Cytological differentiation between benign and malignant perianal gland proliferative lesions in dogs: a preliminary study

CYTOLOGICAL DIFFERENTIATION BETWEEN BENIGN AND MALIGNANT PERIANAL GLAND PROLIFERATIVE LESIONS IN DOGS: A PRELIMINARY STUDY

SUMMARY

Objectives. Castration and conservative surgical removal are the treatments of choice for perianal gland adenomas, whereas carcinomas have no hormone dependency and must be treated with wide margin surgical excision. Given the lack of specific guidelines, the present study aimed to evaluate the diagnostic power of individual cytological criteria and their best combination to allow the differentiation between benign and malignant perianal gland proliferative lesions in dogs.

Methods. A retrospective study was carried out on cytological samples of canine perianal gland proliferative lesions with subsequent histopathological confirmation on surgical samples**.**

Results. Seventy-seven perianal gland nodules from 56 dogs were included in the study. Histologically, lesions were diagnosed as hyperplasia (n = 2), adenoma (n = 53), epithelioma (n = 6) and carcinoma (n = 16). Of the 28 cytological criteria assessed, 13 were statistically significant, including lack of distinction between hepatoid and reserve cells, low proportion of mature hepatoid cells, delocalization of reserve cells inside clusters and reserve cell morphological atypia (coarse nuclear chromatin, anisocytosis, anisokaryosis). A diagnostic algorithm was proposed accordingly, showing an 87% accuracy (sensitivity, 90.9%; specificity, 85.4%).

Clinical Significance. Cytology can provide useful information for the pre-surgical differentiation between benign and malignant hepatoid gland proliferative lesions. Further studies are warranted to validate the proposed algorithm and test its reproducibility on larger case series.

KEYWORDS

Dog; hepatoid glands; perianal glands; perianal gland tumours; cytology.

INTRODUCTION

Canine perianal gland tumours (PGT) are common neoplasms arising from hepatoid glands (perianal glands, circumanal glands), which are modified sebaceous glands. These ductless glands in dogs are located in the perianal region, on the dorsal and ventral aspect of the tail, in the parapreputial area in males, in the abdominal mammary region in females, on the posterior aspect of the hindlimbs, and on the midline of the back and thorax (Nielsen & Aftosmis 1964, Isitor & Weinman 1979, Gross *et al*. 2005, Turek & Withrow 2013, Goldschmidt & Goldschmidt 2017). Histologically, the lobules of perianal glands consist of mature cells (referred to as "hepatoid" because morphologically resembling hepatocytes), peripherally surrounded by a single layer of basal reserve cells (RC) (Isitor & Weinman 1979).

Proliferative lesions of hepatoid glands include hyperplasia, adenoma, epithelioma and carcinoma (Gross *et al*. 2005).

Adenomas represent the majority (58-96%) of canine PGT and are the third most prevalent tumour type in male dogs (Turek & Withrow 2013, Goldschmidt & Goldschmidt 2017). Their development and progression appear to be sex hormone-dependent, with growth stimulated by androgenic hormones and depressed by estrogenic hormones. The older, intact male is at higher risk, while they are rarely observed in females (mostly in ovariohysterectomized animals) or in castrated males (Hayes & Wilson 1977, Wilson & Hayes 1979, Pisani *et al*. 2006).

Perianal gland carcinomas are much less frequent than their benign counterpart, representing 3% to 21% of all tumours in this region, and may occur in castrated or intact males, as well as in females, implying no hormonal dependency (Turek & Withrow 2013, Goldschmidt & Goldschmidt 2017). Carcinomas tend to grow more rapidly, become ulcerated, adhere to underlying tissues, recur following conservative surgery, and are generally larger than their benign counterpart. Metastases are rare (15% of cases), and most likely to develop late in the course of disease, as the primary tumour becomes bigger and locally invasive. The most frequent metastatic sites are

48 regional lymph nodes, but distant metastases may rarely affect lungs, liver, kidney, bone and
49 abdominal lymph nodes (Nielsen *et al*. 1964, Vail *et al*. 1990, Turek & Withrow 2013, McCourt *et*
50 *al*. 2018).

