Urinary Microvesicles in Cats with Chronic Kidney Disease and Systemic Hypertension

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Introduction

Chronic kidney disease (CKD) is a common morbidity and a leading cause of mortality in domestic cats, affecting 3.6% of the UK cat population[1]. CKD is a diverse syndrome with various causes, but they all converge on a final common pathway – tubulointerstitial nephritis and fibrosis.

Current biomarkers have limitations in diagnosing CKD[2]. Urinary microvesicles (MVs) may act as biomarkers for various conditions, including renal disease, and may be implicated in the pathophysiology of the progression of CKD. MVs may potentially address the limitations with current biomarkers and provide the clinician with more information about the state of the feline kidney.

Aims & Hypothesis

Aims

- Investigate whether urinary MVs are present in feline urine
- Describe and optimise isolation method
- Determine whether urinary MV numbers increase in CKD and hypertension

Hypotheses

- Feline urinary MVs are present and readily detectable with flow cytometry.
- Urinary MVs increase in cats with CKD and hypertension.

Materials & Methods

Study design: Retrospective cross-sectional study using frozen stored feline urine samples from geriatric cat clinics held in two London first-opinion practices.

Sample collection: Performed under ethical approval. URN 20131258.

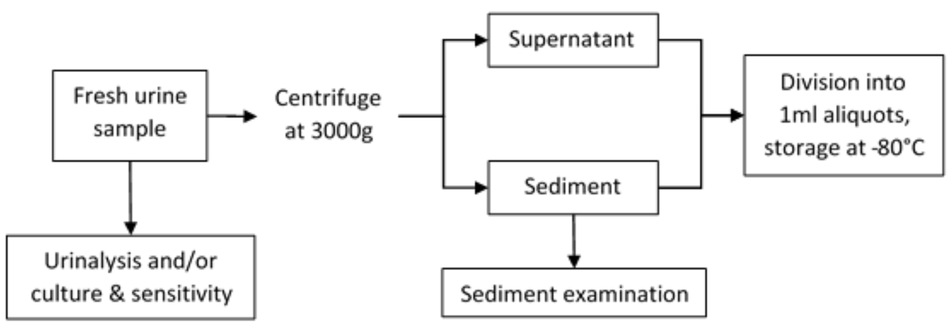

Figure 1: Urine collection and storage protocol at the geriatric cat clinic

Inclusion criteria: Cats ≥ 8 years old, fasted at time of sample collection, samples collected between 2010-18, collected via cystocentesis

Exclusion criteria: Confirmed or suspected hyperthyroidism or urinary tract infection, patients on anti-hypertensive therapy, diabetic patients, contaminated samples

Sample classification:

	Healthy	CKD
sCr/µmol/l	≤177	>177
USG	≥1.035	<1.035
	Healthy	**Hypertensive**
SBP/mmHg	<170	≥170

Study population: Ninety-nine urine samples were identified. Based on the above criteria, samples were classified into four groups: healthy-normotensive (NT; n=25), CKD-NT (n=42), non-azotaemic-hypertensive (HT; n= 16), and CKD-HT (n=16). The breed most represented was the Domestic Shorthair (n=80).

Acknowledgements and references

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[1] O'Neill, D.G. *et al*. (2014) "Prevalence of disorders recorded in cats attending primary-care veterinary practices in England," *The Veterinary Journal*, 202(2), 286–291.

[2] Paepe, D. (2015) "Early recognition of feline chronic kidney disease," *European Journal of Companion Animal Practice*, 25(3), 61–77.

[3] Lawson, C. *et al* (2017) "Extracellular vesicles: evolutionarily conserved mediators of intercellular communication," *Yale Journal of Biology and Medicine*, 90(3), 481-491.

Results

MV count in the non-azotaemic-HT group was significantly higher ($p<0.05$, $p<0.0001$, $p<0.001$ respectively) than that in the healthy-NT, CKD-NT and CKD-HT groups (Figure 6). MV count in the healthy-NT group was also significantly higher ($p<0.05$) than the CKD-NT group. All other comparisons did not differ significantly.

