Journal of Veterinary Internal Medicine

Journal of Veterinary Internal Medicine Open Access
ACVIM American College of Veterinary Internal Medicine

STANDARD ARTICLE OPEN ACCESS
Small Animal Internal Medicine Oncology

Cytologic Evaluation as a Diagnostic Tool to Differentiate Adrenocortical Tumors and Pheochromocytomas

Hunter J. Piegols[1] | Rachel W. Williams[2] | Nirali Pathak[2] | Laura E. Selmic[1] | Giovanni Tremolada[3] | Russell Sakacs[1] | Laurie Millward[1] | Janis Lapsley[1]

[1]The Ohio State University Veterinary Medical Center, The Ohio State University, Columbus, Ohio, USA | [2]UF Small Animal Hospital, University of Florida, Gainesville, Florida, USA | [3]Flint Animal Cancer Center, Colorado State University, Fort Collins, Colorado, USA

Correspondence: Hunter J. Piegols (piegols.3@osu.edu)

Received: 26 July 2024 | **Revised:** 21 January 2025 | **Accepted:** 29 January 2025

Funding: The authors received no specific funding for this work.

Keywords: adenoma | adrenal | aspirate | carcinoma | cytology

ABSTRACT

Background: The utility of cytologic evaluation to distinguish adrenocortical tumors and pheochromocytomas in dogs has not been thoroughly investigated, partly because of the perceived risks of the procedure.

Objectives: Report test characteristics of fine needle aspiration (FNA) and cytologic evaluation for differentiation of adrenocortical tumors and pheochromocytomas in dogs. Complications associated with FNA also were recorded.

Animals: Thirty-eight dogs with 40 adrenal tumors that had FNA and cytologic evaluation performed before adrenalectomy were included in the study from three institutions. Tumors included 17 pheochromocytomas, 21 adrenocortical tumors, 1 concurrent adrenocortical adenoma and pheochromocytoma, and 1 malignant neoplasm.

Results: Of the 40 FNA cytologic aspirations performed, 35 (87.5%) had a predominant cell type identified and therefore were considered of diagnostic quality. Of these, 30 (85.7%) correlated with the final histopathological diagnosis. When all samples were included, FNA and cytologic evaluation had a sensitivity of 77.3%, specificity of 76.5%, positive predictive value of 81.0%, negative predictive value of 72.2%, and accuracy of 76.9% for identifying adrenocortical tumors. For pheochromocytomas, these values were 72.2%, 95.2%, 92.8%, 80.0%, and 84.5%, respectively. Six (15.9%) dogs had self-limiting complications associated with the FNA procedure.

Conclusion and Clinical Importance: Fine needle aspiration and cytologic evaluation of adrenal tumors has a low complication rate and can help differentiate adrenocortical tumors and pheochromocytomas. Thus, cytologic evaluation of adrenal tumors should be considered to help differentiate adrenal tumors and allow more individualized treatment of affected dogs.

1 | Introduction

Advanced imaging has increased the detection of adrenal tumors with incidental tumors identified in up to 9.3% of dogs [1]. The most common tumors reported in dogs undergoing adrenalectomy are pheochromocytomas and adrenocortical tumors [2]. Multiple differentiating tests have been proposed to distinguish adrenocortical tumors and pheochromocytomas including advanced imaging, plasma and urine normetanephrine concentrations, serum inhibin concentration, endogenous adrenocorticotropic hormone concentration, urine cortisol-to-creatinine ratio, and low dose dexamethasone suppression testing [3–5]. Challenges include the potential for non-corticosteroid-producing adrenocortical tumors as well

Abbreviations: CI, confidence interval; cm, centimeter; FNA, fine needle aspiration; IQR, interquartile range; NPV, negative predictive value; PPV, positive predictive value.

as pituitary-dependent hyperadrenocorticism with concurrent adrenal tumors. Furthermore, pheochromocytomas can have episodic secretion leading to variation in normetanephrine concentrations [6]. Other limitations include testing availability and prolonged turnaround time [7].

If an adrenalectomy is pursued, pre-operative knowledge of the tumor type may guide medical management and identify potential risks. For example, pheochromocytomas have been associated with peri-operative instability secondary to excessive secretion of catecholamines [8, 9]. Pre-operative treatment with phenoxybenzamine has been proposed previously to minimize peri-operative mortality in dogs with pheochromocytomas, but the benefit has been questioned [2, 8]. Pathologic changes to the myocardium also have been identified at necropsy in dogs with pheochromocytomas [10]. Carcinomas have been associated with hypercoagulability [11]. Experimental studies in rats found that corticosteroids affect wound healing [12]. Additionally, pre-operative corticosteroid administration has been associated with increased risk of surgical site infection in dogs [13]. It is therefore possible that dogs with increased corticosteroids secondary to hyperadrenocorticism may have increased risk of surgical site infection. Knowledge of the tumor type may identify risks and enable appropriate risk-minimizing mitigations, which has been proposed to allow more individualized medicine in dogs with adrenal tumors [14].

Cytology provides a readily available means to help differentiate adrenal tumors. Historically, perceived risk led to strong recommendations against adrenal gland aspiration, but such risk is not well supported by recent literature [15, 16]. Currently, the accuracy of cytology to distinguish adrenocortical tumors and pheochromocytomas is poorly reported. Accuracy of distinguishing adrenocortical tumors from pheochromocytomas was reported to range from 90%–100% among different pathologists in a recent study, but limitations included having a combined study population of dogs and cats, having only 11 samples acquired by cytology, and having a final histologic diagnosis in only six cases [17]. Another study found 71% of adrenal cytologic samples were diagnostically conclusive, but only eight had a histologic diagnosis for comparison, limiting ability to interpret accuracy of the diagnostically conclusive samples [16].

