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PII: S1090-0233(18)30368-X

DOI: https://doi.org/10.1016/j.tvjl.2019.105358

Article Number: 105358

Reference: YTVJL 105358

To appear in:

Accepted Date: 20 August 2019

Please cite this article as: Giraldi M, Paltrinieri S, Curcio C, Scarpa P, Serum concentration of homocysteine in spontaneous feline chronic kidney disease, *The Veterinary Journal* (2019), doi: https://doi.org/10.1016/j.tvjl.2019.105358

Original Article

Serum concentration of homocysteine in spontaneous feline chronic kidney disease

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Highlights

- Serum homocysteine (Hcy) was measured in healthy cats at risk of chronic kidney disease (CKD) and in cats with CKD
- The enzymatic method was validated and showed high precision and accuracy in feline serum
- Hcy increased in cats with CKD compared with cats at risk and increased with International Renal Interest Society (IRIS) stage
- Preliminary results of our longitudinal study suggested that Hcy might be useful to predict disease progression

Abstract

Serum homocysteine (Hcy) increases in people and dogs with chronic kidney disease (CKD). Hyperhomocysteinemia (HHcy) has also been associated with CKD-related hypertension and proteinuria. The aims of this study were to: (1) validate an enzymatic method for quantification of Hcy in feline serum; (2) evaluate whether HHcy was associated with the presence and severity of CKD, proteinuria or hypertension; and (3) determine whether HHcy could predict disease progression.

The intra- and inter-assay coefficients of variation (CVs) and the recovery rates of linearity under dilution and spiking recovery tests of the enzymatic method were 3.1–6.7%, 11.6–12.5%, 96.9±5.4% and 96.9±5.4%, respectively. Healthy cats at risk of CKD (*n*=17) and cats with CKD (*n*=19) were sampled over a 6-month period (63 samples in total). Cats with CKD had significantly higher Hcy concentrations (*P*=0.005) than cats at risk. The concentration of Hcy was higher (*P*=0.002) in moderate-severe CKD than in mild CKD and correlated moderately with serum creatinine (*P*<0.0001; *r*=0.51). The concentration of Hcy increased with the magnitude of proteinuria and correlated weakly with urinary protein to creatinine ratio (*P*=0.045; *r*=0.26). HHcy was not associated with hypertension. At the time of enrollment, Hcy concentration was significantly higher (*P*=0.046) in cats that developed CKD compared to cats that remained stable. The enzymatic method for Hcy measurement in feline serum was precise and accurate. HHcy was relatively common in cats with advanced CKD and seemed to predict disease progression, but further studies are warranted.

Keywords: Cats; Chronic kidney disease; Homocysteine; Hypertension; Proteinuria

Introduction

Chronic kidney disease (CKD) is relatively common in cats, especially in geriatric cats, and the progressive and irreversible renal damage can profoundly affect quality of life (Bartges, 2012). Despite improved standardization of the diagnostic approach to feline CKD recommended by International Renal Interest Society (IRIS)[1], affected cats are often not identified until late in the disease process and predicting progression is often challenging. Among serum and urinary biomarkers recently evaluated in feline CKD to address this

[1] See: International Renal Interest Society. CKD Early Diagnosis http://www.iris-kidney.com/education/early_diagnosis.html (accessed 8 August 2019)

clinical issue (Hokamp and Nabity, 2016), only SDMA has been recommended for early diagnosis and disease progression.[2]

