



# WINN FELINE FOUNDATION

For the Health and Well-being of All Cats

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637 Wyckoff Ave., Suite 336, Wyckoff, NJ 07481 • [www.winnfelinefoundation.org](http://www.winnfelinefoundation.org)  
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## BIOMARKERS OF PROTEINURIA AND HYPERTENSION IN FELINE CKD

**PROJECT STUDY:** Biomarkers of proteinuria and hypertension as risk factors for development and worsening of feline CKD.

Principal Investigators: Dr. Salvaterio Patrinieri, Dr. Paola Scarpa; University of Milan  
*Preliminary Final report summary, WZ14-009*

The preliminary part of the project was focused on a preliminary evaluation of preanalytical and analytical variability of urinary protein-to-creatinine (UPC) ratio, parameters used in IRIS staging of feline CKD. Specifically, different storage conditions, effect of creatinine predilution, and imprecision and method-dependent difference were evaluated in order to determine whether they could affect proteinuria interpretation in clinical practice. Proteinuria was stable up to 6 hours at room temperature, 1 week at refrigeration temperature, four freeze-thaw cycles and 4 weeks at  $-20^{\circ}\text{C}$ , whereas the use of different analytical methods (such as Pyrogallol Red Molybdated and Brilliant Blue Coomassie) resulted in inaccuracy and suboptimal concordance in classifying samples according to IRIS substaging, that in turn can potentially affect clinical decisions, make questionable the comparison of UPC results between different laboratories, and have significant impact in substaging cats affected by CKD.

The main part of this project was aimed to assess how a group of biomarkers may allow an early diagnosis of CKD, may identify cats at risk of severe worsening of the disease, or may be a predictor of hypertension. The biomarkers were serum big endothelin-1 (big-ET1), homocysteine (Hcy), aldosterone and urinary big-ET1, alpha-1 microglobulin (A1MG) and the presence of tubular proteins in urine (evaluated with sodium dodecyl sulfate-agarose gel electrophoresis, SDS-AGE).

To this aim, privately owned cats (i.e. clinically healthy cats at risk to develop CKD and cats affected with CKD) were prospectively enrolled and sampled every 3 or 6 months (depending on IRIS staging) over 18 months to determine the temporal appearance of azotemia. At each visit, serum and urine sample were collected and blood systolic pressure was measured by a noninvasive method in order to stage these cats according to the IRIS classification system. Moreover, serum and urine samples were stored for biomarker analysis.

Among 175 cats examined in this research institution for the purpose of this research, forty-eight privately owned cats have been included in the study and 99 serum and urinary samples were collected. Thirty of these cats were re-examined and sampled over time.

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The Winn Feline Foundation is a non-profit organization [501(c)(3)] established by The Cat Fancier's Association.  
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Different results were found with the different biomarkers. Big-ET1 did not give satisfying results in serum, whereas in urine the ELISA method yielded satisfying validation results, supporting its introduction in this species. Urinary big-ET1 was associated with the severity of CKD and proteinuria, revealing that it could be a promising aid in nephropathic cats and could shed light on the pathogenesis of tubulo-interstitial and glomerular damage in cats with CKD. The method to measure Hcy can be considered reliable in cats according to the validation tests. Serum Hcy increased progressively with the progressive increase in severity of CKD and the detection of high Hcy in some non-azotemic patients with CKD could add this new marker to those currently available for the identification and staging of the kidney disease of the cat. Conversely, no direct relationship was found between Hcy and hypertension. Aldosterone was not associated with severity of CKD, proteinuria and SBP. Therefore, neither Hcy nor aldosterone can be considered an indicator of hypertension in cats affected with CKD. The ELISA kit used for A1MG measurement failed all the validation tests and results were considered unacceptable; further studies are therefore needed to investigate the presence of A1MG in cats with CKD and tubular damage. SDS-AGE showed the consistent presence of bands with high molecular weight in healthy cats, suggesting that this pattern is normal in this specie and has to be taken into account while evaluating proteinuria in cats; tubular bands were frequent in patients with CKD at any stage, confirming the predominant tubule-interstitial damage of this disease in cats and suggesting that SDS-AGE can be considered a valuable aid in diagnostic approach to feline CKD.

Furthermore 66 cats were valuated also by SDMA in a single determination. This parameter could be a useful diagnostic tool in the evaluation of a nephropathic or potentially nephropathic patient, but the complete evaluation of the renal status includes mandatory blood work, urinalysis and imaging is necessary. Definitely, SDMA alterations were often concurrent with the alteration of at least one of the other parameters mostly used to diagnose nephropathy. Furthermore, a second measurement is necessary especially whether the result is around the cut-off value or in absence of other alterations compatible with CKD.

## **Publications:**

“Evaluation of a commercial ELISA for measurement of feline urinary alpha-1 Microglobulin”

Marco Giraldi, Saverio Paltrinieri, Paola Scarpa

International Journal of Health, Animal Science & Food Safety, Vol 3, n. 1s, 2016

The original article “Evaluation of the analytic variability of urine protein-to-creatinine ratio in cats”

(Marco Giraldi, Gabriele Rossi, Walter Bertazzolo, Stefano Negri, Saverio Paltrinieri and Paola Scarpa) accepted by the Journal Veterinary Clinical Pathology on February 2018

The original article “Serum concentration of homocysteine in feline spontaneous chronic CKD”

(Marco Giraldi, Saverio Paltrinieri, Carola Curcio, Paola Scarpa) is going to be submitted to Veterinary Journal

Our aim is to submit in the next six months paper about SDS, SDMA, aldosterone and hematuria.

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## **Presentations:**

“Evaluation of proteinuria in cats: comparison between Coomassie Brilliant Blue and Pyrogallol Red Molybdate”.

Giraldi M, Rossi G., Bertazzolo W., Paltrinieri S., Scarpa P. ECVIM-CA Congress, Lisbon, September 2015

“Frequency of electrophoretic changes in urine of old cats with or without CKD”

Marco Giraldi, Paola Scarpa, Saverio Paltrinieri. ECVCP Congress, Nantes, October 2016

*Summary prepared © 2018*

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