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Plasma and urinary metanephrine and normetanephrine concentrations using liquid chromatography with tandem mass spectrometry in healthy cats and in a cat with pheochromocytoma

Maria Teresa Prego[1] | Maria Joana Dias[1,2,3] | Rui Lemos Ferreira[1] | Salomé Gonçalves[2,3] | Tiago Dias Domingues[4] | Greet Junius[5] | Evi Van den Steen[5] | Sara Galac[6] | Rodolfo Oliveira Leal[1,2,3]

[1]Veterinary Teaching Hospital, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal

[2]CIISA – Centro de Investigação Interdisciplinar em Sanidade Animal, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal

[3]Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), University of Trás-os-Montes e Alto Douro, Vila Real, Portugal

[4]Department of Statistics and Operations Research, Faculty of Sciences, University of Lisbon, Lisbon, Portugal

[5]AML – Algemeen Medisch Laboratorium, Antwerpen, Belgium

[6]Department of Clinical Sciences, Utrecht University, Utrecht, The Netherlands

Correspondence
Rodolfo Oliveira Leal, Hospital Escolar Veterinário/Departamento de Clínica, Faculdade de Medicina Veterinária, Universidade de Lisboa, 1300-477 Lisbon, Portugal.
Email: rleal@fmv.ulisboa.pt

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Abstract

Background: Pheochromocytoma (PCC) is rare in cats and plasma (PL) and urinary (U) metanephrines (metanephrine [MN]; normetanephrine [NMN]) measurement is rarely described in cats.

Objectives: We evaluated the utility of PL and U MNs measurement in 10 healthy cats and a cat with a confirmed diagnosis of pheochromocytoma (PheoCat), using liquid chromatography with tandem mass spectrometry (LC-MS-MS).

Methods: Urine and EDTA PL samples collected from each of the 10 cats and the PheoCat were promptly stored at −80°C and remained frozen until analysis. To evaluate U MNs stability, an additional urine sample collected from the healthy cats was refrigerated for 24 hours before freezing. Urinary creatinine concentration (Creat) was assessed using the same spot urine samples to calculate U MNs-to-creatinine ratios.

Results: The PL-MN and PL-NMN median concentrations of the healthy cats were 2.73 and 7.02 nmol/L, respectively. The median U-MN/Creat and U-NMN/Creat ratios were 70 and 139 μg/g, respectively. The PheoCat had a PL-MN of 3.68 nmol/L, PL-NMN of 66.27 nmol/L, U-MN/Creat of 179 μg/g, and U-NMN/Creat of 1262 μg/g. The PheoCat had markedly increased concentrations of both PL and U MNs when compared to the healthy cats. No significant difference was found between U MNs measured in urine samples that underwent 24 hours of refrigeration in comparison to those that were frozen immediately.

Abbreviations: LC-MS-MS, liquid chromatography with tandem mass spectrometry; MN, metanephrine; NMN, normetanephrine; PCC, pheochromocytoma; PheoCat, cat with a pheochromocytoma; PL, plasma; U, urinary; UR, refrigerated urine.

Conclusions: We report preliminary reference intervals for PL and U MNs in cats using LC-MS-MS and the potential clinical applicability of these biomarkers for the diagnosis of PCC in cats.

KEYWORDS
feline pheochromocytoma, healthy cats, liquid chromatography with tandem mass spectrometry, plasma and urinary metanephrines