Homocysteine (Hcy) is an amino acid involved in S-adenosyl methionine metabolism. Hcy is remethylated to methionine and then converted to s-Adenosylmethionine in the cytoplasm (Long and Nie, 2016; Stanger et al., 2004). Since remethylation of Hcy to methionine is dependent on cobalamin, deficiency of this vitamin is associated with mild increase of Hcy in people (Stanger et al., 2004). In cats, cobalamin deficiency was not significantly associated to hyperhomocysteinemia (HHcy) in one study and it was hypothesized that the metabolism of Hcy is unique in cats (Ruaux et al., 2001). In people, circulating Hcy strongly correlates with glomerular filtration rate (GFR; Tak et al., 2015), and HHcy is associated with declining GFR and CKD-associated cardiovascular complications (Levi et al., 2014). In dogs, Rossi and colleagues (2008; 2013a) investigated HHcy in the late stages of CKD. It was hypothesized that CKD-associated HHcy relied on two mechanisms: (1) reduced renal excretion, and (2) impaired extra-renal Hcy metabolism due to nephron loss and the toxic effects of retained metabolites (van Guldener, 2006). HHcy has deleterious effects on cellular metabolism, mainly mediated by increased oxidative stress (Fowler, 2005), and it is considered a uremic toxin (Duranton et al., 2012). In humans, Hcy positively correlates in a linear fashion with systolic blood pressure (Nygard et al., 1995), and is increased in patients with CKD-associated hypertension and/or proteinuria (Stehouwer and van Guldener, 2003); it might also be a predictor of hypertension (Wang et al., 2014). Impaired renal function can increase both systolic blood pressure (SBP) and Hcy, but these two factors are not necessarily related. In cats, in-hospital measurement of SBP can be

[2] See: International Renal Interest Society. Symmetric dimethylarginine (SDMA): new biomarker of renal function in dogs and cats http://www.iris-kidney.com/pdf/12_symmetric-dimethylarginine_track-changes.pdf (accessed 8 August 2019)

challenging due to the white-coat effect (Belew et al., 1999). Since hypertension can be a life-threatening complication of CKD (Syme, 2011), a biomarker of hypertension could be useful in feline clinical practice.

To our knowledge, studies investigating Hcy concentration in cats with CKD, with or without hypertension, have not been published. Therefore, the aims of this study were to: (1) validate the enzymatic method for measuring Hcy; (2) evaluate Hcy in cats with CKD and investigate potential relationships between HHcy and the severity of azotemia, proteinuria or hypertension; and (3) evaluate the utility of Hcy for the prediction of disease progression in a longitudinal study.

Materials and methods

Case selection and study design

This study was conducted using blood samples collected from client-owned cats presented for routine health screening at the Veterinary Teaching Hospital of University of Milan from November 2014 to May 2017. Informed consent was signed by the owners and, according to the ethical committee statements of the University of Milan (Approval number 2/2016; Approval date February 15th, 2016), biological samples collected during diagnostic workup were used also for research purposes. Cats included were part of a prospective study of feline patients monitored over 18 months. Currently healthy cats at risk of developing CKD and cats with CKD at any stage were enrolled. Cats >8 years and cats of any age of breeds predisposed to CKD (e.g. Persian, Abyssinian, Maine Coon), were considered at risk of CKD. These cats were clinically healthy and did not have laboratory abnormalities. Cats with CKD were of any age and breed, and were diagnosed and staged in accordance with

IRIS staging guidelines.[3] Cats at risk and cats at IRIS stage 1 were checked every 6 months; cats at IRIS stage 2-4 were checked every 3 months.

Exclusion criteria were as follows: presence of infectious, endocrine, cardiovascular diseases or malignant tumors; administration of drugs affecting SBP (e.g. glucocorticoids, calcium-channel blockers, angiotensin-converting enzyme inhibitors, angiotensin-receptor blockers, aldosterone inhibitors, and opioids); diuretics or anti-inflammatory drugs in the 4 weeks before enrollment. Cats with acute kidney injury or dehydration (evaluated on clinical examination exploring skin turgor and mucous membranes) were excluded.

To assess the relationship between Hcy and azotemia, proteinuria or hypertension, all available samples (regardless of time of collection) were used for analysis. For the longitudinal study, cats with two serum samples collected at time of inclusion (T_0) and 6 months later were classified into two groups: (1) 'stable': cats that remained in the same IRIS stage; and (2) 'progressive': cats that progressed to a higher IRIS stage (e.g. from at risk to IRIS stage 2 or from IRIS stage 3 to IRIS stage 4).