51 Histologically, PGT are composed by both RC and hepatoid cells (HC). In well-differentiated
52 adenomas, RC can be identified surrounding the lobules of HC, to mimic the normal glandular
53 architecture. Conversely, in the majority of malignant PGT, HC are irregularly intermingled with
54 and dissociated by RC (Berrocal *et al.* 1989, Goldschmidt *et al.* 1998, Gross *et al*. 2005,
55 Goldschmidt & Goldschmidt 2017). Epitheliomas are predominantly composed by RC, with fewer
56 HC, and are characterised by low-grade malignancy (Gross *et al*. 2005, Goldschmidt & Goldschmidt
57 2017).
58 The importance of distinguishing between benign and malignant perianal gland tumours is due to
59 the different influence of testosterone on the evolution of these neoplasms, and consequently on
60 the therapeutic strategies that can be applied. Castration and conservative surgical removal
61 remain the treatments of choice for hyperplasia and adenomas, whereas full staging and wide
62 margin surgical excision are recommended for carcinomas and epitheliomas (Wilson & Hayes
63 1979, Turek & Withrow 2013).
64 Fine-needle aspiration cytology (FNAC) offers several advantages over histology, including minimal
65 invasiveness, lower risk of complications, ease of sample collection and rapid results; however,
66 among the main diagnostic limits of cytology is the impossibility of assessing tissue architecture
67 and the infiltrative nature of the lesion (Klaassen 2002, Shelly 2003, Fisher 2014). Additionally,
68 specific cytological criteria to aid the differentiation between perianal gland adenomas and
69 carcinomas are currently lacking.

The aim of this retrospective study was to identify cytological criteria to differentiate benign and malignant perianal gland proliferative lesions and develop a diagnostic algorithm to be applied in clinical practice.

MATERIALS AND METHODS

A retrospective study was carried out on cytological samples of canine PGT with subsequent histopathological confirmation on surgical samples. Cases were recruited at the Pathology Service of the Department of Veterinary Medical Sciences, University of Bologna, Italy.

Histological samples were reviewed by two of the authors (SS and AR2) and cases with a diagnosis of perianal gland hyperplasia, adenoma, epithelioma or carcinoma according to Gross *et al*. (2005) were included.

For each case, the corresponding cytological sample was examined and the smears with inadequate cellularity or with a high percentage of damaged cells were excluded from the analysis. All cytological smears were obtained by fine-needle aspiration of the nodules, allowed to air-dry and stained with May Grünwald-Giemsa.

Background information recorded in all cases included dogs' breed, age, sex and neutering status, tumour location (perianal region or ectopic) and number of days between cytological and histological examination. When available, information on tumour size and ulceration was also retrieved from specimen submission forms.

Evaluated cytological criteria included cellularity, blood contamination, characteristics of cellular clusters, characteristics of HC and RC, squamous and sebaceous differentiation, background findings and inflammation. A full description of the evaluated criteria is provided as supplemental material (Supplemental Table 1). These criteria were blindly assessed by three of the authors (SS, AR1 and AR2) and diagnosis was by consensus.

Cases were divided in two groups (group 1: hyperplasia/adenoma; group 2: epithelioma/carcinoma) based on the final histological diagnosis. Mann-Whitney U test and Fisher's exact test/Chi-square test were used to identify the variables that were significantly different between the two groups. Among the cytological criteria with statistically significant *P* values, those judged more inclusive and easily assessable were combined into various algorithms. These combinations were tested for their sensitivity, specificity and accuracy in the identification of tumours from group 2 by two blinded observers (SS and AR1).

Descriptive statistics were used to present dogs and tumour characteristics. When appropriate, data sets were tested for normality by use of the D'Agostino and Pearson omnibus normality test. Values were expressed as mean ± SD in case of normal distribution, or as median with an interquartile range (IQR) in case of non-normal distribution.

Data were analysed by use of commercial software programs (SPSS Statistics v.19, IBM, Somers, New York, and Prism v.5.0, GraphPad, San Diego, California). P-values < 0.05 were considered significant.

RESULTS

Background information

Seventy-seven hepatoid gland nodules from 56 dogs fulfilled the inclusion criteria. Thirteen dogs with multiple (2-4) nodules were included. Upon histological examination, 55 nodules (71%) were judged as benign or hyperplastic (group 1; 2 hyperplasia and 53 adenomas), while 22 (29%) were diagnosed as malignant (group 2; 6 epitheliomas and 16 carcinomas). In group 2, the malignant areas occupied the majority of the nodule in 11 (50%) cases, while in the remaining 11, only a small portion of the nodule was considered malignant.