We sought to assess the value of fine needle aspiration (FNA) to distinguish primary adrenal tumors in dogs before adrenalectomy. Our primary objective was to assess the diagnostic utility of cytology to distinguish adrenocortical tumors and pheochromocytomas before undergoing an adrenalectomy when compared with the final histopathology report. A secondary aim was to identify complications associated with adrenal gland aspiration.

2 | Methods

2.1 | Study Population

Ours was a multi-institutional, cross-sectional study involving retrospective review of medical records to assess the diagnostic utility of cytologic evaluation for pre-operative diagnosis of adrenal tumors. Institutional Animal Care and Use Committee approval was not required given the retrospective nature of the study. Electronic medical records at three academic veterinary hospitals were searched for dogs undergoing an adrenalectomy that had pre-operative FNA and cytologic evaluation of the adrenal gland being excised. Dogs were included if they underwent adrenalectomy between January 1, 2013 and March 1, 2024. For inclusion, the cytologic evaluation of the adrenal tumor had to be interpreted by a veterinary clinical pathologist. Exclusion criteria were if a pre-operative cytology report or post-operative histopathology report was not available for review. If bilateral adrenalectomy, either single-session or staged, was performed, both tumors were included as separate entities.

2.2 | Data Collection

Collected data included: age at the time of surgery, breed, sex and neuter status, and body weight. Continuous data were evaluated for normality of distribution using the Shapiro–Wilk test and reported as mean ± SD when normally distributed and median (interquartile range [IQR]) when non-normally distributed. The affected side (left or right) was noted as well as maximal diameter of the tumor on pre-operative ultrasound examination or computed tomography. It was recorded if the tumor was sampled under general anesthesia or sedation. The cytologic description was categorized as an epithelial or neuroendocrine cell type based on the predominant cell type identified in the cytology report during medical record evaluation. Alternatively, the sample was categorized as non-diagnostic if a predominant cell type could not be identified other than cells consistent with blood contamination. Other neoplastic or inflammatory cytologic diagnoses of adrenal tumors were not encountered and thus were not recorded in the study. Complications noted in the medical record in association with FNA of the adrenal gland were recorded. Complications were graded using a scheme previously described for adverse events in investigational therapy. Briefly, grade 1 was considered mild, grade 2 moderate, grade 3 severe (clinically relevant but not immediately life threatening), grade 4 life-threatening, and grade 5 death [18]. The surgical procedure performed and histologic diagnosis were recorded.

2.3 | Statistical Analysis

All statistics were performed using Microsoft Excel (Version 2308 Build 16.0.16731.20542) with a StatPlus Add-In (Version 7.8.4). Diagnostic accuracy was calculated for all cases identified as well as for samples having a cytologic description predominated by an epithelial or neuroendocrine cell type, which was considered as diagnostic quality. The number of diagnostic quality and nondiagnostic samples collected under general anesthesia and sedation was recorded with group differences tested using a Pearson Chi-square test. The 95% confidence intervals (CI) were calculated. Age at surgery, body weight, and maximal tumor diameter on imaging were reported for correctly categorized tumors and for those that were either non-diagnostic on cytology or incorrectly categorized with group differences tested using a *t*-test or Mann–Whitney *U* test, depending on if data were normally or non-normally distributed, respectively. Tumor laterality and sex also were reported for correctly categorized

tumors and for those that were either non-diagnostic cytologic samples or incorrectly diagnosed with group differences tested using a Pearson Chi-square test. Tumors were assigned as correctly categorized when the cytologic description identified an epithelial or neuroendocrine cell type and were diagnosed as an adrenocortical tumor or pheochromocytoma on histopathology, respectively. Tumors were assigned as incorrectly categorized when the cytologic diagnosis did not correlate with the histopathologic diagnosis.

Test characteristics including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were calculated to assess the ability of cytology to correctly identify adrenal tumors as epithelial tumors with the histopathology results consistent with an adrenocortical tumor considered the gold standard; 95% CI were also calculated. The same values were calculated to assess the ability of cytology to correctly identify adrenal tumors as neuroendocrine tumors with the histopathology results consistent with a pheochromocytoma considered the gold standard.

The proportion of dogs developing complications was recorded with 95% CI calculated.

3 | Results

3.1 | Clinical Findings

Thirty-eight dogs with 40 adrenal tumors were identified that met the inclusion criteria at three academic institutions. The different institutions contributed 3, 13, and 22 dogs. The most common dog breeds were mixed breed dogs (12), dachshunds (4), bichon frise (2), shih tzu (2), and unspecified terriers (2). Dog breeds with only one dog identified included American Staffordshire terrier, Australian shepherd, Boston terrier, Cavalier King Charles spaniel, French bulldog, golden retriever, Jack Russell terrier, keeshond, Manchester terrier, miniature poodle, Pomeranian, pug, Rhodesian ridgeback, rottweiler, springer spaniel, and Yorkshire terrier. Median body weight was 15.1 kg (IQR, 6.3–27.1). Mean +/− SD age at the time of surgery was 11.3 +/− 1.9 years old. There were 20 spayed female dogs and 18 neutered male dogs. There were 20 left-sided and 20 right-sided tumors. Maximal tumor diameter on imaging was 3.2 +/− 1.4 cm (range, 0.8–7.3 cm). Thirty-six dogs underwent unilateral adrenalectomy, and two dogs underwent bilateral adrenalectomy.