Samples from a representative group of 12 cats were incubated with anti-CD235 and anti-CD61 antibodies to look for MVs of erythrocyte and platelet origin respectively. Results showed that none of these samples were positive for either surface marker (Figure 7).

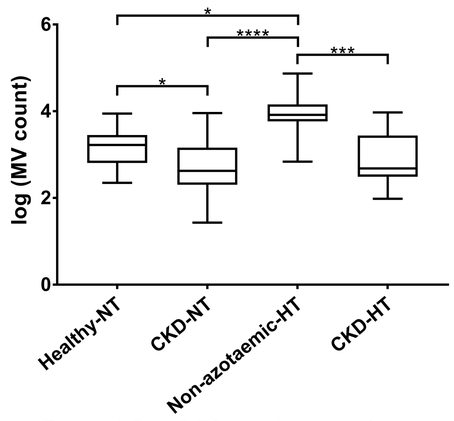

Figure 6: Log (MV count) among the four different groups, showing MV count, median and interquartile ranges

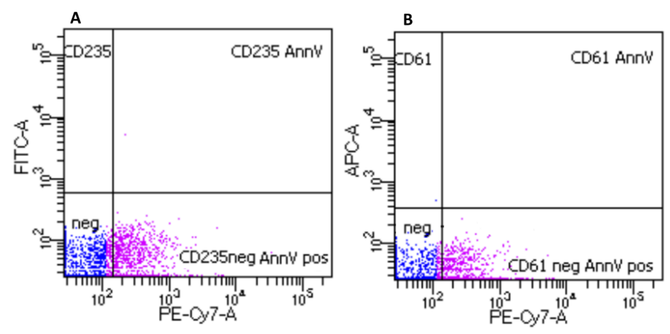

Figure 7: Dual fluorescence analysis of the MV gate from a representative sample showing CD235-FITC (A) and CD61-APC (B) fluorescence.

Discussion

Overall, this study shows that MVs can be readily detected and quantified in stored feline urine from both healthy and non-healthy cats using flow cytometry.

Significance of results

The MV count differed significantly in the CKD-NT and CKD-HT groups compared to the healthy-NT and non-azotaemic-HT groups respectively. This supports the potential use of urinary MVs as biomarkers in feline CKD. Contrary to out initial hypothesis, MV counts were lower in the CKD groups. This may reflect a lower number of functional renal tubular cells in more advanced CKD.

Hypertension appears to also have an independent effect on urinary MV counts in non-azotaemic cats, which again, may reflect target organ damage.

Utility of MVs

Current biomarkers such as serum creatinine and SDMA have their limitations, one of which is limited sensitivity. Urinary MVs may be able to demonstrate the presence of tubulointerstitial inflammation long before increases in these serum biomarkers are seen.

Urinary MVs may also be useful in cats with concurrent CKD and hyperthyroidism, giving us a snapshot of what is happening in the renal parenchyma without the confounding effect of excess circulating thyroid hormone on GFR.

Potential limitations

Our definition of azotaemia differs from the IRIS definition, potentially making it difficult to compare populations and results with other studies.

Doppler blood pressure measurement used in this study has its limitations compared to gold standard direct arterial catheterization.

Patient population in London first-opinion practices may not be representative of all cats in general.

MV physiology in general, and in the kidney in particular, have yet to be elucidated.

Conclusions & future research

MVs are present and readily detectable in frozen stored feline urine sample from both healthy and non-healthy cats using flow cytometry.

Fluorochrome staining identified a subpopulation of MVs positive for AnnV. None of the assayed samples were positive for CD235 or CD61. These monoclonal antibodies have been previously shown to be cross-reactive with feline antigens in plasma[3]. This suggests that circulating plasma MVs were not present and that all MVs likely originated from the post-glomerular space.

MV numbers are associated with CKD and hypertension and this suggests that MVs may be useful as non-invasive biomarkers for these diseases. However, the mechanism by which this occurs is unclear.

Future research may consider looking into the significance of MV surface markers, investigating other surface markers, and prospectively assessing patients over time.

Declaration

The authors have no conflicts of interest to declare.

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