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Plasma-Free Metanephrine and Free Normetanephrine Measurement for the Diagnosis of Pheochromocytoma in Dogs

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Background: Measurement of plasma-free metanephrines is the test of choice to identify pheochromocytoma in human patients.

Objectives: To establish the sensitivity and specificity of plasma-free metanephrine (fMN) and free normetanephrine (fNMN) concentrations to diagnose pheochromocytoma in dogs.

Animals: Forty-five client-owned dogs (8 dogs with pheochromocytoma, 11 dogs with adrenocortical tumors, 15 dogs with nonadrenal disease, and 11 healthy dogs.)

Methods: A prospective study. EDTA plasma was collected from diseased and healthy dogs and submitted for fMN and fNMN measurement by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Results: Free MN concentration (median [range]) was significantly higher in dogs with pheochromocytoma (8.15 [1.73–175.23] nmol/L) than in healthy dogs (0.95 [0.68–3.08] nmol/L; $P < .01$) and dogs with adrenocortical tumors (0.92 [0.25–2.51] nmol/L; $P < .001$), but was not different from dogs with nonadrenal disease (1.91 [0.41–6.57] nmol/L; $P \geq .05$). Free NMN concentration was significantly higher in dogs with pheochromocytoma (63.89 [10.19–190.31] nmol/L) than in healthy dogs (2.54 [1.59–4.17] nmol/L; $P < .001$), dogs with nonadrenal disease (3.30 [1.30–10.10] nmol/L; $P < .001$), and dogs with adrenocortical tumors (2.96 [1.92–5.01] nmol/L); $P < 0.01$). When used to diagnose pheochromocytoma, a fMN concentration of 4.18 nmol/L had a sensitivity of 62.5% and specificity of 97.3%, and a fNMN concentration of 5.52 nmol/L had a sensitivity of 100% and specificity of 97.6%.

Conclusions and Clinical Importance: Plasma fNMN concentration has excellent sensitivity and specificity for the diagnosis of pheochromocytoma in dogs, whereas fMN concentration has moderate sensitivity and excellent specificity. Measurement of plasma-free metanephrines provides an effective, noninvasive, means of identifying dogs with pheochromocytoma.

Key words: Adrenal; Canine; Diagnosis.

Pheochromocytomas are catecholamine-producing tumors derived from chromaffin cells in the adrenal medulla or extra-adrenal sympathetic nervous system, where they are termed paragangliomas.[1] Pheochromocytomas should be considered malignant tumors in dogs. Potential consequences include invasion of the caudal vena cava and surrounding soft tissues, which occurs in over 50% of cases,[2] metastatic spread to local lymph nodes and distant organs,[3] and massive retroperitoneal hemorrhage.[4]

Clinical signs of pheochromocytoma result from excessive secretion of catecholamines from tumor tissue. These signs typically are vague and nonspecific but can be acute and life threatening, making identification of affected patients important. The most commonly reported clinical signs in affected dogs include lethargy, tachyarrhythmias, polyuria and polydipsia, and collapse but also may include cardiac arrest, dyspnea, and hemoabdomen.[3] In human patients, exocytosis of epinephrine and norepinephrine from pheochromocytoma cells often is intermittent, making many pheochromocytomas clinically silent for periods of time.[5] Over 50% of pheochromocytomas in dogs are identified during investigation of a concurrent disease or during necropsy examination,[3] suggesting that many pheochromocytomas in dogs also are unassociated with apparent clinical signs.

Treatment of pheochromocytoma requires adrenalectomy, whereas other adrenal tumors may be biologically inactive, or may respond to medical management alone, such as those causing adrenal-dependent hyperadrenocorticism. However, anesthesia and adrenalectomy in pheochromocytoma patients may be associated with complications such as blood pressure variation, tachyarrhythmias, and intraoperative hemorrhage from the tumor site.[6] Treatment with phenoxybenzamine

Abbreviations:

AUC	area under curve
COMT	catechol O-methyltransferase
CV	coefficient of variation
fMN	free metanephrine
fNMN	free normetanephrine
HPLC	high pressure liquid chromatography
LC-MS/MS	liquid chromatography-tandem mass spectrometry
LDDST	low dose dexamethasone suppression test
MN	metanephrine
NMN	normetanephrine

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before adrenalectomy substantially decreases perioperative mortality in dogs with pheochromocytoma.[6] Therefore, identifying affected patients before adrenalectomy is necessary to avoid needless surgery, allow beneficial antihypertensive medication to be started before adrenalectomy and ensure that sufficient care is given in the pre- and postoperative periods.

