

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

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28 Abstract

1 Gleason grading - the most useful predictor of prognosis for prostate cancer in men - was
2 updated at a 2005 consensus conference by the International Society of Urological
3 Pathology. Since Gleason-like growth patterns have been recognised in dogs, this study
4 aimed to apply the modified Gleason grading to 45 canine prostate carcinomas.
5 A single primary growth pattern was observed in 28 cases, a secondary pattern in 11 cases
6 and a tertiary pattern in 6 cases. Cribriform, solid and small acinar/ductal were the most
7 common primary, secondary and tertiary morphological patterns, respectively.
8 The highest Gleason score (GS10) was obtained in 46.7% of cases. Nine of 14
9 metastasizing cases were classified as GS10. Gleason pattern 5 was present in 33 of cases.
10 This study suggests that the modified Gleason grading, based on specific histological growth
11 patterns existing in canine prostate carcinomas, may be accepted th as a grading system for
12 histopathology in the practice settings in order to complete the clinical assessment for the
13 best management of the patient.

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15 Keywords: dog, prostate, carcinoma, Gleason grading

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23 Introduction

24 Human prostatic carcinomas (PCs) are graded by pathologists using the Gleason system
25 (Gleason, 1966), which remains one of the most powerful prognostic indicators in PC
26 (Humphrey, 2004; Young et al., 2000). An important feature of the Gleason grading is that it
27 does not rely on detailed assessment of nuclear morphology, but it assigns numerical grades
28 (1-5) based upon the architectural patterns of the tumour that are best evaluated at low

1 power magnification. Patterns 1, 2 and 3 represent tumours that most closely resemble
2 normal prostate gland, and patterns 4 and 5 are tumours showing increasingly abnormal
3 glandular architecture (Gleason, 1966). A primary grade is then assigned to the most
4 prevalent pattern, while the second most prevalent pattern is the secondary grade and the
5 sum of these grades provides the overall Gleason score (GS). If there is only one pattern, its
6 grade is simply doubled to reach the score (Gleason, 1966).

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

25 Recently, in dog prostate cancers, we have recognized patterns of growth corresponding to
26 those described by Gleason in human prostate cancer (Palmieri et al., 2014). The mixture of
27 growth pattern we have seen in canine PC closely resembles the mixture of growth pattern
28 seen in human androgen refractory prostate cancer, whose majority of cases showed a

1 mixture of Gleason grades 4 and 5 (Shah et al., 2004). Since canine PC shares several
2 similarities with human PC and may serve as a valuable model for human prostate cancer,
3 this study aims to apply the modified Gleason grading system to score canine prostate
4 carcinomas.

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6 Materials and methods

7 Histologic evaluation, classification and grading

8 This study cohort included 45 formalin-fixed, paraffin-embedded samples of canine prostatic
9 carcinoma retrieved from the archives of the School of Veterinary Science – Diagnostic
10 Pathology Service of the University of Queensland and the Department of Veterinary Science
11 and Public Health, the University of Milan. Specimens were represented by tissue samples
12 collected during necropsy (n = 20), prostatectomy (n= 4) or biopsy (by ultrasound or
13 exploratory laparotomy; n = 20). In one case, the information about the type of sample was
14 not available. Clinical data were obtained from the histological reports. Prostatic samples
15 were collected from dogs with the following clinical signs: haematuria (3/20 biopsies; 3/20
16 necropsies; 1/4 prostatectomy), tenesmus (1/20 biopsies; 2/20 necropsies), hind limb
17 weakness (1/20 biopsy; 4/20 necropsies), enlarged prostate (14/20 biopsies; 6/20
18 necropsies), dysuria (7/20 biopsies; 1/20 necropsy), anuria (2/20 biopsies), abdominal pain
19 (1/20 necropsy), and stranguria (1/20 biopsy; 5/20 necrosies; 1/4 prostatectomy). No history
20 was available in 12 cases.

21 Five-micron-thick sections were stained with haematoxylin and eosin (H&E) for the
22 histopathological examination.

23 The classification of prostate cancer was based on the human WHO classification of Tumors
24 of the Urinary System and Male Genital Organs (Eble et al., 2004) and growth patterns
25 recently described in dogs (Palmieri et al., 2014).

26 Gleason grading was performed according to the 2005 ISUP modified Gleason grading
27 system (Table 1, Fig. 1) (Epstein et al., 2005). Gleason score was obtained by adding the
28 primary and secondary grades together. A tertiary pattern higher than the primary and

1 secondary grades has been included in the final Gleason score as the secondary grade. This
2 means that if a biopsy contained multiple patterns with 3, 4 and 5 in various proportions and
3 pattern 5 being the least prevalent (tertiary grade), then the Gleason pattern 5 component
4 was upgraded to a secondary grade before assigning the final Gleason score. The
5 component of intraductal spread (cribriform or papillary) was counted as Gleason pattern 5 if
6 it contained intraluminal comedonecrosis. Any amount of Gleason pattern 5 (even <5% of
7 PC), as long as recognisable at low-power examination, was considered significant and
8 included for analysis.