Data collection

History was recorded and complete physical examination was performed on each cat at each visit. SBP was measured according to the current guidelines (Taylor et al., 2017), using a non-invasive Doppler technique (Minidop ES-100VX, Hadeco) and recording the mean of three consecutive and consistent values (<20% variability). For non-cooperative cats or those that appeared to be stressed, SBP was re-measured within 7 days in a less stressful clinical setting. To exclude white-coat hypertension, SBP was also evaluated 7 days later in

[3] See: International Renal Interest Society. IRIS Staging system. http://www.iris-kidney.com/education/staging_system.html (accessed 8 August 2019)

cats SBP >150 mmHg; this result was the one counted for the study. Blood (approximately 3 mL) was collected from the cephalic or jugular vein after 12 h of fasting and split between tubes with and without EDTA. At least 7 mL of urine was collected using ultrasound-guided cystocentesis. Urine collection was attempted again within 7 days if the bladder was empty; concurrent blood collection to measure serum urea and creatinine concentration was repeated at that time in cats with CKD with unstable disease.

Samples were analyzed within 2 h at an in-house clinical pathology laboratory. A routine CBC was performed using a laser-based analyzer (Sysmex XT 2000 iV, Sysmex Co) followed by microscopic slide review. A panel of serum biochemical parameters (including creatinine concentration measured using the Jaffe method; Stockham and Scott, 2008) was performed using an automated spectrophotometer (Cobas Mira, Roche Diagnostic). In the case of macroscopic clots in the EDTA tube or hemolysis/lipemia in serum, samples were excluded and blood collection was repeated within 7 days. Complete urinalysis was performed following a standard protocol (Rossi et al, 2015) and the urinary protein to creatinine (UPC) ratio was calculated on supernatants using pyrogallol red-molybdate for quantification of total urinary protein, as described by Rossi et al. (2012). Aliquots of serum (140 µL) were stored at -20 °C for subsequent analysis.

Measurement of homocysteine

An enzymatic method (Diazyme Europe) was performed using the Cobas Mira analyzer. The method was calibrated and controlled before each session using the human-based calibrator and control solutions provided by the manufacturer.

Hcy was measured using batched stored serum samples, after no more than 18 month storage at −20 °C. Aliquots were thawed overnight at 4 °C and warmed at room temperature 1 h before analysis. Samples tested in the first analysis were also used to prepare reference materials for a preliminary method validation. Specifically, a 'high pool' and a 'low pool' were prepared by mixing five samples with the highest Hcy concentrations and five samples with the lowest Hcy concentrations, respectively. Intra-assay variability was determined by measuring the Hcy concentration of the two pools 20 times in the same run. Inter-assay variability was determined by measuring each pool on 5 consecutive days, keeping serum in closed tubes at 4 °C. The linearity under dilution (LUD) test was performed by measuring Hcy in triplicate in serial two-fold dilutions (i.e. 1:2, 1:4 and 1:8) of the 'high pool' using distilled water and calculating mean values. Three more samples with high Hcy concentrations and three samples with low Hcy concentrations were used to prepare another 'high pool' and another 'low pool', respectively, to perform a spiking recovery (SR) test. This 'high pool' was diluted 20%, 40%, 60% and 80% with the corresponding 'low pool' and the resulting mixed samples were assayed in triplicate.

Statistical analysis

Commercially available software (GraphPad Prism 5.0, GraphPad Software) was used for statistical analysis. *P* values <0.05 were considered statistically significant. The distribution of data for each variable was assessed using the Kolmogorov-Smirnov test and non-parametric statistics were applied for non-normally distributed data. Mean values, standard deviations (SD) and coefficients of variation obtained in both intra- and inter-assay tests were determined. For both LUD and SR tests, linear regression between expected and observed results was investigated and the percentage of recovery was calculated as follows:

Recovery = Mean observed/expected x 100.

The Mann-Whitney *U* test was used to compare the Hcy concentrations recorded (1) in all cats with CKD (including all cats, independent of IRIS stage) vs. cats at risk; (2) in proteinuric cats (UPC≥0.4) vs. non-proteinuric and borderline proteinuric cats (UPC<0.4); and (3) in moderately to severely hypertensive cats (SBP≥160 mmHg) vs. normotensive and borderline hypertensive cats (SBP<160 mmHg). The thresholds for UPC and SBP were used because anti-proteinuric and anti-hypertensive treatments, respectively, were necessary above these values.[4,5] Kruskal-Wallis tests were used to compare Hcy concentrations as follows: (1) by IRIS stage, based on serum creatinine and (2) by IRIS substage, based on UPC ratios. Since the number of samples from cats with IRIS stage 3 and 4 was low, these samples were pooled in a single group ('IRIS 3-4'). Mann-Whitney *U* tests were also used to compare Hcy concentrations at T_0 in 'stable' and 'progressive' groups in the longitudinal study, for all cats and for the sub-set of cats classified as at risk at T_0. Correlations between Hcy and serum creatinine, UPC ratio and SBP were assessed using the Spearman correlation test.