There is no widely used test for the diagnosis of pheochromocytoma in dogs, and affected patients show no consistent abnormalities on CBC, biochemical profiles, or urinalysis. Affected dogs tend to be middle aged or older.[1] Diagnosis is made more challenging by the fact that affected patients commonly have concurrent diseases, which can include both pituitary-dependent and adrenal-dependent hyperadrenocorticism.[3,7,8] Definitive diagnosis of pheochromocytoma in dogs currently relies on histopathology of adrenal tissue after adrenalectomy or necropsy. In human patients, detection of increased concentrations of catecholamines and their breakdown products in urine or plasma is the standard test for pheochromocytoma.[9] These breakdown products include metanephrine (MN) and normetanephrine (NMN), which collectively are referred to as "metanephrines". Measurement of plasma-free metanephrines generally is considered to be superior to measurement of other catecholamine products, and a multicenter, prospective study reported a sensitivity of 99% and specificity of 89% when using plasma-free metanephrine (fMN) and free normetanephrine (fNMN) concentrations to identify humans with pheochromocytoma.[10]

Measurement of metanephrines rarely is performed in dogs. Urinary NMN-to-creatinine ratio has been shown to be significantly increased in dogs with pheochromocytoma compared with dogs with hyperadrenocorticism and healthy dogs,[11,12] but plasma-free metanephrines previously have only been measured in healthy laboratory Beagles.[13] This study aimed to measure plasma fMN and fNMN concentrations in dogs with pheochromocytoma, adrenocortical tumors, nonadrenal disease, and healthy dogs by liquid chromatography-tandem mass spectrometry (LC-MS/MS) and to determine the sensitivity and specificity of plasma fMN and fNMN concentration for the diagnosis of pheochromocytoma in dogs.

Materials and Methods

Dogs were recruited prospectively between March 2007 and November 2010. Dogs undergoing investigation of an adrenal mass and dogs with nonadrenal disease were recruited from the Royal Veterinary College and other first-opinion and referral practices. Healthy dogs were recruited from patients presenting for elective surgery or health-check appointments at the Royal Veterinary College or first-opinion practices.

Background information recorded for each dog included signalment, body weight, underlying disease, and presence of metastasis or local invasion of adjacent structures by adrenal tumors. Local tissue invasion or metastasis was identified by diagnostic imaging, exploratory laparotomy, or postmortem examination. Recent treatment with trilostane,[a] corticosteroids, or medications known to affect plasma MN and NMN concentrations, such as monoamine oxidase inhibitors, beta-receptor antagonists, calcium-channel blockers, and alpha-adrenoreceptor antagonists,[14,15] also was recorded. All dogs were assigned a disease severity score based on the American Society of Anesthesiologists Physical Status Classification System[16] (Table 1). All disease severity scores were assigned by a single clinician (RG) after a review of each patient's hospital record.

Recruited dogs were categorized as healthy, having nonadrenal disease, pheochromocytoma, or an adrenocortical tumor. Dogs were classified as healthy based on normal physical examination findings, unremarkable serum biochemical results, and the absence of owner-reported clinical signs. Adrenocortical tumors were diagnosed on the basis of histology, or owing to documentation of a good clinical response to trilostane therapy after endocrine test results that supported a diagnosis of adrenal-dependent hyperadrenocorticism in a patient with compatible historical and physical examination findings. All fMN and fNMN measurements were performed before the start of trilostane therapy in these dogs. All pheochromocytomas were diagnosed histologically.

Free Metanephrine and Free Normetanephrine Measurement

Three to four milliliters of blood were collected in EDTA from each dog for fMN and fNMN measurement. Plasma was separated within 6 hours of collection and was stored at −80°C before measurement of fMN and fNMN concentrations by LC-MS/MS at the Central Laboratory for Clinical Chemistry at the University of Groningen. All samples were shipped overnight on dry ice. The study was approved by the Ethics Committee of the Royal Veterinary College and all samples were obtained with owner consent.