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

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12 Results

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

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

20 The most common primary grades were, in decreasing order of prevalence, cribriform with
21 (11 out of 45) and without (6 out of 45) comedonecrosis, solid (13 out of 45), small
22 acinar/ductal (10 out of 45), papillary with (1 out of 45) and without comedonecrosis (2 out
23 45), mucinous (1 out of 45), and signet ring (1 out of 45).

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

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

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

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

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

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

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

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

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24 Discussion

25 Prostate biopsy and histopathological assessment are the key steps in PC diagnosis in both
26 humans and dogs and PC grading may be essential for an appropriate treatment decision-
27 making also in the canine species, especially when used at an early stage.

1 In this study, we have demonstrated that canine prostatic carcinoma may show variable
2 morphological features and Gleason-like growth patterns that would aid in the acceptance of
3 the modified Gleason score as a grading system for histopathology obtained from prostate
4 biopsy or radical prostatectomy (RP).

5 As in humans, in most canine PC more than one histological pattern is present and may be
6 as assigned to a primary, secondary or tertiary grade.

7 In men, the presence of a tertiary grade is associated more frequently with Prostate Specific
8 Antigen (PSA) recurrence, extraprostatic extension, surgical margin positivity, seminal
9 vesicle infiltration and lymph node metastases (Delahunt et al., 2012). In our cases, the
10 tertiary grade was observed in 6 cases and associated with the highest GS (GS10) in 4 out
11 of 6. Therefore, the presence of a tertiary grade may be a marker of more aggressive
12 disease, although the limited number of cases coupled with the lack of follow-up information
13 hindered a correct prognostic assessment.

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

22 Regarding sample collection, the highest GS has been observed in necropsy or
23 prostatectomy specimens, although for the prostatectomy follow-up, only a subset of all
24 needle core cases was studied as only a minority of the patients had undergone radical
25 prostatectomy. The Gleason score of biopsy, prostatectomy specimens or samples obtained
26 from necropsy may be not the same for several reasons. Borderline grades (tumours
27 displaying features that are intermediate between two gleason score) and pathological errors
28 are possible explanations (Montironi et al., 2005). Most frequently, a sampling error (i.e.

1 when a higher grade is missed on biopsy) is the most likely, since only a very small amount
2 of the total prostate tissue is sampled for histological analysis during biopsy. Further
3 techniques to improve PC detection rate and GS accuracy should be introduced in dogs, one
4 of which is increasing the number of biopsy samples.

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

8 In men, Gleason pattern 5 predicts a worse outcome compared with that in patients at high
9 risk without pattern 5 in terms of biochemical recurrence, metastasis and cancer-specific
10 death (Sabolch et al., 2011). Therefore, the diagnostic recognition of high Gleason patterns
11 4 and 5 is vital, as these are the patterns that constitute the most aggressive and potentially
12 lethal prostate cancers (Bastian et al., 2006; Vira et al., 2008). To reflect its unique clinical
13 significance, the 2005 ISUP modification of the Gleason grading system recommended
14 upgrading of tertiary Gleason pattern 5 PC in prostate biopsies to a secondary Gleason
15 pattern, regardless of how small the amount of Gleason pattern 5 might be (Epstein et al.,
16 2005). The morphological subpatterns of Gleason pattern 5 PC in relation to its amount and
17 pattern distribution should be systematically analysed since Gleason pattern 5 is most
18 frequently missed by pathologists in consultation practice when it represented secondary or
19 tertiary component of carcinoma (Fajardo et al., 2011). The most common morphological
20 presentation associated with Gleason pattern 5 in our study is the solid undifferentiated
21 carcinoma, followed by the cribriform/papillary subtype with comedonecrosis, similar to what
22 is reported in humans (Fajardo et al., 2011; Shah and Tadros, 2014). Therefore, increased
23 awareness of these morphologic presentations of Gleason pattern 5 is important to minimize
24 interobserver diagnostic variability.

25 In conclusion, the recognition of Gleason-like growth patterns in canine prostatic
26 carcinoma emphasises the variable morphological features showed by this type of tumour
27 and the potential usefulness of the histopathological grading in the canine practice setting.
28 The recent definition of the different histomorphological patterns of canine PC (Palmieri et al.,

1 2014) – which veterinary pathologists should be aware of - represents the basic foundation to
2 enhance the diagnostic recognition of high-grade patterns 4 and 5. The Gleason grading
3 system should be used in all prostate tissue samples, including needle-core biopsies and
4 prostatectomy specimens in order to improve the categorization of tumour features, the
5 extent of glandular differentiation and the pattern of neoplastic growth. We suggest that once
6 carcinoma is detected and the different morphological patterns recognized, the Gleason
7 grading system may be potentially applied in the practice settings in order to complete the
8 clinical assessment for the best management of the patient, assessing the potential for local
9 cure and the risk for distant metastasis.

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11 Conflict of interest

12 Conflicts of interest: none.

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19 Figures legend

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

23