3.2 | Diagnostic Findings

Twenty tumors were sampled with the dog under general anesthesia and 18 were sampled with the dog sedated; this information was not recorded for two tumors. Fine needle aspiration was performed under ultrasound guidance in all cases. Of the cytologic samples obtained, 87.5% ($n = 35$; 95% CI, 73.2%–95.8%) had an identifiable predominant cell type and were considered of diagnostic quality. All 20 FNA samples collected under general anesthesia were considered as diagnostic quality whereas 72% ($n = 13$) of FNA samples collected under sedation were considered as diagnostic quality ($p = 0.01$). Of the 35 cytologic samples considered as diagnostic quality, 60% ($n = 21$) were consistent with an epithelial cell type (Figure 1) and 40% ($n = 14$) were consistent with a neuroendocrine cell type (Figure 2). On histologic evaluation 42.5% ($n = 17$) of tumors were consistent with a pheochromocytoma, 52.5% ($n = 21$) were consistent with adrenocortical tumors, 1 was consistent with adrenocortical adenoma with concurrent pheochromocytoma, and 1 was consistent with a malignant neoplasm. Five pheochromocytomas were positive on Grimelius stain for argyrophilic granules; the tumor diagnosed as an adrenocortical adenoma with concurrent pheochromocytoma also had regions positive on Grimelius stain for argyrophilic granules. One adrenocortical tumor was negative on Grimelius stain, and 1 adrenocortical tumor was positive for melan-A. The malignant neoplasm was analyzed by immunohistochemistry and was negative for inhibin-alpha, paired box 8, and factor VIII-related antigen with the cell of origin not determined. Of the adrenocortical tumors, 66.7% ($n = 14$) were adrenocortical adenomas, 28.6% ($n = 6$) were adrenocortical carcinomas, and 4.8% ($n = 1$) were adrenocortical hyperplasia.

3.3 | Testing Characteristics of Cytology to Diagnose Adrenal Tumors

Overall, cytology of all the samples had a diagnostic accuracy of 75% (CI, 58.8%–87.3%). Accuracy was defined by identifying either an epithelial or neuroendocrine cell type on cytology with the respective cell types being identified on histopathology. The malignant neoplasm on histopathology was non-diagnostic on cytology and thus was included in the inaccurate category for the calculation of overall diagnostic accuracy; it was excluded from subsequent calculations because a more definitive diagnosis was not achieved. The tumor consistent with an adrenocortical adenoma and pheochromocytoma on histopathology was consistent with a neuroendocrine cell type on cytology and was considered correctly categorized because a pheochromocytoma was present. When considering only cytologic samples of diagnostic quality, cytology had an accuracy of 85.6% (CI, 69.7%–95.2%; Table 1).

Baseline characteristics associated with each tumor were divided into correctly categorized tumors and those that were non-diagnostic cytologic samples or incorrectly categorized (Table 2). Of the variables assessed, age was the only factor that was significantly different between correctly categorized cytologic samples and non-diagnostic or incorrectly categorized cytologic samples.

To assess diagnostic utility, test characteristics consisting of sensitivity, specificity, PPV, NPV, and accuracy were determined to assess the ability of cytology to identify a mass as consistent with an adrenocortical tumor (Table 3) and subsequently pheochromocytoma (Table 4). When performing these calculations, the tumor that had neuroendocrine cell type on cytologic evaluation and a concurrent pheochromocytoma and adrenocortical adenoma on histopathology was considered a false negative for the adrenocortical tumor calculations and a true positive for the pheochromocytoma calculations because the adrenocortical adenoma component failed to be