Free MN and fNMN were measured by LC-MS/MS. Solid-phase extraction, high pressure liquid chromatography (HPLC), and tandem mass spectrometry stages all were performed by a Spark Holland system.[b] This system previously had been validated for human plasma samples[17] and a brief validation for canine plasma samples was performed as part of the current study. Two separate groups of 6 EDTA plasma samples were used for calculation of intra- and interassay coefficient of variation (CV). The intra-assay CV for fMN measurement was 1.7% at a mean fMN concentration of 0.74 nmol/L, and the interassay CV was 3.15% at a mean fMN concentration of 1.53 nmol/L. Validation for fNMN measurement produced an intra-assay coefficient of variation (CV) of 1.4% at a mean fNMN concentration of 2.00 nmol/L, and an interassay CV of 2.4% at a mean fNMN concentration of 4.56 nmol/L.

Table 1. Explanation of disease severity scores assigned to patients.

Score	Description
1	Healthy patient not presented for disease process, eg, neutering, vaccination appointment
2	Localized disease, without systemic effects and causing no biochemical abnormalities, eg, orthopedic injury, wounds, mild gastrointestinal disease
3	Patient appears clinically well but has systemic disease causing mild-to-moderate biochemical abnormalities
4	Compromised patient with systemic disease
5	Unstable patient or disease that may cause rapid deterioration if untreated

Statistical Analysis

Kruskal–Wallis tests followed by Dunn's multiple comparison analysis were used to compare variables, including fMN and fNMN concentrations, among the 4 groups of dogs. Significance was set at $P < .05$ and all results were reported as median and range.

The results of fMN and fNMN measurements were used to calculate the sensitivity and specificity of fMN and fNMN concentration for the diagnosis of pheochromocytoma in dogs. Free MN and fNMN results were combined for all dogs without pheochromocytoma (ie, those in the healthy, nonadrenal disease and adrenocortical tumor groups) to create the reference group. The 2.5th and 97.5th percentiles were calculated for both fMN and fNMN concentrations within this group by nonparametric methods to give ranges incorporating 95% of measured values. The 97.5th percentile for fMN and fNMN concentration in this group was used as a cut-off point to determine the sensitivity and specificity of fMN and fNMN concentrations for the diagnosis of pheochromocytoma. Receiver operating characteristic curves for fMN and fNMN were plotted and linear regression was used to investigate a possible relationship between tumor size and fMN, and fNMN, concentrations in dogs with pheochromocytoma. All analyses were performed by commercial statistical software packages.[c,d]

Results

Eight dogs with pheochromocytoma, 11 dogs with adrenocortical tumors, 15 dogs with nonadrenal disease, and 11 healthy dogs were recruited. Dogs with nonadrenal disease consisted of 2 dogs with gastrointestinal foreign bodies, 2 dogs with inflammatory bowel disease, 2 dogs with neurological disease, and 1 dog with each of the following: hereditary hypocobalaminemia, renal dysplasia, protein-losing nephropathy, exercise intolerance, chronic bronchitis, primary hyperparathyroidism, portosystemic shunt, extensive skin wounds, and psychogenic polydipsia.

All pheochromocytomas or adrenocortical tumors were unilateral. Ultrasonographic tumor measurements were available for all pheochromocytomas and 9 adrenocortical tumors. Median maximum tumor diameter was 5.0 cm (range 3.0–7.0 cm) for pheochromocytomas and 4.1 cm (range 2.2–7.0 cm) for adrenocortical tumors. Distant metastasis was not documented for any tumor. Local invasion was present in 1 adrenocortical tumor and 6 pheochromocytomas. Linear regression analysis indicated a significant correlation between pheochromocytoma diameter and fMN concentration ($P = 0.04$), but no significant correlation between pheochromocytoma diameter and fNMN concentration ($P = 0.31$).

Nine adrenocortical tumors were diagnosed histologically. These consisted of 2 adenomas, 4 carcinomas, and 3 tumors classified as adenomas with borderline carcinomatous change. Histology was performed after adrenalectomy in 5 of these cases. Two of these dogs had hyperadrenocorticism, 2 had nonfunctional tumors, and 1 dog had an adrenocortical tumor causing signs of mineralocorticoid excess caused by 11-deoxycorticosterone secretion. Histology was performed after postmortem examination in the other 4 cases. One of these dogs was suspected of having hyperadrenocorticism because of consistent clinical signs and ACTH stimulation test results, but the functional status of the other 3 tumors was unknown.