1 | INTRODUCTION

Pheochromocytoma (PCC) is a catecholamine-producing neuroendocrine tumor arising from chromaffin cells of the adrenal medulla.[1,2] They are extremely rare in cats and knowledge is limited to 6 cases with histological confirmation in the veterinary literature.[3-6] The antemortem diagnosis of PCC in cats is usually challenging because clinical signs are often nonspecific, episodic, and not noticed by the owners.[7,8] In the previous case reports of PCC in cats, polydipsia and polyuria and systemic hypertension were the most common clinical findings.[9] Therefore, PCC should be considered in cats exhibiting polyuria and polydipsia, weakness, hypertension, or some combination of these signs, especially if an adrenal mass is identified. Computed tomography is the examination of choice for the assessment of primary adrenal masses, local vascular invasion and intra-abdominal metastasis.[10,11] Initial screening for PCC requires biochemical evidence of inappropriate catecholamine production. However, a definitive diagnosis relies on histopathology of the adrenal mass.[12] Epinephrine and norepinephrine are converted into metanephrine (MN) and normetanephrine (NMN) in the adrenal medulla, respectively.[13,14] In contrast to catecholamines, these metabolites are continuously released into the circulation.[8] For this reason, plasma (PL) and urinary (U) metanephrines (MN and NMN) provide better diagnostic sensitivity than PL- or U-catecholamines.[15] In humans, measurement of PL-free and U fractioned MNs is most sensitive for diagnosis and most suitable for reliable exclusion of PCC.[15,16] The question of whether urine or PL is best is controversial, but PL MN concentrations are recommended more often as a test of choice in humans,[17] whereas it has been suggested that urine is superior to PL in dogs.[18] Regardless of the sample, there is a strong preference for NMN determination over MN both in dogs and humans.[15,19] Measurement of U MNs in dogs has been established using spot urine samples and their concentrations are correlated with U creatinine concentration.[20]

In cats, only 2 published studies report measurement of MNs. One assessed PL-free MNs using high performance liquid chromatography (HPLC),[21] and the other evaluated an ELISA for MNs in feline urine.[22] Liquid chromatography with tandem mass spectrometry (LC-MS-MS) has become more popular in human medicine, because it offers higher analytical specificity when compared to immunoassays or conventional HPLC.[23] Liquid chromatography with tandem mass spectrometry formerly was approved for PL-MN/NMN and U-MN/NMN measurement in dogs[20,24] and humans.[25] To the best of our knowledge, this methodology has not yet been used in cats for the measurement of MNs.

We evaluated the utility of PL-MN/NMN and U-MN/NMN measurement using LC-MS-MS in adult healthy cats and compared the concentrations obtained from healthy cats with those from a cat with a confirmed diagnosis of PCC (PheoCat). To evaluate the stability of U MNs in cats, we also assessed whether different storage conditions affected results by comparing measurements obtained from urine samples immediately stored at −80°C with samples refrigerated for 24 hours at +4°C before storage at −80°C.

2 | MATERIALS AND METHODS

2.1 | Animals

A cross-sectional study was conducted, including 10 clinically healthy adult cats, recruited from students and staff at the Veterinary Teaching Hospital, Faculty of Veterinary Medicine, University of Lisbon. The study was approved by the local ethical committee. With the owners' consent, all cats underwent physical examination, blood testing (including symmetric dimethylarginine, total T4 and electrolyte concentrations), urinalysis with protein-to-creatinine ratio, abdominal ultrasound examination, and systolic blood pressure (SBP) assessment. Cats were excluded if hypertension was detected (SBP mean >160 mm Hg measured using an oscillometric device), or if azotemia, hyperthyroidism, electrolyte imbalances, proteinuria or abnormal findings on abdominal ultrasound examination were detected. Ultrasonographic evaluation of the size of the adrenal glands in 2 body weight categories, performed by an experienced sonographer, was an inclusion criterion, with an accepted maximum of 3.9 mm thickness for cats weighing ≤4 kg and 4.8 mm for those weighing >4-8 kg.[26]

2.2 | PL-free and U MNs

Blood and urine samples (2 mL each) were collected from each cat for measurement of fractionated PL-free MNs and U MNs. Urine samples were collected by cystocentesis and were not submitted to an acidification process. Urine and EDTA blood samples were centrifuged and promptly stored at −80°C (U and PL, respectively).

2.3 | Urinary stability assessment

A portion of the urine collected from each cat was kept refrigerated for 24 hours at +4°C before storage at −80°C (UR).