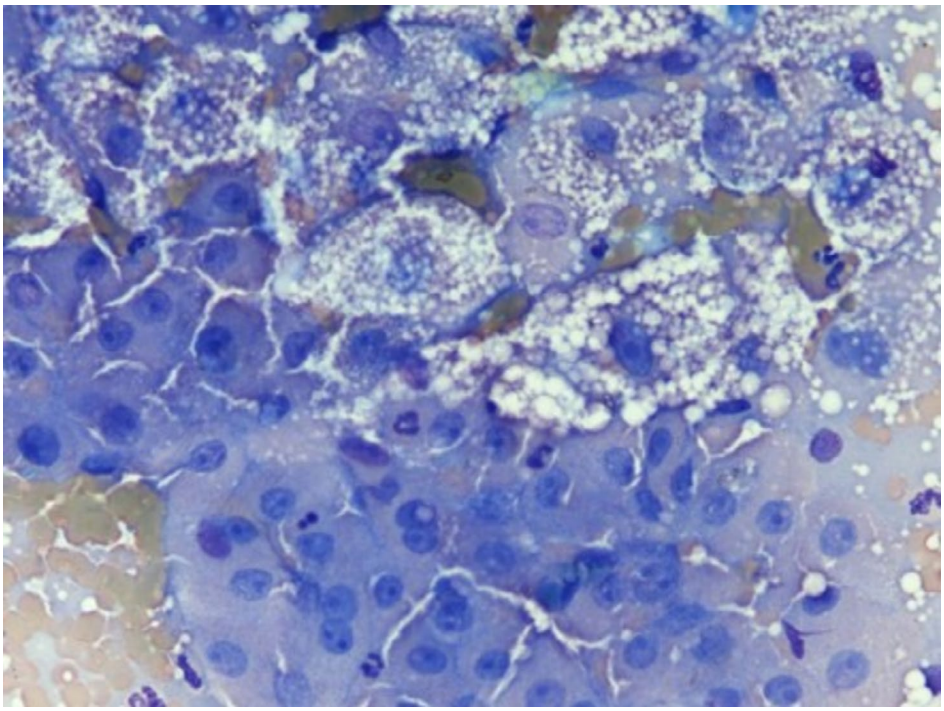

FIGURE 1 | Cytology of canine adrenocortical adenoma. Fine needle aspirate of an adrenal mass was markedly cellular and contained numerous variably sized clusters of relatively uniform large oval to polygonal epithelial cells. The nuclear to cytoplasmic ratio was low. Cells exhibited mild to rarely moderate anisocytosis and anisokaryosis. The cells contained round nuclei with occasional one to two prominent nucleoli and finely to coarsely stippled chromatin. The cytoplasm is abundant and pale pink to amphophilic in color. Occasional cells contained numerous distinct small cytoplasmic vacuoles. (Wright-Giemsa; 50× oil objective).

detected on cytology whereas the pheochromocytoma was detected on cytology.

3.4 | Complications Associated With FNA Procedure

Of the dogs undergoing FNA procedures, 15.9% ($n = 6$; CI, 6.0%–31.3%) had complications identified in the medical records in association with the FNA procedure. The following complications were identified: grade 1 bradycardia ($n = 2$), grade 2 intermittent second-degree atrioventricular block ($n = 1$), grade 2 hypercapnia ($n = 1$), grade 2 hypothermia ($n = 1$), and grade 3 apnea ($n = 1$). Only 18 dogs had blood pressure monitoring during the FNA procedure, but no hypertensive episodes were recorded. No other complications were identified, and no care beyond monitoring recovery from sedation or general anesthesia was required for treatment. Of the six dogs with complications, four (66.7%) had pheochromocytomas and two (33.3%) had adrenocortical adenomas. One dog with bilateral adrenocortical tumors was receiving prazosin and one dog with a pheochromocytoma was receiving amlodipine at the time of the FNA procedure; no other anti-hypertensive medications were being administered at the time of sampling.

4 | Discussion

Although multiple diagnostic tests to distinguish adrenocortical tumors and pheochromocytomas are reported, the utility of cytologic evaluation has received limited evaluation. Of the FNA performed, 87.5% were considered of diagnostic quality, based on having a predominant cell type identified. A previous study with 58 adrenal gland FNA across 15 institutions found 71% of cytologic samples to be diagnostic [16]. One potential explanation for this difference is that regular performance of the procedure may increase competency and subsequent obtainment of diagnostic samples with two of the centers in our study performing 37 of the 40 FNA. Sampling performed under general anesthesia was associated with more samples being of diagnostic quality, which is suspected to be associated with general anesthesia potentially facilitating ultrasound-guided FNA as well as the potential to collect multiple samples. Given an overall accuracy of 75% for all cytologic samples and 85.6% when confined to diagnostic cytologic samples, when using histopathology as a gold standard, the results of our study suggest that cytology could be considered as a diagnostic test for distinguishing adrenocortical tumors and pheochromocytomas. The accuracy found is similar but slightly less than previously reported accuracies of 87.5% and 90%–100% for comparing a cytologic diagnosis to histopathology. The specificity of 95.2% and PPV of 92.9% of cytology for identification of pheochromocytomas is of particular interest because a positive result highly supports a dog truly having a pheochromocytoma. It is important to note that PPV and NPV are dependent on disease prevalence with PPV increasing and NPV decreasing as the prevalence increases, respectively. Thus, the PPV found would be lower in a population of dogs with a lower prevalence of adrenal neoplasia, such as a general population of dogs. There was a complication rate of 15.9% with overall low severity of complications documented in the included cases, further suggesting that the risk of the procedure is relatively low while providing useful

