

## Evaluation of Predictors of the Development of Azotemia in Cats

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**Background:** Chronic kidney disease (CKD) is a common condition in geriatric cats. Diagnosis is based on the development of persistent azotemia with inadequate urine concentrating ability. Biomarkers are sought for early identification.

**Hypothesis:** Clinical variables, urine concentrating ability, proteinuria, and *N*-acetyl- $\beta$ -D-glucosaminidase (NAG) index will be predictive of cats at risk of developing azotemia within 12 months.

**Animals:** Client-owned nonazotemic geriatric ( $\geq 9$  years) cats.

**Methods:** Prospective longitudinal cohort study monitoring a population of healthy nonazotemic geriatric cats every 6 months until development of azotemia, death, or the study end point (September 30, 2007). Multivariable logistic regression analysis was used to assess baseline clinical, biochemical, and urinalysis variables, urine protein to creatinine ratio (UP/C), urine albumin to creatinine (UA/C) ratio, and urinary NAG index as predictors of development of azotemia.

**Results:** One hundred and eighteen cats were recruited with a median age of 13 years. Ninety-five cats (80.5%) had been followed or reached the study end point by 12 months of which 30.5% (29/95) developed azotemia. Age, systolic blood pressure, plasma creatinine concentration, urine specific gravity, UP/C, UA/C, and NAG index were significantly associated with development of azotemia in the univariable analysis ( $P \leq .05$ ). However, in the multivariable analysis, only plasma creatinine concentration with either UP/C (Model 1) or UA/C (Model 2) remained significant.

**Conclusions and Clinical Importance:** This study demonstrates a high incidence of azotemia in a population of previously healthy geriatric cats. Proteinuria at presentation was significantly associated with development of azotemia although causal association cannot be inferred. Evaluation of NAG index offered no additional benefit.

**Key words:** Cat; Chronic renal failure; Kidney; Renal; Urinary tract.

Chronic kidney disease (CKD) is a commonly diagnosed condition in the geriatric feline population. An early retrospective report suggested that 15% of cats >15 years of age may have evidence of renal impairment.<sup>1</sup> The mean age of cats with CKD is reported as between 9.2 and 12 years depending on the population of cats examined and prevalence is considered to increase with age.<sup>1–3</sup> Data from the USA suggest that the prevalence of CKD in cats may be increasing, although the role of increased clinical diagnosis is uncertain and studies are composed predominantly of 2nd opinion case material, which therefore may not represent the true prevalence in the general population.<sup>1,4</sup> In cats, CKD usually is diagnosed on the basis of a persistently increased plasma creatinine concentration, but it is frequently reported that up to 75% of functional renal mass may be lost before azotemia is detected.<sup>5</sup> In addition, a proportion of nonazotemic and otherwise healthy cats are diagnosed and treated for idiopathic hypertension.<sup>a</sup> Glomerular filtration rate (GFR) measurements are rarely available in such cases to fully evaluate renal function, and longitudinal studies to determine if such

### Abbreviations:

|      |  |
|------|--|
| BAH  | Beaumont Animals' Hospital                   |
| CI   | confidence interval                          |
| CKD  | chronic kidney disease                       |
| ESRD | end-stage renal disease                      |
| GFR  | glomerular filtration rate                   |
| HT   | hypertensive                                 |
| –LR  | negative likelihood ratio                    |
| +LR  | positive likelihood ratio                    |
| LTF  | lost to follow-up                            |
| NAG  | <i>N</i> -acetyl- $\beta$ -D-glucosaminidase |
| NPV  | negative predictive value                    |
| NT   | normotensive                                 |
| OR   | odds ratio                                   |
| PDSA | People's Dispensary for Sick Animals         |
| PPV  | positive predictive value                    |
| SBP  | systolic blood pressure                      |
| UA/C | urine albumin to creatinine ratio            |
| UP/C | urine protein to creatinine ratio            |
| USG  | urine specific gravity                       |

cats are at increased risk of the developing azotemia are lacking.