The 55 tumours from Group 1 (benign proliferative lesions) belonged to 32 intact males (82%), 3 castrated males (8%), 1 intact female (2%) and 3 spayed females (8%). Eighteen dogs were crossbred; the most represented breeds were Siberian husky, Yorkshire terrier and German shepherd (two cases each). The mean age was 11 ± 2.2 years.

Forty-nine (89%) nodules were located around the anal ring, whereas 6 (11%) were ectopic (tail, *n* = 3; inguinal region, *n* = 1; abdomen, *n* = 2).

The 22 tumours from Group 2 (malignant proliferative lesions) belonged to 16 intact males (94%) and 1 spayed female (6%). Nine dogs were crossbred; the most represented breed was Siberian husky (*n* = 3). The mean age was 13 ± 2.4 years.

Eighteen (82%) nodules were located around the anal ring, whereas 4 (18%) were ectopic (tail, *n* = 3; trunk, *n* = 1).

None of the above demographic features was significantly different between groups 1 and 2. Information on nodule diameter and ulceration was available in 60 (78%) and 57 (74%) cases, respectively. Median tumour diameter was significantly larger in group 2 (2 cm; IQR, 2-3 cm) compared with group 1 (1.3 cm; IQR, 0.7-2 cm; *P* = 0.003). The percentage of ulcerated nodules was 93% in group 2 and 65% in group 1 (difference not statistically significant; *P* = 0.08).

Cytological examination

The median time elapsed between cytological and histological examination was 21 days (IQR, 9-31 days). The cytological criteria significantly associated with histological malignancy included larger cell clusters, irregular cluster margins, lack of distinction between HC and RC, low proportion of mature HC, nodular foci of RC inside clusters, dissociation of HC by RC, increased proportion of cells with N:C ratio > 1, HC anisocytosis/anisokaryosis, RC anisocytosis/anisokaryosis and coarse

chromatin pattern and, high numbers of cells with cytoplasmic vacuoles, sebaceous differentiation and lymphoplasmacytic inflammation (Table 1; Figure 1).

Based on these results, a diagnostic algorithm was developed to differentiate benign and malignant proliferative lesions (Figure 2).

This algorithm allowed the identification of malignant tumours (group 2) with 90.9% sensitivity (95% CI, 70.8-98.9%), 85.4% specificity (95% CI, 73.3-93.5%) and 87% accuracy (95% CI, 77.4-93.6%). Overall, 2 (9%) malignant tumours were misdiagnosed as benign with this method, all of which only had a minor portion of the nodule containing malignant cells.

DISCUSSION

While a generic cytological diagnosis of PGT is straightforward, the cytological distinction between benign and malignant PGT can be more prone to error. Indeed, no specific guidelines for the cytological identification of these neoplasms have been proposed and a recent report found no evidence that the proportion of basal cells in canine PGT cytology is an indication of malignancy (Evans et al. 2018). Even histologically, the differential diagnosis between adenoma and well-differentiated carcinoma can be difficult and mostly relied on architectural elements, like evidence of infiltrative growth and haphazardous cellular arrangement (Gross *et al*. 2005, Goldschmidt & Goldschmidt 2017).

Herein, we present the results of a study aimed at evaluating the best combination of cytological criteria to differentiate benign and malignant hepatoid gland proliferative lesions.

Despite the indications provided in the literature, demographic information and macroscopic features were not always helpful in this distinction, as 13% of benign lesions were observed in castrated males and in spayed/intact females, and the presence of ulceration was not an hallmark of malignancy. This highlights even more the importance of a presurgical diagnosis for these tumours.

Of the 28 cytological criteria assessed, as many as 13 were statistically significant, including cellular morphological features, arrangement of cells inside clusters and numerical proportion between cellular types.