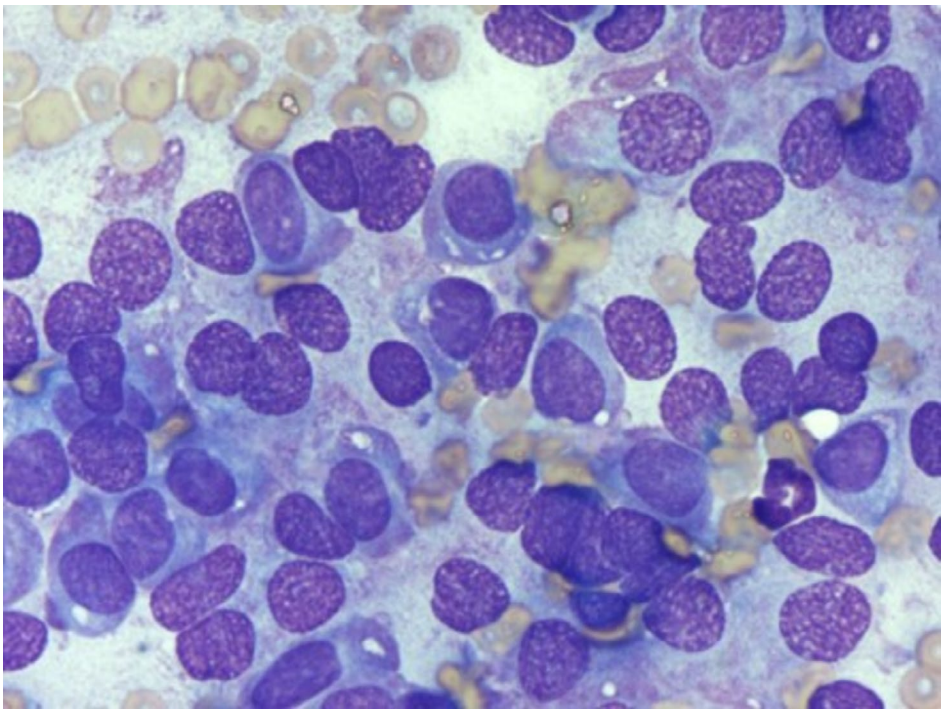

FIGURE 2 | Cytology of canine pheochromocytoma. Fine needle aspirate of an adrenal mass was markedly cellular with numerous naked nuclei and rare to occasional intact cells consistent with endocrine/neuroendocrine origin. Cells were arranged individually and in small clusters. Intact cells displayed mild anisocytosis and anisokaryosis. The nuclear to cytoplasmic ratio was high. Cells were oval to polygonal with a small amount of medium blue cytoplasm that occasionally contained moderate numbers of fine pink granules. The nuclei were oval and centrally placed with coarsely stippled chromatin and inconspicuous nucleoli. (Wright-Giemsa; 100× oil objective).

TABLE 1 | Comparison of cytology and histopathology for diagnostic cytologic samples of adrenal masses ($n = 35$).

		Histopathology		
		Adrenocortical tumor	**Pheochromocytoma**	**Total**
Cytology	Epithelial cell type	17	4	21
	Neuroendocrine cell type	1	13	14
	Total	18	17	35

Note: Overall accuracy of diagnostic cytologic samples = 85.6% (95% CI, 69.7%, 95.2%).
Abbreviation: CI, confidence interval.

information. Only a single grade 3 adverse event was noted with all others being mild or moderate [18].

Using cytology as a diagnostic test for distinguishing adrenal tumors has several potential benefits. Most adrenal tumors are diagnosed using imaging, and ultrasound-guided FNA of the tumor can be readily performed. The cytologic findings also can be interpreted in combination with advanced imaging findings. For example, pheochromocytomas tend to be more hyperattenuating, are associated with a larger contralateral adrenal gland, and have more prominent tumor thrombi on computed tomography than do carcinomas [3]. Cytologic evaluation by a veterinary clinical pathologist is rapid, relatively inexpensive, and widely available, further facilitating this diagnostic option. Cytologic features to help distinguish adrenocortical tumors and pheochromocytomas have been described including adrenocortical tumors being more likely to have intact cells, cells that either occur singly or in cohesive clusters, low nuclear to cytoplasmic ratio, basophilic and variably vacuolated cytoplasm, and coarse or condensed chromatin [17]. Other diagnostic tests such as evaluation of plasma and urine metanephrine concentrations require high-pressure liquid chromatography with electrochemical detection or liquid chromatography–tandem mass spectrometry [4]. The need for specialized equipment for determination of metanephrine concentrations results in limited availability and increased costs as compared with cytologic evaluation. Limitations for the two previous studies on adrenal cytology include that eight or fewer cases in each study had histopathology results [16, 17]. Additionally, one of the studies included imprint cytology, masses collected during necropsy, and cats. Thus, this study may not represent the use of cytology for distinction of adrenocortical tumors and pheochromocytomas in dogs when used in a clinical setting.

Cytology had an overall accuracy of 84.6% for identifying pheochromocytomas with a PPV of 92.9% and specificity of 95.2%. Although measurement of metanephrines in plasma, urine, and saliva is documented to diagnose pheochromocytomas in dogs,

TABLE 2 | Baseline characteristics of dogs undergoing pre-operative adrenal cytology with subsequent adrenalectomy.

Characteristic	Correctly categorized cytologic samples ($n=30$)	Non-diagnostic or incorrectly categorized cytologic samples ($n=10$)	p
Weight (kg; IQR)	15.8 (7.7–24)	7.4 (6.0–27.9)	$p=0.39^{A}$
Age (years) +/− SD	11.0 +/− 1.6	12.8 +/− 2.01	T-test: $p<0.01^{B}$
Sex (n)			$p=0.36^{C}$
Female spayed	17	4	
Male neutered	13	6	
Tumor laterality			$p=0.14^{C}$
Left	17	3	
Right	13	7	
Maximal tumor diameter (cm) +/− SD	3.0 +/− 1.1	3.9 +/− 1.8	T-test: $p=0.09^{B}$

Note: Comparison tests used were Mann–Whitney U test (A), T-test (B), and Pearson's chi-square (C).
Abbreviations: cm, centimeter; IQR, interquartile range; kg, kilogram; SD, standard deviation.

TABLE 3 | Test characteristics for ability of cytology to identify adrenocortical tumors.

		Histologic diagnosis consistent with adrenocortical tumor		
		Present	Absent	
Cytologic diagnosis consistent with adrenocortical tumor	Present	17	4	PPV: 81.0% (95% CI 58.1%, 94.6%)
	Absent	5	13	NPV: 72.2% (95% CI 46.5%, 90.3%)
		Sensitivity: 77.3% (95% CI 54.6%, 92.2%)	Specificity: 76.5% (95% CI 50.1%, 93.2%)	

Note: Accuracy in identifying adrenocortical tumors: 76.9% (95% CI, 60.7%, 88.9%).
Abbreviations: CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value.