Two adrenocortical tumors were diagnosed on the basis of endocrine testing and a good response to trilostane therapy. Both of these dogs had clinical signs consistent with hyperadrenocorticism, which resolved with trilostane therapy. One dog underwent an ACTH stimulation test, which was not consistent with hyperadrenocorticism, and an LDDST, which was consistent with the diagnosis. The 2nd dog presented for polydipsia, lethargy, and polyphagia. This dog had normal responses to a routine ACTH stimulation test and LDDST, but showed an increased serum 17-hydroxyprogesterone concentration after ACTH stimulation. This dog was clinically suspected of having hyperadrenocorticism, and its clinical signs resolved with trilostane therapy.

Seven pheochromocytomas were associated with clinical signs, including shaking, vomiting, and panting. One pheochromocytoma was diagnosed incidentally, although this dog had a history of hypertension, which later was thought to have been caused by the tumor. Endocrine testing performed in dogs with pheochromocytomas included ACTH stimulation testing alone (n = 3), LDDST administration alone (n = 2), or both ACTH stimulation testing and LDDST administration (n = 1). All of these results were unremarkable, except for 1 ACTH stimulation test, which yielded both an increased basal cortisol concentration (724 nmol/L; reference interval, <250 nmol/L) and poststimulation cortisol concentration (961 nmol/L; reference interval, <500 nmol/L). This occurred in a dog with a pheochromocytoma that was diagnosed at postmortem examination, and no evidence of adrenocortical neoplasia was found. Two dogs with pheochromocytoma had no endocrine testing performed, apart from fMN and fNMN measurements. Four pheochromocytomas were considered to be histologically malignant. No immunohistochemical analysis was performed on any tumor. One dog with pheochromocytoma was receiving phenoxybenzamine PO at the time of sampling and had a fMN concentration of 11.64 nmol/L and a fNMN concentration of 61.6 nmol/L. One dog with an adrenocortical tumor underwent a LDDST 12 hours before fMN and fNMN measurement and had a fMN concentration of 2.19 nmol/L and a fNMN concentration of 3.99 nmol/L. No other dogs were receiving medication known to alter plasma fMN or fNMN concentrations.

Patient characteristics and the results of fMN and fNMN measurement are shown in Table 2. There was no significant difference in body weight among the 4 groups ($P = .8$). Dogs with pheochromocytoma were significantly older ($P < .01$) and had a higher disease severity score ($P < .001$) than healthy dogs, but there was no significant difference in age or disease severity between dogs with pheochromocytoma and dogs with nonadrenal disease or adrenocortical tumors (all $P \geq .05$).

Plasma fMN concentration was significantly higher in dogs with pheochromocytoma than in healthy dogs ($P < .01$) and dogs with adrenocortical tumors ($P < .001$), but was not significantly different from dogs with nonadrenal disease ($P \geq .05$) (Table 2, Fig 1). Plasma fNMN concentration was significantly higher in dogs with pheochromocytoma than in healthy dogs ($P < .001$), dogs with nonadrenal illness ($P < .001$), and dogs with adrenocortical tumors ($P < .01$) (Table 2, Fig 2). Free NMN concentration in pheochromocytoma showed less overlap with fNMN concentrations measured in other groups than was shown for fMN concentration (Figs 1 and 2). A significant difference was found between fMN concentration in dogs with nonadrenal disease and dogs with adrenocortical tumors ($P < .05$), but no other difference in fMN or fNMN concentration was detected among healthy dogs, dogs with nonadrenal disease, or dogs with adrenocortical tumors.

The 2.5th and 97.5th percentiles of fMN concentration in all dogs without pheochromocytoma were 0.29 nmol/L and 4.18 nmol/L, respectively. Using the 97.5th percentiles as a cut-off value gave a sensitivity of 62.5% (95% confidence interval [CI], 30.6–86.3%) and specificity of 97.3% (95% CI, 86.2–99.5%) for the diagnosis of pheochromocytoma using fMN concentration. The 2.5th and 97.5th percentiles of fNMN concentration in all dogs without pheochromocytoma were 1.56 nmol/L and 5.52 nmol/L, respectively. Using the 97.5th percentile as a cut-off value gave a sensitivity of 100% (95% CI, 67.6–100%) and specificity of 97.6% (95% CI, 86.2–99.5%) for diagnosis of pheochromocytoma using fNMN concentration.