2.4 | PheoCat

With the owner's consent, stored (−80°C) surplus PL and urine samples collected from an 8-year-old neutered male domestic shorthair cat previously diagnosed with PCC (confirmed by histopathology and immunohistochemistry) were submitted for analysis. Plasma and urine samples were collected before any treatment.

All samples were stored at −80°C until measurement of PL-MN/NMN and U-MN/NMN by LC-MS-MS at the AML-Algemeen Medisch Laboratorium. Samples were shipped on dry ice. Urine creatinine (Creat) was measured (Jaffe method on Architect Abbott C16000) in the same spot urine samples so as to calculate the U-MN/Creatinine and U-NMN/Creatinine ratios.

2.5 | Statistical analysis

All collected data were recorded in Microsoft Office Excel. Descriptive statistics and statistical tests were performed using the commercial statistical software IBM SPSS Statistics for Windows, version 28.0.1.0. For all statistical tests, *P* values <.05 were considered significant. Because of sample size, non-parametric tests were used. Median and interquartile range (IQR) were calculated to obtain a summary of the distribution of scores, apart from age of healthy cats which was presented as median and range. Statistical difference between refrigerated and immediately frozen urine samples was determined using the Wilcoxon signed-rank test for paired samples.

3 | RESULTS

3.1 | Sample population

Ten healthy cats were recruited, with a median age of 6 years (range, 4-10 years). Of these, 3 cats were males and 7 were females. All cats were neutered. Physical examination, blood test results and urinalysis were normal in all cats. All cats had normal-sized adrenal glands and unremarkable findings on abdominal ultrasound examination.

3.2 | PL-free and U MN and NMN

Frozen urine and PL were used. Samples from healthy cats were stored for a period of 1-3 months whereas those from the PheoCat were stored for 1 year until analysis.

The PL-MN and PL-NMN median concentrations of the healthy cats were 2.73 nmol/L (IQR, 2.37) and 7.02 nmol/L (IQR, 5.2), respectively. The U-MN/Creat ratio median value was 70 µg/g (IQR, 70) whereas the U-NMN/Creat ratio median value was 139 µg/g (IQR, 77).

Results obtained from the PheoCat indicated a PL-MN of 3.68 nmol/L and a PL-NMN of 66.27 nmol/L, which correspond to 1.3 and 9.4 times the median concentrations of the healthy cats. The PheoCat U-MN/Creat ratio was 179 µg/g and the U-NMN/Creat ratio was 1262 µg/g, corresponding to 2.55 and 9.07 times the median values observed in the healthy cats.

None of the results obtained from the PheoCat overlapped with the median values determined for the healthy cats (Figure 1). No overlap occurred between PL-NMN and U-NMN/Creat measurements of all healthy cats when compared to the PheoCat. Nevertheless, overlap between the results of the PheoCat and those of the 10 healthy cats occurred in 4/10 cats for PL-MN and in 1/10 cat for U-MN/Creat.

3.3 | Urinary MNs stability

The U-MN and UR-MN median concentrations of the healthy cats were 246 ng/mL (IQR, 316.75) and 253 ng/mL (IQR, 313.25), respectively. The U-NMN and UR-NMN median concentrations were 420 ng/mL (IQR, 401.25) and 429 ng/mL (IQR, 405.5), respectively. A Wilcoxon signed-rank test for paired samples was used to evaluate U MN stability after 24 hours of refrigeration. Both U MN and NMN proved to be stable for at least 24 hours at +4°C, and no significant difference was found between U-MN vs UR-MN (p .33) and U-NMN vs UR-NMN (p .81).

4 | DISCUSSION

We report the measurement of PL and U MNs by LC-MS-MS in healthy cats and in a cat with a confirmed diagnosis of PCC. The affected cat had a marked increase in all of the measured variables, particularly with respect to NMN, highlighting the clinical applicability of these biomarkers in the diagnosis of PCC in cats.