urinary normetanephrine is perceived to be the most used and described in dogs [4, 19–21]. A cut-off ratio of four-times the highest urine normetanephrine to creatinine ratio measured in control samples, a ratio of > 364, was previously proposed, but it had a low sensitivity of 57.1% with three of the seven dogs with pheochromocytomas failing to be detected using this cut-off [19]. This contrasts with the use of cytology which has a sensitivity of 72.2%. Furthermore, a confounding factor is that dogs with hyperadrenocorticism also have increased normetanephrine, suspected to be from increased catecholamine production secondary to increased glucocorticoids, which results in the need for a high threshold for urine normetanephrine to creatinine ratios to have a diagnostically useful PPV [19]. In contrast, cytologic evaluation has a sensitivity of 72.2% while still maintaining a PPV of 92.9%. Plasma free normetanephrine concentration using a cut-off of 5.52 nmol/L has a sensitivity and specificity of 100% and 97.6%, but the study only had eight dogs, all of which had clinical signs or a history of hypertension and a median tumor size of 5.0 cm [7]. It is unclear if the reported sensitivity and specificity would remain the same in a larger population that contained dogs with incidentally found pheochromocytomas because such dogs may have lower normetanephrine concentrations or more episodic secretion than dogs with reported clinical signs. Additionally, urine normetanephrine to creatinine ratio may be influenced by the location of sample collection, with collection in a clinic associated with higher ratios than when samples are collected at home [21]. Overall, cytologic evaluation of pheochromocytomas is not influenced by many of the confounding factors associated with measurements of urine normetanephrine concentrations.

Cytology had an accuracy of 76.9% for diagnosing adrenocortical tumors. Many diagnostic tests to support an adrenocortical tumor, such as a low-dose dexamethasone suppression test and endogenous adrenocorticotropin releasing hormone, are dependent on the tumor secreting cortisol. Serum inhibin concentrations are increased in dogs with hyperadrenocorticism because of production of inhibin by tumor cells, and limited evaluation of inhibin in dogs with non-cortisol secreting adrenocortical tumors is available. In four dogs however the serum concentration was highly variable [5]. A more systematic characterization of adrenal corticosteroid production beyond cortisol in dogs with primary adrenal tumors is warranted. Dogs also can have concurrent pituitary and adrenal lesions, which is reported in 5% of dogs with hypercortisolism and in 10% of

TABLE 4 | Test characteristics for ability of cytology to identify pheochromocytomas.

		Histologic diagnosis consistent with pheochromocytoma		
		Present	**Absent**	
Cytologic diagnosis consistent with pheochromo-cytoma	Present	13	1	PPV: 92.9% (95% CI 66.1%, 99.8%)
	Absent	5	20	NPV: 80.0% (95% CI 59.3%, 93.2%)
		Sensitivity: 72.2% (95% CI, 46.5%, 90.3%)	Specificity: 95.2% (95% CI, 76.2%, 99.9%)	

Note: Accuracy in identifying pheochromocytomas: 84.6% (95% CI, 69.5%, 94.1%).
Abbreviations: CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value.

dexamethasone-resistant dogs [22]. Of dogs with pituitary-dependent hyperadrenocorticism, 39% and 25% fail to suppress using a low- and high-dose dexamethasone tests, respectively [23]. Thus, it is possible to have dogs with pituitary-dependent hyperadrenocorticism and a concurrent non-functional adrenocortical tumor or pheochromocytoma, potentially complicating the interpretation using a suppression test. Diagnostic tests based on functionality of the adrenocortical tumor present a challenge with diagnosing adrenocortical tumors. Because cytology is not dependent on tumor function for its diagnostic utility, it provides an opportunity to identify non-functional adrenocortical tumors that may be otherwise difficult to identify. Another limitation for adrenal tumor differentiation is that endocrine testing for adrenocortical tumors is only indicated when clinical signs of hyperadrenocorticism are present [24]. The utility of such diagnostic tests in dogs with adrenal tumors that do not produce signs of hyperadrenocorticism is unclear. Cytology may allow diagnosis and differentiation of non-functional adrenocortical tumors that may fail to be diagnosed using other methods.

Of the dogs in our study that underwent FNA of the adrenal gland, 15.9% had complications related to the procedure. Most of the complications noted may have been secondary to general anesthesia or sedation required for sample procurement rather than the FNA procedure itself. In a similar safety study, only 5% ($n=1$) of dogs developed a complication, ventricular tachycardia, at the time of the procedure [15]. Another study estimated a death rate of approximately 1% associated with the procedure, based primarily on radiologist recollection, which is at risk for availability bias [16]. No dogs in our study died, had hemorrhage noted in the record, or required post-procedural supportive care beyond recovery from general anesthesia and sedation. Thus, the risk associated with performing a FNA was not perceived to be excessive.

Fine needle aspiration of the adrenal gland is performed in humans using computed tomography guidance or endoscopic ultrasound guidance with adequate samples retrieved in 90.4% of cases [25]. This result is similar to the 87.5% of samples having a predominant cell type identified that were considered of diagnostic quality in our study. Complications associated with computed tomography-guided needle biopsy in humans include pneumothorax, pain, hemorrhage, pancreatitis, and needle tract metastasis [26]. The complication rate reported in humans varies from 0% to 12% [25, 26]. Although we found a complication rate of 15.9%, it did not include any of these complications noted in humans. Confirmation of metastatic disease is reported in 53% of adrenal FNA in humans [25]. This finding contrasts with dogs in which only 2% of adrenal FNA are consistent with metastatic disease, and most are consistent with primary adrenal tumors [16]. Adrenal FNA in humans is regarded as a diagnostic that is useful for differentiating primary adrenal cortical and medullary tumors with complications considered uncommon, further suggesting utility in dogs [25, 26].