Results

Cats

Thirty-six cats, 16 male (all neutered except for one) and 20 female (all spayed) fulfilled the inclusion criteria. Breeds included Domestic shorthair (*n*=26); Exotic shorthair, Persian, Siberian and Norwegian Forest (*n*=2 each); Maine Coon and Birman cats (*n*=1 each). Age ranged from 5.6 to 18.8 years (mean ± SD, 11.3 ± 3.6 years). At T_0, 17 cats were at risk,

[4] See: International Renal Interest Society. Proteinuria http://www.iris-kidney.com/education/proteinuria.html (accessed 8 August 2019)
[5] See: International Renal Interest Society. Hypertension http://www.iris-kidney.com/education/hypertension.html (accessed 8 August 2019)

and 19 were diagnosed with CKD (four at IRIS stage 1, 10 at stage 2, three at stage 3 and two at stage 4).

Preliminary method validation

The intra-assay CV for the 'low pool' (mean ± SD, 9.17 ± 0.61 μmol/L) and the 'high pool' (mean ± SD, 26.14 ± 0.81 μmol/L) were 6.7% and 3.1%, respectively, excluding one outlier for each pool. The inter-assay CV for the 'low pool' and the 'high pool' were 12.5% and 11.6%, respectively. Linear regression analysis demonstrated excellent linearity for both the LUD test (P=0.001; r^2 = 0.998; slope = 0.999; confidence interval (CI) of slope, 0.856 - 1.142) and the SR test (P=0.001; r^2 = 0.988; slope = 0.989; CI of slope = 0.842 - 1.138), and recovery rates were 96.98 ± 5.36% and 90.05 ± 11.23% for LUD and SR tests, respectively.

Hcy by group/subgroup

Sixty-three serum samples collected at either the first visit or during the follow up were included in this part of the study. Twenty-eight samples were from cats at risk; nine were from cats in IRIS stage 1; 17 were from cats in IRIS stage 2; six were from cats in IRIS stage 3; and three were from cats in IRIS stage 4. UPC ratio and SBP were not available in all cases because of loss to follow up; data were available for 58 and 50 cases, respectively. Details of age, serum creatinine, UPC ratio, SBP and serum Hcy concentration recorded at the time of sampling in cats at risk or in cats with CKD are reported in Table 1.

Hcy was significantly lower (P=0.005) in cats at risk (median: 8.46 μmol/L; minimum-maximum [min-max], 3.37-40.24 μmol/L) compared to the whole group of cats with CKD (independent of the IRIS stage; median, 12.12 μmol/L; min-max, 4.94-23.42 μmol/L). Hcy concentration gradually increased with IRIS stage and was significantly higher

(*P*=0.002) in cats with IRIS stage 3-4 compared to cats at risk (Fig. 1a). Hcy concentration was also moderately and positively correlated with serum creatinine (*P*<0.0001; *r*=0.51; Fig. 2a). Hcy concentration was not significantly different (*P*=0.091) among the three IRIS stages of proteinuria (Fig. 1b) or when results of proteinuric cats (*n*=6; median, 13.36 μmol/L; min-max, 9.98-23.42 μmol/L) were compared with those of a group composed of non-proteinuric and borderline proteinuric cats (*n*=52; median, 10.70 μmol/L; min-max, 3.37-40.24 μmol/L; *P*=0.089). However, Hcy increased with the UPC ratio and was significantly but weakly correlated with UPC (*P*=0.045; *r*=0.26; Fig. 2b). No significant differences in Hcy concentration were demonstrated between normotensive or borderline hypertensive cats (*n*=37; median, 9.61 μmol/L; min-max, 3.37-40.24 μmol/L) and moderately to severely hypertensive cats (*n*=13; median, 9.98 μmol/L; min-max, 4.27-30.73 μmol/L; *P*=0.451; Fig. 1c) and there was no correlation between Hcy concentration and SBP (*P*=0.71; *r*=0.05; Fig. 2c).