Receiver operating characteristic curves for the diagnosis of pheochromocytoma in dogs using fMN and fNMN concentration yielded an AUC of 0.9 for fMN concentration (95% CI, 0.79–1.00) and an AUC of 1.0 (95% CI, 1.0–1.0) for fNMN concentration (Fig 3). These values indicate that, provided appropriate cut-off points are chosen, fMN concentration shows very good discrimination, and fNMN concentration shows excellent discrimination, for the diagnosis of pheochromocytoma in dogs.

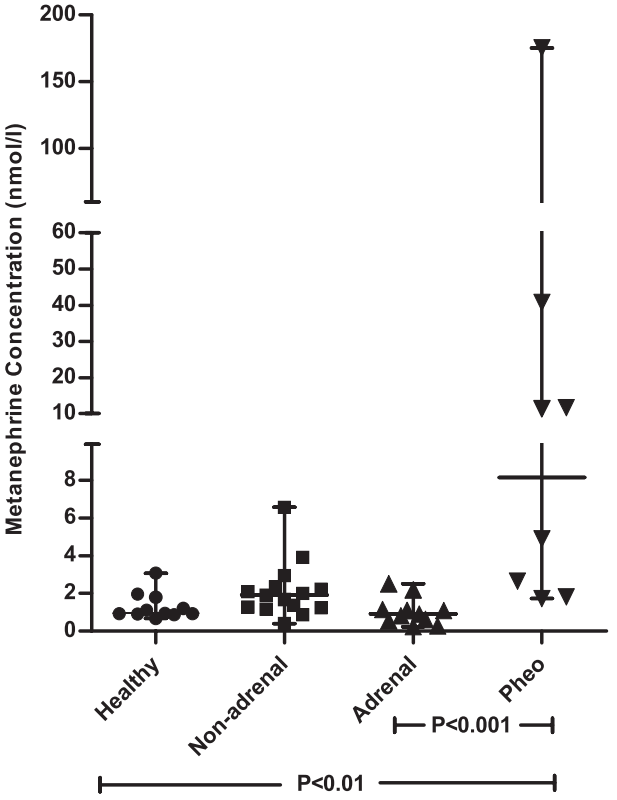

Fig 1. Scatterplot of free plasma metanephrine concentrations in healthy dogs (Healthy, n = 11, •), dogs with nonadrenal disease (Nonadrenal, n = 15, ■), dogs with adrenocortical tumors (Adrenal, n = 11, ▲), and dogs with pheochromocytoma (Pheo, n = 8, ▼). Horizontal bar represents median concentration. Statistical significance between pheochromocytoma group and other groups is shown below the graph.

Discussion

The aim of this study was to compare plasma fMN and fNMN concentrations in dogs with pheochromocytoma, adrenocortical tumors, nonadrenal disease, and healthy dogs, and to assess the sensitivity and specificity of plasma fMN and fNMN concentration

Table 2. Median plasma-free metanephrine and normetanephrine concentrations and physical characteristics of healthy dogs, dogs with nonadrenal disease, dogs with adrenocortical tumors, and dogs with pheochromocytoma.

	Healthy	Nonadrenal Disease	Adrenocortical Tumor	Pheochromocytoma
Number (n)	11	15	11	8
Age (years)	3.3** (1.5–7.0)	6.5 (0.3–14.2)	10.6 (6.2–14.0)	11.1 (8.3–14.3)
Bodyweight (kg)	22.0 (5.0–35.3)	20.3 (5.3–61.5)	23.6 (9.2–46.7)	26.3 (5.5–38.5)
Number of males	4 (All MN)	8 (4 MN, 4 ME)	3 (2 MN, 1 ME)	7 (5 MN, 2 ME)
Number of females	7 (6 FN, 1 FE)	7 (6 FN, 1 FE)	8 (All FN)	1 (FN)
Disease Severity Score	1*** (1–1)	2 (2–4)	3 (3–5)	4 (3–5)
MN Concentration (nmol/L)	0.95** (0.68–3.08)	1.91 (0.41–6.57)	0.92*** (0.25–2.51)	8.15 (1.73–175.23)
NMN concentration (nmol/L)	2.54*** (1.59–4.17)	3.30*** (1.30–10.1)	2.96** (1.92–5.01)	63.89 (10.19–190.31)

MN, Male neutered; ME, Male entire; FN, Female neutered; FE, Female entire.
** and *** indicate a statistically significant difference compared with pheochromocytoma group (**P<.01, ***P<.001).