Limitations of our study are mostly related to its retrospective nature. Despite being a multi-institutional study, a limited number of dogs had pre-operative cytology of an adrenal tumor that ultimately was removed by adrenalectomy. Type II error is possible with failed identification of further group differences. Concurrent differentiating tests were not routinely performed, and direct comparisons regarding accuracy compared with endocrine testing such as low-dose dexamethasone suppression tests and urine normetanephrine concentrations could not be made. The clinical decision to perform FNA of the adrenal gland was determined by the clinician, which is a potential source of bias. One case identified had an undefined malignant neoplasm on histopathology and a non-diagnostic cytology. The role of cytology in such cases is unclear given the limited number of cases in our study. Another limitation is that differentiation between adrenocortical hyperplasia, adenomas, and adenocarcinomas was not performed because of the limited number of cases. In a larger data set, ability of cytology to further differentiate adrenocortical tumors would be of interest. Variation in pathologist certainty of diagnosis also was not assessed. Immunohistochemistry to confirm the diagnosis of a pheochromocytoma or adrenocortical tumor was not routinely performed. Additionally, protocols for sedation and general anesthesia, monitoring during the procedure, and performing the FNA were not standardized. Complications associated with the FNA procedure may have been secondary to the sedation or general anesthesia rather than FNA itself. Finally, there was a lack of long-term follow-up for development of complications such as needle-tract metastasis.

Overall, diagnostic cytologic samples have an accuracy of 85.6% in the population studied. The high PPV for identifying a pheochromocytoma within our study population suggests that a cytologic diagnosis of a neuroendocrine tumor should be viewed as

having a high likelihood of the dog having a pheochromocytoma diagnosed on histopathology. Information on differentiating adrenal tumor type may allow for a patient-focused approach in medical management of such dogs, especially in the pre-operative period, as well as may play a role in the decision to pursue surgical management. In a recent retrospective study of 302 dogs, 87% survived to discharge after adrenalectomy, with tumor-related survival of 3.96 years [2]. Pre-operative treatment has been best described for pheochromocytomas. Pre-treatment with phenoxybenzamine previously was reported to decrease the risk of mortality in dogs with pheochromocytomas, but this benefit has not been demonstrated in more recent studies [2, 6, 8, 27]. Many of the medications used for pre-operative management would best be used with knowledge of the specific adrenal tumor type and the associated risks of that tumor. Performance of a FNA with subsequent cytologic evaluation appears to be a relatively low risk procedure that may supplement information from other diagnostic tests and advanced imaging to help the clinician differentiate adrenocortical tumors and pheochromocytomas. This information may allow more individualized management of dogs with adrenal tumors and disease-specific risk assessment, which may benefit clinical management.

Acknowledgments

The authors have nothing to report.

Disclosure

The authors declare no off-label use of antimicrobials.

Ethics Statement

The authors declare no Institutional Animal Care and Use Committee or other approval was needed. The authors declare human ethics approval was not needed.

Conflicts of Interest

The authors declare no conflicts of interest.

References

1. J. I. Baum, S. E. Boston, and J. B. Case, "Prevalence of Adrenal Gland Masses as Incidental Findings During Abdominal Computed Tomography in Dogs: 270 Cases (2013-2014)," *Journal of the American Veterinary Medical Association* 249, no. 10 (2016): 1165–1169.

2. H. J. Piegols, B. E. Abrams, J. M. Lapsley, et al., "Risk Factors Influencing Death Prior to Discharge in 302 Dogs Undergoing Unilateral Adrenalectomy for Treatment of Primary Adrenal Gland Tumours," *Veterinary and Comparative Oncology* 21, no. 4 (2023): 673–684.

3. P. Pey, S. Specchi, F. Rossi, et al., "Prediction of Vascular Invasion Using a 7-Point Scale Computed Tomography Grading System in Adrenal Tumors in Dogs," *Journal of Veterinary Internal Medicine* 36, no. 2 (2022): 713–725.

4. M. F. van den Berg, H. S. Kooistra, G. C. M. Grinwis, et al., "Reference Intervals for Plasma, Urinary, and Salivary Concentrations of Free Metanephrines in Dogs: Relevance to the Diagnosis of Pheochromocytoma," *Journal of Veterinary Internal Medicine* 37, no. 1 (2023): 173–183.

5. C. Brömel, R. W. Nelson, E. C. Feldman, et al., "Serum Inhibin Concentration in Dogs With Adrenal Gland Disease and in Healthy Dogs," *Journal of Veterinary Internal Medicine* 27, no. 1 (2013): 76–82.

6. D. Enright, V. M. Dickerson, J. A. Grimes, S. Townsend, and K. M. Thieman Mankin, "Short- and Long-Term Survival After Adrenalectomy in 53 Dogs With Pheochromocytomas With or Without Alpha-Blocker Therapy," *Veterinary Surgery* 51, no. 3 (2022): 438–446.

7. R. Gostelow, N. Bridger, and H. M. Syme, "Plasma-Free Metanephrine and Free Normetanephrine Measurement for the Diagnosis of Pheochromocytoma in Dogs," *Journal of Veterinary Internal Medicine* 27, no. 1 (2013): 83–90.

8. M. A. Herrera, M. L. Mehl, P. H. Kass, P. J. Pascoe, E. C. Feldman, and R. W. Nelson, "Predictive Factors and the Effect of Phenoxybenzamine on Outcome in Dogs Undergoing Adrenalectomy for Pheochromocytoma," *Journal of Veterinary Internal Medicine* 22, no. 6 (2008): 1333–1339.