Longitudinal study

Twenty-seven cats were included in the longitudinal study. Seventeen were at risk at T_0; nine of those remained stable for 6 months and eight progressed to CKD (four to IRIS stage 1 and four to IRIS stage 2). Ten cats were diagnosed CKD at T_0; eight of those remained stable (two at stage 1, five at stage 2 and one at stage 3) and two progressed (one from stage 2 to stage 3 and one from stage 3 to stage 4).

There were no significant differences in Hcy concentration between cats that remained stable (*n*=17; median, 10.84 μmol/L; interquartile range [IQR], 5.74-14.07 μmol/L; min-max, 3.37-17.59 μmol/L) and cats that progressed to higher IRIS stages (*n*=10; median, 12.14 μmol/L; IQR, 8.35-19.44 μmol/L; min-max, 7.19-40.24 μmol/L; *P*=0.282, Fig. 3a).

Considering only cats at risk, Hcy concentration was significantly lower (P=0.046) in cats that remained stable for 6 months (n=9; median, 6.68 μmol/L; IQR, 4.98-11.11 μmol/L; min-max, 3.37-13.30 μmol/L) than cats that developed CKD within the same period (n=8; median, 12.14 μmol/L; IQR, 8.58-26.97 μmol/L; min-max, 7.95-40.24 μmol/L; Fig. 3b).

Discussion

This is the first study evaluating Hcy in cats with CKD. The validation study demonstrated that the enzymatic method is precise and accurate, as it is in other species (Rossi et al., 2008), although CVs were higher at low Hcy values and vice versa. Indeed, when analyte concentration was low, slight shifts in measurements from the mean value produced a profound change in CV (Westgard, 2003). The higher CVs recorded in inter-assay test compared to those for the intra-assay test could relate to the preservation of samples (Kale et al., 2012). However, a visual inspection of results from the two pools did not show any increasing or decreasing trends for Hcy concentration over time (data not shown). This suggests that Hcy could be considered stable in serum samples when stored at 4 °C for 5 days and that the higher inter-assay CVs were probably due to the intrinsic variability of the analytical method. LUD and SR tests confirmed that the method had excellent accuracy, similar to that reported in other species (Rossi et al., 2008). A more complete accuracy assessment would require comparisons with a reference standard test (not available for the cat), and the inclusion of data commonly included in validation studies, such as quantification and detection limits, and the investigation of the effects of storage and interfering substances (Kjelgaard-Hansen and Jensen, 2010). In dogs, no clinically relevant variations were observed after storage or in the presence of interfering substances at concentrations commonly found in clinical practice, or after storage (Rossi et al., 2008). Therefore, we speculate that the situation is the same for feline specimens. From this perspective, the

method also provided easily interpretable results when there were small differences among groups.

In our study, Hcy increased in cats with CKD compared to cats at risk and Hcy increased with the IRIS stage. However, differences among groups were often not statistically significant, perhaps due to some overlap among groups, and to the different number of samples per group. Hcy concentration was significantly higher only in late stage CKD by contrast with one canine report, where Hcy was higher at IRIS stage 2 compared with control dogs or dogs at IRIS stage 1 (Rossi et al., 2013a). This difference between species might stem from metabolic differences; Hcy concentrations in cats are not affected by enteropathies (McMichael et al., 2000) or by cobalamin deficiency (Ruaux et al., 2001; Rossi et al., 2013b). Alternatively, the composition of the control group in our study could explain this discrepancy; the canine study included young adults (Rossi et al., 2013a) whereas our study included mainly older cats. CKD is common in elderly cats and we cannot exclude the possibility that cats at risk that were enrolled as controls had mild and slowly progressive CKD that affected Hcy concentration. Cats at risk could also have increased Hcy concentrations, explaining the lack of statistical difference between groups at early stages of CKD.