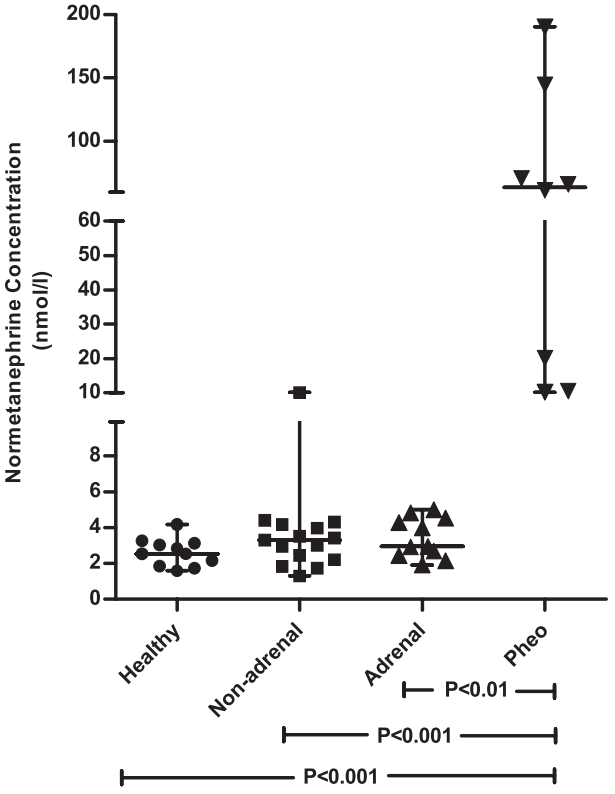

Fig 2. Scatterplot of free plasma normetanephrine concentrations in healthy dogs (Healthy, n = 11, •), dogs with nonadrenal disease (Nonadrenal, n = 15, ■), dogs with adrenocortical tumors (Adrenal, n = 11, ▲), and dogs with pheochromocytoma (Pheo, n = 8, ▼). Horizontal bar represents median concentration. Statistical significance between pheochromocytoma group and other groups is shown below the graph.

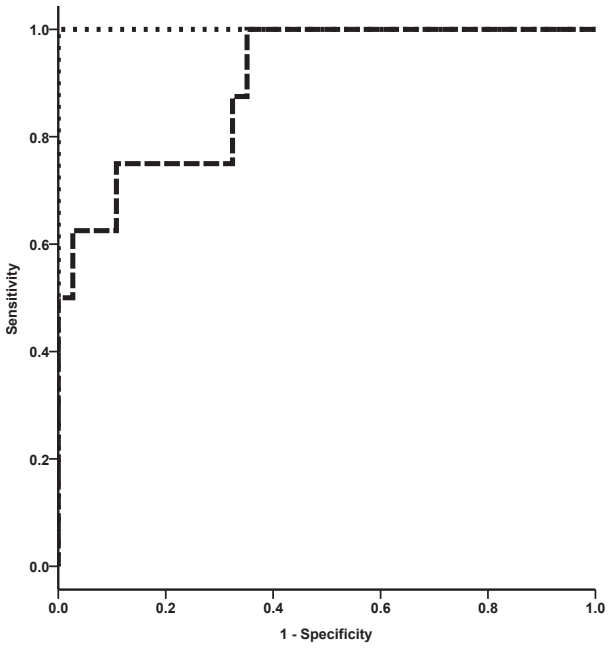

Fig 3. Receiver operating characteristic curves to compare the discriminatory value of plasma-free metanephrine concentration (dashed line) and normetanephrine concentration (dotted line) for the diagnosis of pheochromocytoma in dogs.

for the diagnosis of pheochromocytoma in dogs. Plasma fMN concentration was significantly higher in dogs with pheochromocytoma than in healthy dogs and dogs with adrenocortical tumors, but was not significantly different from dogs with nonadrenal disease. Free NMN concentrations in dogs with pheochromocytoma were significantly higher than in dogs with adrenocortical tumors, nonadrenal disease, and healthy dogs. Free NMN concentration showed high sensitivity and specificity for the diagnosis of pheochromocytoma, whereas fMN concentration showed moderate sensitivity and high specificity for the diagnosis of pheochromocytoma. Plasma fNMN concentration in pheochromocytoma patients showed less overlap with fNMN concentrations measured in the other 3 groups than did fMN concentration.

