Plasma and urinary normetanephrine measurement in dogs with adrenal mass and a cytologic diagnosis of medullary tumor

State of the art

Pheochromocytomas (PCCs) are tumours arising from the chromaffin cells in the adrenal medulla, accounting for approximately 0.01–0.1%[1] of all canine tumours and for approximately 16% of adrenal tumours.[2] Clinical signs result most often from excessive secretion of catecholamines, and rarely from the space-occupying or invasive nature of the tumor.[3-9] Hormone secretion from tumors is highly variable, and consequently, the clinical picture varies considerably. The most frequent clinical signs are weakness, lethargy, tachypnea/panting, anorexia, vomiting, collapse and polyuria and polydipsia (PU/PD).[10] Due to the vague nature of the clinical signs and the similarities in clinical features with other conditions (e.g., PU/PD in dogs with hypercortisolism), canine PCC represents a diagnostic challenge. Additionally, to further complicate the picture, over 50% of PCCs in dogs are identified during the investigation of concurrent disease or during necropsy examination,[3] suggesting that many PCCs in dogs are unassociated with apparent clinical signs. Definitive diagnosis of PCC in dogs currently relies on histopathology of adrenal tissue after adrenalectomy or necropsy. The identification of the affected patients before adrenalectomy aids in appropriate perioperative management as pre-operative treatment with phenoxybenzamine significantly reduces perioperative mortality.[11] The diagnosis is based on a combination of clinical suspicion, finding an adrenal mass, together with the increased plasma, and/or urine concentrations of catecholamine metabolites. In particular, the determination of metanephrine and normetanephrine (plasma and urine) was superior in differentiating dogs with PCC from dogs with hypercortisolism and non-adrenal illness compared to epinephrine and norepinephrine.[12-14] Limitations of these tests include the limited availability of techniques, lack of reference ranges and critical sampling conditions. Fine needle aspiration of the adrenal lesion has been suggested as an alternative rapid and easy method to correctly address an adrenal tumor.[15] Indeed, cytologic examination of adrenal masses allows the distinction between cortical and medullary tumors with high diagnostic accuracy (90%-100%).[15] For this reason,

nowadays, in dogs with adrenal masses with a clinical suspicion of PCC, the FNA of the adrenal masses is routinely performed and it is considered a potential alternative to the metanephrine and normetanephrine measurements. However, if the adrenal cytology is suggestive of a medullary tumor, it does not necessarily imply that the tumor is hormonally active. Indeed, in humans, on rare occasions, PCCs may be non-secretory, or they can only produce dopamine (dopaminergic phenotype). Those tumors are caused by a lack of expression of the enzyme dopamine β hydroxylase, which converts dopamine to norepinephrine; typically, they are not associated with hypertension.[16,17]

Objectives and expected results (max 3000 characters spaces included)

The aim of this study is to measure urinary and plasma normetanephrine in dogs with clinical suspect PCC undergoing FNA of the adrenal mass and in which the cytology is compatible with a medullary tumor.

As in humans, clinical signs of PCC in dogs can occur paroxysmal pointing to sporadic (and unpredictable) catecholamine secretion. Some dogs reveal more constant clinical signs, which is most likely associated with continuous catecholamine release at a lower rate. There are also dogs that are clinically asymptomatic (or have extremely mild signs), and PCC is an incidental finding. Therefore, we hypothesize that the secretory patterns of dogs with medullary tumors might vary according to the severity of clinical signs. Additionally, there seems to be a correlation between the severity and presence of clinical signs and tumor size. Very small PCCs are more often an incidental finding than large tumors, and severe clinical signs are more often associated with large than small tumors.[18] Therefore, another hypothesis is that the normetanephrine levels can vary according to the tumor size.

Methods (max 3000 characters spaces included)

Study design:

- Prospective study
- Duration 24 months

- Number of dogs: 20
- Inclusion criteria:
    - Client-owned dogs with clinical suspicion of PCC undergoing FNA of the adrenal mass in which the cytology is compatible with a medullary tumor.
        - The clinical suspicion of PCC will be based on the presence of suggestive clinical signs (e.g., hypertension, cardiac arrhythmias, PU/PD, collapse, weakness/lethargy, anorexia, vomiting) associated with the presence of an adrenal mass
        - Dogs with an incidental ultrasonographic finding of an adrenal mass without clinical signs (incidentaloma) will also be eligible for inclusion into the study
- Exclusion criteria:
    - Dogs with cortisol-producing adrenal masses or other hormonal-producing cortical tumors (e.g. aldosterone, progesterone)
    - Dogs undergoing FNA of the adrenal group in which the cytology is not suggestive of a medullary tumor
    - Dogs with important comorbidities that preclude the anesthetic procedure for the FNA
- Study design:
    - T0 (time of the inclusion)
        - Obtain owner consent for inclusion in the study
        - Anamnesis
        - Physical examination
        - Blood pressure measurement using an oscillometric device. Blood pressure will be measured according to the current guidelines.[19]

    - Blood and urine sample collection for hematology, biochemistry, standard coagulation profile (PT, aPTT]) and urinalysis, as well as for plasmatic and urinary normetanephrine determination
    - Abdominal US and US-guided FNA of the adrenal mass under general anesthesia.