The preliminary results from our longitudinal study suggested that high Hcy in cats without signs of disease that could potentially induce HHcy may predict the occurrence of CKD. However, the distribution of results in Fig. 3 suggests that the statistical difference could have been affected by two high values in the ‘progressive’ group. These values were not affected by analytical errors, since these two cats were also sampled during the follow-up and Hcy concentration was maintained over time (data not shown). However, no increases in

Hcy were recorded in many cats that developed CKD. Therefore, this preliminary result needs to be confirmed by further studies using larger sample sizes followed over time.

When cats were grouped based on the degree of proteinuria, increasing Hcy concentration was not statistically significant, similar to what was demonstrated using IRIS stages based on serum creatinine. This is different to what has been reported for dogs, since in one published study, proteinuric dogs had significantly higher Hcy than non-proteinuric dogs (Rossi et al., 2013a). This difference could be explained by the pathogenesis of canine CKD, which is frequently due to immune complex glomerulonephritis, while feline CKD is rarely associated with glomerular injury (Chakrabarti et al., 2013). Proteinuria in cats is generally mild and increases in the later stages of CKD (Syme et al., 2006), suggesting that it may be a consequence rather than a cause of kidney injury. Therefore, any Hcy increases in proteinuric cats could be confounded by the severity of CKD. A multivariate analysis with a larger sample set is required to investigate this.

Serum Hcy concentration in cats was not correlated with SBP, by contrast with humans (Stanger et al., 2004), but not dogs (Rossi et al., 2013a). Therefore, serum Hcy concentration should not be considered as a biomarker of hypertension in cats.

Conclusion

The enzymatic method investigated for Hcy measurement was precise and accurate in cats. However, the diagnostic potential of Hcy in cats was lower than that reported for other species. The demonstration of increasing Hcy concentrations associated with the progression of CKD and the detection of high Hcy concentrations in some non-azotemic cats with CKD in this study could lead to recommendations for Hcy to be used as a biomarker for early

identification and staging of CKD; however, serum creatinine provides more reliable information than Hcy concentration for these purposes. Since no direct relationship with hypertension was demonstrated, Hcy was not considered a biomarker of hypertension in cat in this study.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

Acknowledgments

The authors are grateful to Dr. Tiziana Vitiello that performed routine biochemistry analysis. Most of the samples included in this study were part of a study funded by the Winn Feline Foundation (Grant number WZ14-009).

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Figure legends

Fig. 1. A) Box and whisker plot illustrating the serum homocysteine (Hcy) concentration in groups of cats with CKD and in cats at risk (AR) according to International Renal Interest Society (IRIS) staging system; B) Box and whisker plot illustrating the serum homocysteine (Hcy) concentration in groups of cats divided according to IRIS substaging based on proteinuria (NP = non proteinuric; BP = borderline proteinuric; P = proteinuric); C) Box and whisker plot illustrating the serum homocysteine (Hcy) concentration in groups of cats divided according to systolic blood pressure (SBP; cats with SBP <160 mmHg vs cats with SBP ≥160 mmHg). Horizontal lines represent median, boxes represent the interquartile range (IQR); whiskers extend to ± 1.5 IQRs of the first or third quartile and outliers are shown as dots. The bolded asterisk indicates significant difference (*P*=0.002) of the IRIS 3-4 group compared to At risk group.