This is the first study to measure plasma fMN and fNMN concentrations in diseased dogs, including those with pheochromocytoma, although previous studies have evaluated the use of urinary metanephrine- and catecholamine-to-creatinine ratios in diagnosing pheochromocytoma in dogs.[11,12] Those studies determined that urinary normetanephrine-to-creatinine ratio is significantly increased in dogs with pheochromocytoma compared with dogs with hyperadrenocorticism and normal dogs. Plasma fMN and fNMN concentrations generally are considered to be the optimal test for diagnosing pheochromocytoma in humans and are reported to be more accurate than other biochemical tests, including urinary metanephrine-to-creatinine ratios.[10,18] However, 1 study reported similar sensitivity and specificity using free plasma metanephrines and urinary metanephrines for the diagnosis of pheochromocytoma in people.[19] The high sensitivity and specificity of plasma fNMN concentration for the diagnosis of pheochromocytoma found in this study are similar to that reported for measurement of plasma-free metanephrines in human patients.[10,18,19] The sensitivity of fNM concentration in this study is lower than these results. However, studies in humans frequently examine the diagnostic utility of the sum of fMN and fNMN, making comparison difficult.

Epinephrine and norepinephrine released into the bloodstream from the adrenal medulla are metabolized to fMN and fNMN, respectively, by catechol O-methyltransferase (COMT) enzyme activities in body tissues. However, pheochromocytoma tissue from human patients has been shown to contain a high concentration of COMT. This leads to a high rate of fMN and fNMN production within the tumor tissue itself, which is independent of daily variations in epinephrine and norepinephrine secretion into the circulation. The continuous, intratumoral production of fMN and fNMN in pheochromocytoma patients makes measurement of fMN and fNMN more accurate in diagnosing pheochromocytoma than measurement of the parent catecholamines.[20] Most pheochromocytomas in human patients predominantly secrete norepinephrine,[21] which is metabolized to fNMN, making fNMN concentration

typically higher than fMN concentration.[10] There currently is no study evaluating the secretory pattern of pheochromocytoma in dogs, but the results of this study and previous studies evaluating urinary metanephrines[11,12] suggest that fNMN also is produced in greater quantities than fMN in dogs with pheochromocytoma and therefore is more useful in identifying affected dogs. Plasma fMN and fNMN concentrations are strongly correlated with tumor diameter in human pheochromocytoma patients[22] and a similar correlation between fMN concentration and pheochromocytoma diameter was found in the dogs in the current study.

This study is limited by a relatively small sample size because of the rarity of pheochromocytoma in dogs. The small sample size means that the reference intervals for fMN and fNMN concentration calculated in this study contain fewer patients than is ideal. Reference intervals typically are generated using only healthy individuals. In this study, we included sick patients, including those with other adrenal tumors when generating reference intervals, so these intervals better represent dogs that may be tested for pheochromocytoma. Urinary catecholamine-to-creatinine ratios, and metanephrine-to-creatinine ratios, have been shown to significantly increase in critically ill dogs compared with healthy controls.[23] An increase in plasma fMN concentration during illness may explain the finding that fMN concentration in dogs with nonadrenal disease did not differ significantly from that of dogs with pheochromocytoma in this study. Alternatively, this finding could be an effect of small sample size. The median disease severity in dogs with nonadrenal illness in this study was relatively mild. The disease severity score used in this study was based on The American Society of Anesthesiologists Physical Status Classification System because published illness severity scores for hospitalized dogs require the results of CBC, and other diagnostic tests, and thus would not have been suitable for healthy dogs in this study.[24,25] All disease severity scores were assigned by a single clinician, but the scoring system used is inherently subjective. Additional studies should be performed to evaluate fMN and fNMN concentrations in dogs with critical illness, ideally utilizing published canine illness severity scores.