By providing an indirect indication of tissue architecture, the arrangement of RC within clusters represents a major diagnostic element for the cytological evaluation of PGT. In fine-needle aspirates of perianal gland adenomas, the typical finding is that of three-dimensional clusters of HC entirely surrounded by RC, impairing a clear identification of cellular components. However, examination should focus on the areas of the smear where glandular adenomeres are broken into bidimensional clusters and cell morphology becomes appreciable. Even if a complete peripheral layer of RC surrounding clusters is hard to be found, seeing just a part of the rim in the absence of RC inside clusters is highly suggestive of a benign form. These clusters are also generally characterised by clean, smooth margins. In contrast, in the majority of malignant PGT, RC are irregularly admixed with HC, with dissociation of the latter, or cluster of proliferation of RC alone are observed. However, delocalization of RC may also occur in benign tumours and should not be, in itself, sufficient to diagnose a perianal gland carcinoma. According to the proposed algorithm, this criterion must be associated with evidence of RC anisocytosis/anisokaryosis or nuclear activation. Poorly-differentiated carcinomas can be identified by the presence of an increased number of cells with scant cytoplasm, resembling RC, but with larger nuclei and prominent nucleoli (cells with intermediate differentiation). These cells are the results of dysregulations in the maturation pattern from RC to HC. As malignancy increases, tumours may be entirely composed by poorly-differentiated cells, with total disappearance of HC (Goldschmidt & Goldschmidt 2017); in these cases, the differential diagnosis from anal sac carcinomas can be difficult.

In the impossibility to directly assess tissue architecture or tumour invasiveness, cellular morphology remains the most powerful criterion for the identification of malignant neoplasms in cytological samples. In perianal glands, however, atypia must be judged with caution, because the physiological presence of a dimorphic cell population can generate a false impression of pleomorphism. In the examined cases, the true cellular pleomorphism was regularly associated with nuclear activation (anisokaryosis, coarse chromatin) in cells other than HC.

An additional morphological element significantly associated with carcinomas was the presence of cytoplasmic vacuoles: while not representing a true cytological criterion of malignancy, it might reflect a state of cell degeneration and fragility more often found in malignant tumours, or an initial stage of sebaceous metaplasia, which was frequently observed in hepatoid gland carcinomas in this and other studies (Gross *et al*. 2005).

A further difficulty in the cytological assessment of PGT is that these tumours are frequently surrounded by areas of perianal gland hyperplasia; so the presence of well-differentiated adenomeres is a constant finding, even in carcinoma smears. Additionally, in half of the carcinomas in this study, the malignant areas were limited to a small portion of the nodule, with the remaining part being referable to adenoma. So these areas may not be sampled, or just a few less differentiated cell clusters may be seen within an otherwise benign population. This has most likely reduced the sensitivity of the proposed algorithm in this study.

Finally, the malignant transformation of a benign PGT during the time elapsed between cytological and histological sampling cannot be completely excluded.

The main limitations of this study include the relatively small number of malignant PGT, which reflects their lower frequency, and the fact that the algorithm has not been tested on *de novo* neoplasms.

In conclusion, the present study proposes an algorithm to aid the pre-surgical differentiation between benign and malignant perianal gland proliferative lesions by cytology. Further research will be necessary to confirm these preliminary data, assess the accuracy of the algorithm and evaluate its inter-observer reproducibility.

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258 **CONFLICTS OF INTEREST**

259 No conflicts of interest have been declared.

260

261 **REFERENCES**

262 **Figure 1.** A – Fine-needle aspirate of a well-differentiated perianal gland adenoma; three-
263 dimensional cluster of cells with a smooth border, for the presence of a complete peripheral layer
264 of small reserve cells (RC) with hyperchromatic nuclei (arrow) enclosing hepatoid cells (HC). May
265 Grünwald-Giemsa (MGG) stain. B – Corresponding histological sample showing multiple
266 adenomeres of mature HC surrounded by a rim of basaloid RC. Haematoxylin and eosin (HE) stain.
267 C – HC irregularly admixed with RC. MGG. D – The same cellular arrangement can be observed in
268 the corresponding histological sample. HE. E – HC dissociated by RC. Being RC nuclei small and
269 hyperchromatic, this should not be considered a sign of malignancy. MGG. F – HC dissociated by
270 enlarged RC with nuclear activation in a well-differentiated perianal gland carcinoma. Note the
271 vacuolation of HC. MGG. G – Cluster predominantly composed by cells with intermediate
272 differentiation, with disappearance of mature HC, suggestive of a poorly-differentiated perianal

gland carcinoma. MGG. H – The same elements can be observed in the corresponding histological sample, where the dimorphism between HC and RC can no longer be appreciated. Note the prominent mitotic activity of neoplastic cells. HE.

Figure 2. Algorithm to differentiate canine perianal gland hyperplasia/adenoma from their malignant counterpart (epithelioma/carcinoma) in cytological smears obtained by fine-needle aspiration cytology.