In human and veterinary medicine, initial testing for PCC should include measurement of fractionated MNs in urine or PL or both.[12,15] The purification and extraction steps in the HPLC assay are relatively laborious and slow, because urine samples are subjected to an acidification process and analytes are separated and pre-concentrated from urine using solid-phase extraction. The LC-MS-MS method therefore has become progressively more popular in biochemical analysis because of its simplicity, but also because of its high detection sensitivity and specificity.[23] This methodology previously has been used in dogs and humans for the measurement of PL and U MNs, and reference ranges are reported in these species.[20,24,25] To our knowledge, LC-MS-MS has not been performed previously in cats for the diagnosis of a PCC.

We intended to assess the normal concentrations of PL and U MNs in cats using LC-MS-MS, and to compare these results with those from a cat with a definitive diagnosis of PCC. Our results are

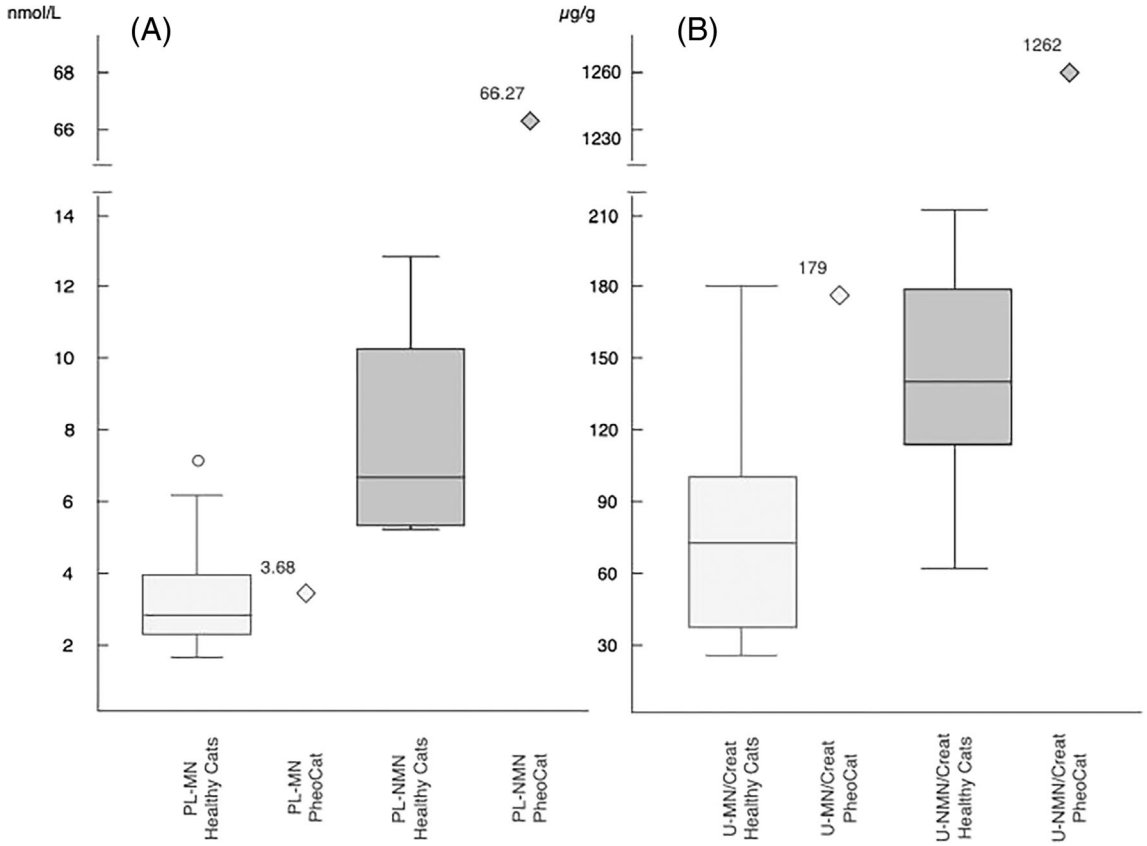

FIGURE 1 (A) Boxplot representation of the distribution of plasma metanephrine (PL-MN) and plasma normetanephrine (PL-NMN) in 10 healthy cats in comparison to the PL-MN/NMN values of the PheoCat. (B) Boxplot representation of the distribution of urinary metanephrine-to-creatinine (U-MN/Creat) and urinary normetanephrine-to-creatinine (U-NMN/Creat) ratios in 10 healthy cats in comparison to the U-MN/Creat and U-NMN/Creat ratios of the PheoCat.

consistent with studies of the biochemical diagnosis of PCC in humans and dogs, supporting the utility of PL-MN/NMN and U-MN/NMN measurement in cats by LC-MS-MS.

We showed that PL and U MNs in healthy cats differed from those reported in healthy dogs, because the median concentrations observed in this population of cats were almost 2 and 3 times higher than the median concentrations reported in healthy dogs for U and PL MNs, respectively.[20,24] These results reinforce the relevance of determining species-specific reference ranges. Results obtained from the PheoCat were considerably higher when compared to those of the healthy cats, particularly with respect to PL-NMN and U-NMN/Creat ratio, supporting the clinical applicability of these biomarkers. Overlap with the PheoCat occurred in 4/10 cats for PL-MN, in 1/10 cat for U-MN/Creat, and in none of the cats for both PL-NMN and U-NMN/Creat, emphasizing that NMN is a potentially better biomarker than MN for the diagnosis of PCC in cats. These results are in agreement with those of a previous study supporting that NMN is more useful than MN in the diagnosis of PCC in dogs, either in PL or urine.[19] In our study, no clear superiority of PL over urine or urine over PL was found. However, a larger population of cats would be needed for a definitive conclusion to be drawn.