Phenoxybenzamine treatment can increase MN and NMN concentrations in human patients.[15] Corticosteroid treatment can trigger catecholamine release from pheochromocytoma tissue,[26] which also could result in increased MN and NMN concentrations. In this study, 1 dog received phenoxybenzamine treatment before being sampled for metanephrine measurement and another dog had an LDDST performed before metanephrine measurement. The dog receiving phenoxybenzamine was found to have fMN and fNMN concentrations in the midrange of concentrations documented in dogs with pheochromocytoma in this study. The dog that underwent an LDDST had fMN and fNMN concentrations in the upper range of those recorded from dogs with adrenocortical tumors. Free MN and fNMN concentrations in both of these dogs may have been increased by their medications. Prior phenoxybenzamine treatment or a recent LDDST are likely to be common occurrences in dogs undergoing investigation of pheochromocytoma and thus were not a surprising limitation of this study. However, it would have been ideal for no dog in this study to receive medications that might affect fMN and fNMN concentrations. Interestingly, the highest fNMN concentration (10.1 nmol/L) in the nonadrenal disease group was recorded in the dog affected by renal dysplasia and severe azotemia. Total plasma MN and NMN concentrations are known to increase in patients with kidney failure attributable to decreased renal clearance. Plasma fMN and fNMN concentrations are thought to be less affected by kidney failure because they are mainly cleared from the circulation by sulfation in the gastrointestinal tract.[27] The high fNMN concentration recorded in this dog suggests that kidney failure could increase free metanephrine concentrations in dogs, but this would require further investigation to be confirmed.

Plasma fMN and fNMN measurement by LC-MS/MS appears to be an accurate method of identifying affected dogs and is minimally invasive. However, availability is limited to centers capable of performing LC-MS/MS for metanephrines, and samples are likely to require frozen or refrigerated shipping to prevent degradation of metanephrines while in transit. The difficulties in diagnosing pheochromocytoma in dogs mean that a widely available test for canine plasma-free metanephrines would be of great benefit in investigating dogs with adrenal masses. A radioimmunoassay for plasma-free metanephrines has been validated in human patients[28] and has been used in dogs,[12] and free plasma metanephrines have been measured by ELISA in humans.[29] However, LC-MS/MS or HPLC is likely to remain the gold standard for fMN and fNMN measurement until immunoassays have been validated further.

A limitation of plasma fMN and fNMN measurement in human patients is that plasma concentrations in healthy individuals can be very low, making detection difficult. The upper limit for plasma fMN concentration in healthy humans is approximately 0.3 nmol/L with concentrations greater than approximately 1.2 nmol/L strongly suggestive of pheochromocytoma. Plasma fNMN concentration in healthy humans is typically <0.6 nmol/L with concentrations greater than approximately 2.0 nmol/L strongly suggestive of pheochromocytoma.[10,30] The findings of our study therefore suggest that dogs have higher plasma fMN and fNMN concentrations than humans and that this difference is especially marked in patients with pheochromocytoma. This means that detection of plasma fMN and fNMN by LC-MS/MS or HPLC is likely to be easier in canine plasma than in human plasma. However, the very high concentrations of free metanephrines found in the plasma of dogs with pheochromocytoma means that samples may require multiple dilutions for an accurate measurement to be obtained. Also, species-specific reference intervals should be utilized when interpreting canine plasma fMN and fNMN concentrations because using a reference interval from humans

is likely to wrongly raise suspicion for pheochromocytoma.

In conclusion, plasma fNMN concentration is significantly increased in dogs with pheochromocytoma compared with healthy dogs, dogs with nonadrenal disease, and dogs with adrenocortical tumors, and fMN concentration is significantly increased in dogs with pheochromocytoma compared with healthy dogs and dogs with adrenocortical tumors. Free NMN concentration showed high sensitivity and specificity for diagnosis of pheochromocytoma, whereas fMN concentration showed moderate sensitivity and high specificity. These results suggest that measurement of plasma-free metanephrines is an effective means of identifying pheochromocytoma in dogs. Canine fMN and fNMN concentrations are much higher than those encountered in human patients, making it crucial to use a species-specific reference interval when interpreting results.

Footnotes

[a] Vetoryl, Dechra Veterinary Products Ltd, Shrewsbury, UK
[b] Spark Holland Symbiosis, Spark Holland Inc, New Jersey
[c] GraphPad Prism, GraphPad Software Inc, California
[d] SPSS, IBM Corporation, Armonk, New York

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Conflict of Interest: Authors have no conflict of interest to disclose.

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