In human medicine, considerable emphasis has been placed on identifying factors that may be associated with the progression of CKD to end-stage renal disease (ESRD) and the requirement for renal replacement therapy. The 2 factors that have perhaps received the most attention are systemic hypertension and proteinuria, but other factors such as estimated GFR, hyperphosphatemia and secondary hyperparathyroidism, anemia, decreased high-density lipoprotein cholesterol concentration, increased fasting glucose concentration, increased body mass index, and cigarette smoking also have been implicated.<sup>6–12</sup> Because of the low prevalence of CKD in the human population, few studies have evaluated factors that may be implicated in the initial

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development of CKD in a prospective manner. However, in those studies that have been performed, factors similar to those influencing progression are highlighted.<sup>13–17</sup>

More recently in human medicine, additional biomarkers have been sought that may indicate early tubular damage before development of azotemia. Although these studies largely have focused on the presence of urinary low-molecular-weight proteins (eg, retinol binding protein,  $\beta_2$ -microglobulin), there also has been interest in the use of urinary enzymes that may indicate tubular damage or increased tubular protein processing. One such enzyme (*N*-acetyl- $\beta$ -D-glucosaminidase [NAG]) is predominately found within the lysosomes of the proximal tubular cells and plays an integral role in the tubular processing of protein. NAG previously has been evaluated in small groups of healthy cats and in those with various types of urinary tract disease.<sup>18,19</sup> It also has been evaluated as a biomarker for the development of azotemia in hyperthyroid cats treated with methimazole.<sup>20</sup> In that study, NAG indexed to urinary creatinine was unable to differentiate cats at risk of developing azotemia. Recent data from our group indicated that the correlation between NAG index and plasma creatinine concentration is poor, but that NAG index may correlate with the magnitude of proteinuria.<sup>b</sup>

Previous studies have shown that magnitude of azotemia and proteinuria are significantly associated with the survival of cats with CKD.<sup>21,22</sup> However, no studies have evaluated factors associated with the development of CKD, and such studies would be useful to expand our understanding of the pathogenesis of CKD in cats. The aim of the current study was to assess the prevalence of development of azotemia within 12 months of presentation in a population of geriatric healthy cats and to assess clinical biochemical and urinalysis variables as risk factors for development of azotemia. Baseline clinical, biochemical, and urinalysis variables, UP/C, UA/C, and NAG index were evaluated as prospective predictors for the development of azotemia in cats followed over a 12-month period.

## Methods

### *Protocol for Case Recruitment*

A prospective cohort study of geriatric healthy cats was undertaken. Cats were recruited from 2 geriatric feline clinics held at first opinion practices in central London (Beaumont Animals' Hospital [BAH], Royal Veterinary College, Camden, and People's Dispensary for Sick Animals [PDSA], Bow). For entry into the study all cats were required to be  $\geq 9$  years of age and perceived as healthy by their owners. Cats with previously diagnosed metabolic or clinically relevant cardiovascular disease or those receiving any long-term treatment other than parasiticides were excluded from the study.

Before entry into the study, a full history was obtained and all cats received a complete physical examination. Systolic blood pressure (SBP) was measured in all cats after a period of acclimatization by the Doppler<sup>c</sup> technique as described previously.<sup>23</sup> Systemic hypertension was defined as SBP  $> 170$  mmHg on multiple occasions or on 1 occasion if in association with evidence of hypertensive choroidopathy. A fundic examination was performed in all cats with SBP  $> 160$  mmHg. One drop of tropicamide 1%<sup>d</sup> was applied to both eyes and indirect ophthalmoscopy was performed at the end

of the consultation period. Systemic hypertension was not an exclusion criterion but the subsequent management of these cats differed from normotensive cats.

### *Protocol for Collection and Storage of Blood and Urine Samples*

The Ethics and Welfare Committee of the Royal Veterinary College and the WALTHAM ethics committee approved the study protocol. Collection and storage of blood and urine samples were performed with the informed consent of the cats' owners. Blood samples were obtained by jugular venipuncture. Routinely, owners were asked to withhold food for 8 hours before visiting the practice. Blood samples were collected into lithium heparin and plain tubes. Heparinized plasma was used for immediate biochemical analysis.<sup>c</sup> For entry into the study cats were required to be nonazotemic with a plasma creatinine concentration  $< 2.0$  mg/dL. Plasma total thyroxine (T4) concentration was evaluated at entry into the study with either serum<sup>f</sup> or heparinized<sup>c</sup> plasma. Cats with total T4 concentrations  $> 55$  nmol/L were considered hyperthyroid and excluded from the study.