Concerning the stability of U MNs in cats under different storage conditions, we found that U MNs were not significantly different in urine samples that underwent 24 hours refrigeration in comparison to those that were immediately frozen at −80°C. These findings suggest that U MNs in cats are stable for at least 24 hours at refrigeration temperatures, when measured by LC-MS-MS. Similar results have been reported for humans and dogs.[20,27] Because MN and NMN assays currently are not widely available in veterinary medicine, the stability of U MNs during transport to a specialized diagnostic facility is important. Moreover, given the observed stability of the refrigerated samples for 24 hours, urine collection at home may be a practical approach to explore in the future. Urine samples were not subjected to an acidification process because it has been suggested in humans that acidification is not necessary for MNs if samples are immediately analyzed or frozen.[27] This feature can be considered an additional advantage of LC-MS-MS over HPLC.

In conclusion, our study supports the utility of PL-MN/NMN and U-MN/NMN measurement in cats by LC-MS-MS and suggests that U MNs are stable for a period of 24 hours under refrigeration. The PheoCat had a marked increase in all of the measured variables, particularly PL-NMN and the U-NMN/Creat ratio, when compared to results in the healthy cats, emphasizing the clinical applicability of these biomarkers in the diagnosis of PCC in cats in the future. Additional investigations into PL and U MNs in a larger population of healthy cats and in cats with non-adrenal disease are required to establish reference ranges for this species.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

CEIE (Comissão de Ética para a Investigação e Ensino/Ethical Committee for Research and Teaching), after evaluation of the activities that involve animal manipulation and welfare, within the scope of the research project, considered that the ethical and animal welfare subjects are safeguarded, according to the current legislation and the code of good practices. Therefore, the CEIE approved the implementation of the experimental protocol that held at the Faculty of Veterinary Medicine facilities. Evaluation of the Research project—N/Ref 002/2022.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

ORCID

Sara Galac https://orcid.org/0000-0001-9159-5596
Rodolfo Oliveira Leal https://orcid.org/0000-0002-2463-4062

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