9. F. Zublena, C. De Gennaro, and F. Corletto, "Retrospective Evaluation of Labetalol as Antihypertensive Agent in Dogs," *BMC Veterinary Research* 16, no. 1 (2020): 256.

10. F. F. Argenta, M. Slaviero, L. S. de Mello, et al., "Pathological Aspects and Immunohistochemical Evaluation of Troponin C in the Cardiovascular System of Dogs With Pheochromocytoma," *Topics in Companion Animal Medicine* 53-54 (2023): 100777.

11. P. Vilar Saavedra, A. L. Garcia, S. Z. Lopez, et al., "Hemostatic Abnormalities in Dogs With Carcinoma: A Thromboelastographic Characterization of Hypercoagulability," *Veterinary Journal* 190, no. 2 (2011): 378–383.

12. H. P. Ehrlich and T. K. Hunt, "Effects of Cortisone and Vitamin A on Wound Healing," *Annals of Surgery* 167 (1968): 324–328.

13. J. Espinel-Rupérez, M. D. Marín-Ríos, V. Salazar, et al., "Incidence of Surgical Site Infection in Dogs Undergoing Soft Tissue Surgery: Risk Factors and Economic Impact," *Veterinary Record. Open* 6, no. 1 (2019): e000233.

14. G. Hayes, "Update of Adrenalectomy," *Veterinary Clinics of North America. Small Animal Practice* 52, no. 2 (2022): 473–487.

15. J. A. Sumner, L. Lacorcia, A. M. Rose, A. P. Woodward, and J. E. Carter, "Clinical Safety of Percutaneous Ultrasound-Guided Fine-Needle Aspiration of Adrenal Gland Lesions in 19 Dogs," *Journal of Small Animal Practice* 59, no. 6 (2018): 357–363.

16. P. Pey, A. Diana, F. Rossi, et al., "Safety of Percutaneous Ultrasound-Guided Fine-Needle Aspiration of Adrenal Lesions in Dogs: Perception of the Procedure by Radiologists and Presentation of 50 Cases," *Journal of Veterinary Internal Medicine* 34, no. 2 (2020): 626–635.

17. W. Bertazzolo, M. Didier, M. E. Gelain, et al., "Accuracy of Cytology in Distinguishing Adrenocortical Tumors From Pheochromocytoma in Companion Animals," *Veterinary Clinical Pathology* 43, no. 3 (2014): 453–459.

18. A. K. LeBlanc, M. Atherton, R. T. Bentley, et al., "Veterinary Cooperative Oncology Group-Common Terminology Criteria for Adverse Events (VCOG-CTCAE v2) Following Investigational Therapy in Dogs and Cats," *Veterinary and Comparative Oncology* 19, no. 2 (2021): 311–352.

19. S. Quante, F. S. Boretti, P. H. Kook, et al., "Urinary Catecholamine and Metanephrine to Creatinine Ratios in Dogs With Hyperadrenocorticism or Pheochromocytoma, and in Healthy Dogs," *Journal of Veterinary Internal Medicine* 24, no. 5 (2010): 1093–1097.

20. N. Sasaki, Y. Ikenaka, Y. Inoue, et al., "Urinary Free Metanephrines Measurement in Dogs With Adrenal Gland Diseases Using a New Simple Liquid Chromatography Tandem Mass Spectrometry Method," *Journal of Veterinary Medical Science* 83, no. 4 (2021): 648–655.

21. K. Höglund, H. Palmqvist, S. Ringmark, and A. Svensson, "Quantification of Normetanephrine in Canine Urine Using ELISA: Evaluation of Factors Affecting Results," *Journal of Veterinary Diagnostic Investigation* 34, no. 1 (2022): 28–35.

22. K. L. van Bokhorst, H. S. Kooistra, S. A. E. B. Boroffka, and S. Galac, "Concurrent Pituitary and Adrenocortical Lesions on Computed

Tomography Imaging in Dogs With Spontaneous Hypercortisolism," *Journal of Veterinary Internal Medicine* 33, no. 1 (2019): 72–78.

23. E. C. Feldman, R. W. Nelson, and M. S. Feldman, "Use of Low- and High-Dose Dexamethasone Tests for Distinguishing Pituitary-Dependent From Adrenal Tumor Hyperadrenocorticism in Dogs," *Journal of the American Veterinary Medical Association* 209, no. 4 (1996): 772–775.

24. E. N. Behrend, H. S. Kooistra, R. Nelson, C. E. Reusch, and J. C. Scott-Moncrieff, "Diagnosis of Spontaneous Canine Hyperadrenocorticism: 2012 ACVIM Consensus Statement (Small Animal)," *Journal of Veterinary Internal Medicine* 27, no. 6 (2013): 1292–1304.

25. A. G. Novotny, J. P. Reynolds, A. A. Shah, et al., "Fine-Needle Aspiration of Adrenal Lesions: A 20-Year Single Institution Experience With Comparison of Percutaneous and Endoscopic Ultrasound Guided Approaches," *Diagnostic Cytopathology* 47, no. 10 (2019): 986–992.

26. M. K. Mody, E. A. Kazerooni, and M. Korobkin, "Percutaneous CT-Guided Biopsy of Adrenal Masses: Immediate and Delayed Complications," *Journal of Computer Assisted Tomography* 19, no. 3 (1995): 434–439.

27. C. Appelgrein, G. Hosgood, E. Drynan, and A. Nesbitt, "Short-Term Outcome of Adrenalectomy in Dogs With Adrenal Gland Tumours That Did Not Receive Pre-Operative Medical Management," *Australian Veterinary Journal* 98, no. 9 (2020): 449–454.