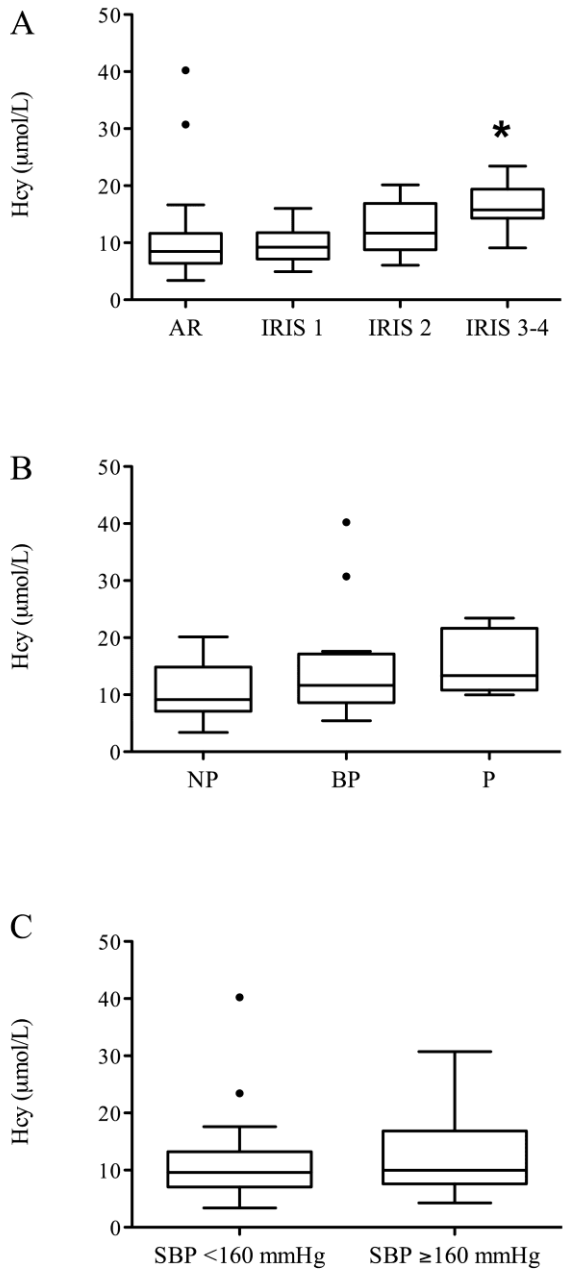

Fig. 2. A) Relationship between homocysteine (Hcy) and serum creatinine (sCrea) concentrations in 63 cats (28 cats at risk and 35 cats with chronic kidney disease, CKD), showing a moderate positive correlation (P<0.0001; r=0.51). B) Relationship between homocysteine (Hcy) and urinary protein-to-creatinine (UPC) ratio in 58 cats (27 cats at risk and 31 cats with CKD), showing a weak positive correlation (P=0.045; r=0.26). C) Relationship between homocysteine (Hcy) and systolic blood pressure (SBP) concentrations

in 50 cats (22 cats at risk and 28 cats with CKD); no correlation was found (P=0.71; r=0.05). The line shows the best-fit and the dotted lines represent the 95% confidence interval.