- Blood samples (1.5 ml) for the CBC will be collected into EDTA-coated plastic tubes.*
- Blood samples (2 ml) for the biochemistry profile will be collected in plastic tubes without anticoagulant.*
- Blood samples (2 ml) for the coagulation profile will be collected into tubes containing 3.2% trisodium citrate (1 part citrate: 9 parts blood).*

*All the samples will be anallyzed within 1 to 3 hours.

- Urine and plasma samples for the determination of normetanephrine will be collected within 30 minutes during workup in the hospital as the stress increases metanephrine excretion.[20] The urine sample will be immediately placed in a tube and stored at −80°C until analysis. Plasma samples will be collected in chilled heparin tubes, centrifuged at 4°C, and stored at −80°C. Samples will be shipped on dry ice to the respective laboratory and thawed immediately before analysis.
- Analytical methods
    - Complete blood count (Advia 2120, Siemens Healthcare Diagnostics, Erlangen, Germany), chemistry profile (AU 480, Beckman Coulter/Olympus, Brea, CA), coagulation profile (BFT II, Siemens, Munich, Germany) and urinalyses will be performed by standard laboratory methods at the medical laboratory of the referral institution.
    - Urinary and plasmatic normetanephrines determination will be performed at the AML BV laboratory (Belgium) using liquid chromatography-tandem mass spectrometry

(LC/MSMS)(SCIEX Triple Quad™ 5500 LC-MS/MS System) as previously described.[14]

Potential applications, scientific and/or laboratory impact, technology advancements, brief remarks on ethical issues or security (max 2000 characters spaces included)

The study protocol will be submitted for approval by the Scientific Ethics Committee of the University of Bologna. This study does not involve procedures different (or additional) compared to those that would be carried out in dogs with a clinical suspicion of PCC. Therefore, there should be no problems with approval by the ethics committee.

Knowing the hormonal activity of a PCC before adrenalectomy aids in appropriate perioperative management. Indeed, as stated above, treatment with phenoxybenzamine before adrenalectomy significantly reduces perioperative mortality in dogs with PCC.[11] Cytology of the adrenal glands is advocated as a diagnostic tool to confirm the tumor origin before the definitive diagnosis with histopathology is carried out.[15] However, not all medullary tumors might be hormonally active. Up to date, no studies have investigated if tumors classified as of medullary origin by cytology are associated with a parallel increase in NMN. Therefore, it is unclear if the cytological diagnosis of a medullary tumor can substitute the urinary and/or plasmatic NMN measurement. This data is relevant because treating pre-operatively a dog with phenoxybenzamine only based on cytology results, could result in unnecessary pre-operative management with costs for the owners and potential side effects for the patient (hypotension, vomiting, tachycardia). The result of this study can help to clarify if the cytology can be used as a substitute diagnostic method to the MNM measurement or if NMN determination would always be advisable in order to establish if PCC is hormonally active. If the cytological evidence of adrenal tumors is always associated with increasing levels of urinary/plasmatic NMN, these two methods might be used interchangeably. Moreover, if the NMN

levels will vary according to the severity of clinical signs and tumor size, these features might help to predict the hormonal activity of the tumors and to aid in appropriate preoperative planning.

Activities and insurance

The fellow will be involved in all operational phases of the research project starting with the selection and inclusion of cases. He/she will be primarily involved in data collection. He/she will then contribute to the processing and analysis of the results obtained with the aim of promoting scientific dissemination through oral communication at international conferences and articles published in peer-reviewed journals.

The fellow will also be involved in the clinical activity of the Veterinary Teaching Hospital of The University of Bologna (maximum 860 hours) and included in its continuing education, actively participating in weekly journal club meetings, book reading and interactive discussion of clinical cases.

Please note that in order to carry out this type of activity, the person concerned must have adequate insurance coverage. In particular:

- The contribution for membership in the University's professional insurance plan will be deducted from the first available payment.
- It is also advisable for the person concerned to verify their insurance coverage for gross negligence and activities carried out in the public sector.

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