a sampling error (i.e. when a higher grade is missed on biopsy) is the most likely, since only a very small amount of the total prostate tissue is sampled for histological analysis during biopsy. Further techniques to improve PC detection rate and GS accuracy should be introduced in dogs, one of which is increasing the number of biopsy samples.

Our study demonstrates that Gleason pattern 5 is a relatively frequent presentation in a contemporary practice setting. This pattern has been observed in 33 out of 45 cases, usually as a primary grade but also as a tertiary grade in one case.

In men, Gleason pattern 5 predicts a worse outcome compared with that in patients at high risk without pattern 5 in terms of biochemical recurrence, metastasis and cancer-specific death (Sabolch et al., 2011). Therefore, the diagnostic recognition of high Gleason patterns 4 and 5 is vital, as these are the patterns that constitute the most aggressive and potentially lethal prostate cancers (Bastian et al., 2006; Vira et al., 2008). To reflect its unique clinical significance, the 2005 ISUP modification of the Gleason grading system recommended upgrading of tertiary Gleason pattern 5 PC in prostate biopsies to a secondary Gleason pattern, regardless of how small the amount of Gleason pattern 5 might be (Epstein et al., 2005). The morphological subpatterns of Gleason pattern 5 PC in relation to its amount and pattern distribution should be systematically analysed since Gleason pattern 5 is most frequently missed by pathologists in consultation practice when it represented secondary or tertiary component of carcinoma (Fajardo et al., 2011). The most common morphological presentation associated with Gleason pattern 5 in our study is the solid undifferentiated carcinoma, followed by the cribriform/papillary subtype with comedonecrosis, similar to what is reported in humans (Fajardo et al., 2011; Shah and Tadros, 2014). Therefore, increased awareness of these morphologic presentations of Gleason pattern 5 is important to minimize interobserver diagnostic variability.
In conclusion, the recognition of Gleason-like growth patterns in canine prostatic carcinoma emphasises the variable morphological features showed by this type of tumour and the potential usefulness of the histopathological grading in the canine practice setting. The recent definition of the different histomorphological patterns of canine PC (Palmieri et al., 2014) – which veterinary pathologists should be aware of - represents the basic foundation to enhance the diagnostic recognition of high-grade patterns 4 and 5. The Gleason grading system should be used in all prostate tissue samples, including needle-core biopsies and prostatectomy specimens in order to improve the categorization of tumour features, the extent of glandular differentiation and the pattern of neoplastic growth. We suggest that once carcinoma is detected and the different morphological patterns recognized, the Gleason grading system may be potentially applied in the practice settings in order to complete the clinical assessment for the best management of the patient, assessing the potential for local cure and the risk for distant metastasis.

Conflict of interest
Conflicts of interest: none.

References


pattern distribution based on the modified Gleason grading system. Human Pathology 45, pp. 2263-2269.


Figures legend

Figure 1. Schematic diagram of modified Gleason grading system. Compared to the conventional system, most cribriform patterns and also poorly defined glands are included in pattern 4 (modified from: Epstein et al., 2005).

Figure 2. Two different architectural patterns of canine cribriform prostate carcinoma. a) Gleason pattern 4 consists of variable-sized, irregular cribriform glands, larger than normal benign glands. H&E. Magnification 200x. b) Gleason pattern 5 with cribriform glands containing central comedonecrosis. H&E. Magnification 200x.

Figure 3. Two different architectural patterns of canine small acinar/ductal prostate carcinoma. a) Gleason pattern 3 consisting of small discrete glands, more variable in size and shape than normal benign glands. H&E. Magnification 200x. b) Gleason pattern 4 with discrete yet poorly formed glands. H&E. Magnification 200x.

Figure 4. a) Gleason pattern 4 of papillary carcinoma. H&E. Magnification 100x. B) Signet-ring cell carcinoma (Gleason pattern 5). H&E. Magnification 200x. c) Gleason pattern 5 of solid carcinoma with solid sheets without glandular differentiation. H&E. Magnification 100x.
Figure 1
Figure 3A
Figure 3B
Figure 4C
### Table 1. The architectural patterns according to the modified Gleason grading system proposed by ISUP in 2005 (modified from: Shah and Zhou, 2012)

<table>
<thead>
<tr>
<th>Gleason pattern</th>
<th>Key features</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Contemporary</strong></td>
<td><strong>Gleason pattern 1</strong></td>
</tr>
<tr>
<td><strong>Contemporary</strong></td>
<td><strong>Gleason pattern 2</strong></td>
</tr>
<tr>
<td><strong>Contemporary</strong></td>
<td><strong>Gleason pattern 3</strong></td>
</tr>
<tr>
<td><strong>Contemporary</strong></td>
<td><strong>Gleason pattern 4</strong></td>
</tr>
<tr>
<td><strong>Contemporary</strong></td>
<td><strong>Gleason pattern 5</strong></td>
</tr>
</tbody>
</table>
Table 2. Gleason score in relation to the type of sample collection

<table>
<thead>
<tr>
<th></th>
<th>Biopsy (n=20)</th>
<th>Necropsy (n=20)</th>
<th>Prostatectomy (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score 6</td>
<td>4 (20%)</td>
<td>2 (10%)</td>
<td>1 (25%)</td>
</tr>
<tr>
<td>Score 7</td>
<td>0</td>
<td>1 (5%)</td>
<td>0</td>
</tr>
<tr>
<td>Score 8</td>
<td>7 (35%)</td>
<td>2 (10%)</td>
<td>0</td>
</tr>
<tr>
<td>Score 9</td>
<td>3 (15%)</td>
<td>2 (10%)</td>
<td>1 (25%)</td>
</tr>
<tr>
<td>Score 10</td>
<td>6 (30%)</td>
<td>13 (65%)</td>
<td>2 (50%)</td>
</tr>
</tbody>
</table>
Table 3. Morphologic presentation based on distribution of Gleason pattern 5

<table>
<thead>
<tr>
<th>Architectural pattern</th>
<th>Primary pattern 5 (n=24)</th>
<th>Secondary pattern 5 (n=8)</th>
<th>Tertiary pattern 5 (n=1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comedocarcinoma</td>
<td>11 (45.8%)</td>
<td>2 (25%)</td>
<td>0</td>
</tr>
<tr>
<td>Solid sheets</td>
<td>12 (50%)</td>
<td>5 (62.50%)</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>Signet ring</td>
<td>1 (4.20%)</td>
<td>1 (12.50%)</td>
<td>0</td>
</tr>
</tbody>
</table>
Highlights
- We applied the new Gleason grading to canine prostate carcinomas
- Most cases obtained the highest Gleason score (GS10)
- As in humans, canine prostate carcinoma has different Gleason-like growth patterns
- Gleason grading may be potentially applied in the practice settings