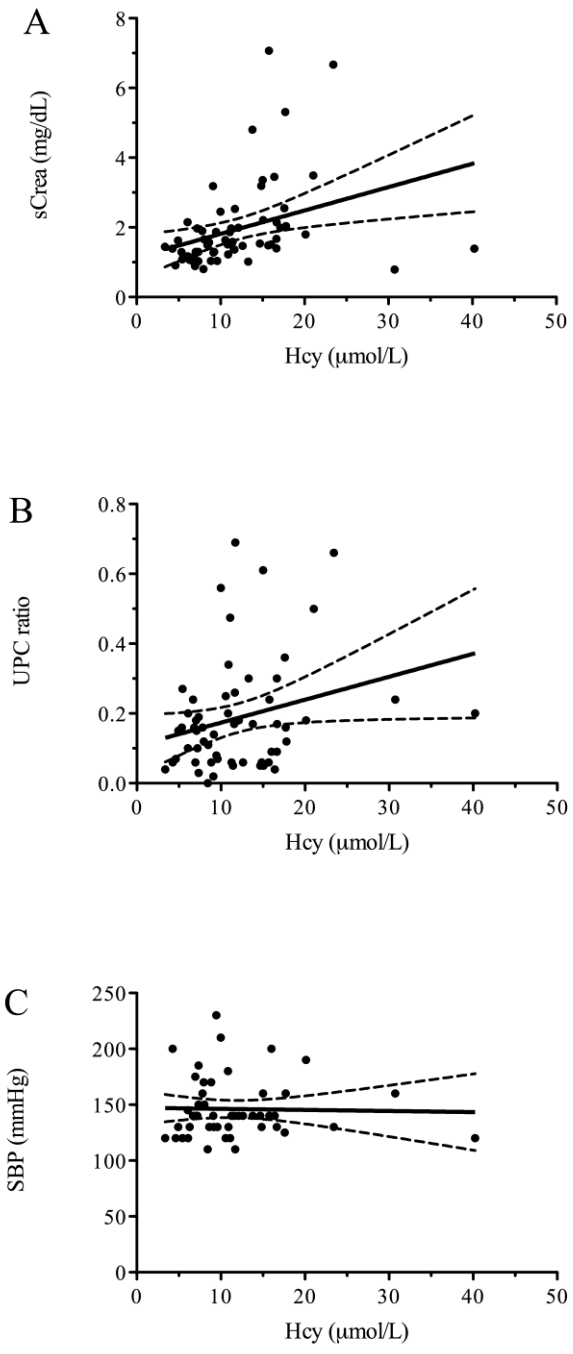

Fig. 3. Scatterplot illustrating serum homocysteine (Hcy) concentration measured at the time of inclusion, in cats that remained stable for 6 months ('Stable' group) and cats that progressed to a higher International Renal Interest Society (IRIS) stage within 6 months

('Progressive' group). A) all cats included in the study, irrespective of the IRIS stage. B) cats classified as at risk at the time of inclusion. The horizontal line represents the median and the whiskers represent 95% confidence interval of the median. The bolded asterisk indicates significant difference (P<0.05) between groups.

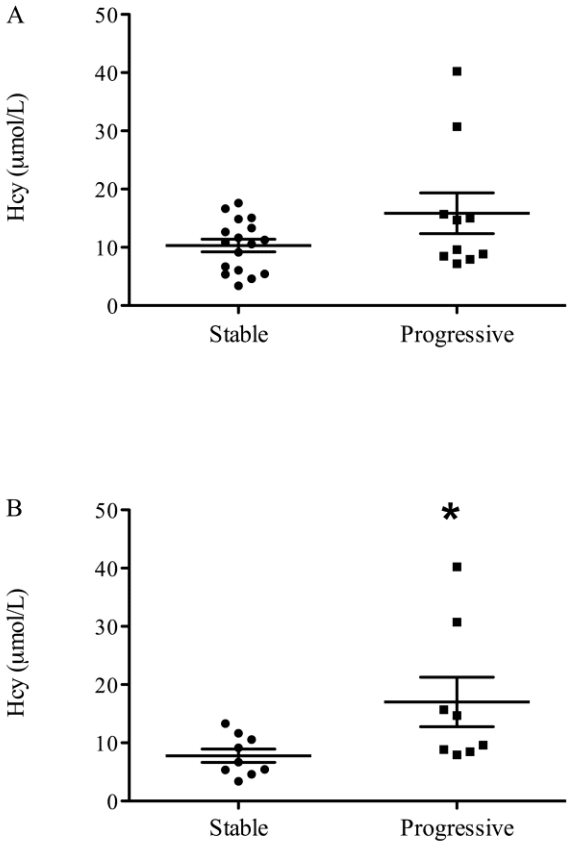

Table 1 Characteristics of feline patients at the time of sampling. Continuous variables were represented as the median (interquartile range)

Variables	Cats at risk (*n*=28)	Cats with CKD (*n*=35)
Age (years)	11.0 (8.7-13.2)	11.6 (8.7-15.3)
Serum creatinine (mg/dL)	1.29 (1.04-1.43)	1.99 (1.62-3.18)
IRIS stage		Stage 1 = 9
		Stage 2 = 17
		Stage 3 = 6
		Stage 4 = 3
UPC[a]	0.14 (0.06-0.20)	0.17 (0.08-0.34)
IRIS substage (proteinuria)[a]	NP = 19;	NP = 20;
	BP = 8	BP = 5;
		P = 6
SBP (mmHg)[b]	135 (120.0-145.0)	140 (130.0-160.0)
IRIS substage (hypertension)[b]	NT = 18;	NT = 17;
	HT = 2;	BHT = 2;
	SHT = 2	HT = 6;
		SHT = 3

CKD, Chronic kidney disease; IRIS, International Renal Interest Society; UPC, Urinary protein-to-creatinine ratio; NP, non proteinuric; BP, borderline proteinuric; P, proteinuric; SBP, systolic blood pressure; NT, normotensive; BHT, borderline hypertensive; HT, hypertensive; SHT severely hypertensive.
[a] UPC was not available in one cat at risk and four cats with CKD.
[b] SBP was not available in six cats at risk and seven cats with CKD.