Collection of a urine sample by cystocentesis was a requirement for entry into the study. Urinalysis included measurement of specific gravity, pH,<sup>g</sup> dipstick chemistry analysis,<sup>h</sup> and microscopic examination of the sediment. Any plasma, serum, and urine sample, collected in excess of immediate diagnostic requirements, was centrifuged<sup>i</sup> at 4 °C for 10 minutes, aliquoted, and stored at  $-80^{\circ}\text{C}$ . These stored urine samples were then used for the batched measurement of urine protein to creatinine ratio (UP/C), urine albumin to creatinine ratio (UA/C), and urine NAG index. UP/C was performed at an external commercial laboratory<sup>e</sup> by a colorimetric pyrogallol red method to determine urine protein concentration and a colorimetric picric acid method to determine urine creatinine concentration. Urine albumin concentration was evaluated by a quantitative albumin ELISA previously validated for use with feline urine.<sup>21</sup> Urine NAG activity was measured with a commercially available colorimetric assay,<sup>j</sup> which uses 3-cresolsulfonphthaleinyl-*N*-acetyl- $\beta$ -glucosaminide as a substrate. Previous validation studies using human urine have shown NAG activity to be stable at  $-70^{\circ}\text{C}$  for a period of 1 year.<sup>24</sup> Urine NAG activity (U/L) was standardized to urine creatinine concentration to give the NAG index. Urine samples were excluded from analysis of proteinuria, albuminuria, and NAG index, if there was evidence of a urinary tract infection, bacteriuria, pyuria, or gross hematuria.

### *Longitudinal Case Follow-Up and Case Management*

Owners of all cats were invited to return the cats for repeat examinations every 6 months. At each visit, cats received a complete physical examination, measurement of SBP, and plasma biochemistry as described previously for the initial visit. Total T4 was evaluated annually in all nonazotemic cats or more frequently when the history (eg, polyphagia, weight loss), clinical examination (eg, palpable goiter, tachycardia, low body condition score), and biochemical analysis (increased alanine aminotransferase or alkaline phosphatase activity) were consistent with a potential diagnosis of hyperthyroidism. Total T4 was not evaluated in all cats at the time of diagnosis of azotemia. When the bladder was palpable, urine samples were obtained at each subsequent visit by cystocentesis and analyzed as described previously. Nonazotemic cats diagnosed with systemic hypertension were treated with amlodipine besylate<sup>t</sup> at an initial dose of 0.625 mg once daily. Hypertensive cats were reexamined 1–2 weeks after initiating antihypertensive medication. In cats in which SBP remained  $> 160$  mmHg, the amlodipine dose was increased to 1.25 mg once daily. After stabilization of SBP, treated hypertensive cats were offered reexamination visits every 8 weeks with physical examination and SBP assessment at each visit. Plasma

biochemistry and, whenever possible, urinalyses were performed at every other visit.

After enrollment into the prospective study, any additional veterinary attention required for conditions unrelated to hypertension, renal disease, or hyperthyroidism was provided by veterinary surgeons at either PDSA or BAH. Any medications used during this study were prescribed on an individual basis in accordance with underlying disease conditions and were not an exclusion criterion once cats were enrolled.

All cats included in the study had the potential to be followed for a minimum of 12 months and were monitored until the development of azotemia (defined as a plasma creatinine concentration  $> 2.0$  mg/dL), death or the study end point (September 30, 2007). Cats that were not presented for reexamination despite multiple telephone messages and a written invitation to return to the geriatric cat clinic were considered lost to follow-up (LTF).

### Statistical Analysis

All statistical analyses were performed by computerized software<sup>1,m</sup> and  $P \leq .05$  was considered significant. Descriptive statistics are presented to define the population, clinical findings, biochemical variables, and urinalysis results for cats at entry into the study and during longitudinal follow-up. Quantitative data were assessed graphically for normality and results are presented as median [25th, 75th percentiles] unless otherwise stated. The population of cats was examined for disease development over the study period and to describe any long-term medications used. In particular, the development of azotemia within the population was examined. A Mann-Whitney *U*-test was used to compare quantitative clinical variables at entry into the study between normotensive and hypertensive cats and also between cats that remained nonazotemic and those that developed azotemia by 12 months. A Wilcoxon signed-rank test was used to compare biochemical variables in cats that developed azotemia at entry into the study and at 12 months and also in cats that remained nonazotemic at entry into the study and at 12 months.

### Multivariable Logistic Regression

Data were evaluated in a multivariable logistic regression model to determine clinical variables associated with development of azotemia. Univariable logistic regression analysis was performed and biologically important factors and variables significant at the 20% level ( $P < .2$ ) were included in a multivariable regression model using a manual forward selection approach. Continuous variables were assessed for linearity. Colinearity of NAG index, UA/C, and UP/C were tested by performing nonparametric Spearman's rank correlation.<sup>25</sup> Because of the lack of independence, UP/C and UA/C were not evaluated within the same model. First order interactions were assessed and goodness of fit of each model was assessed by the Hosmer-Lemeshow test.<sup>26</sup> A nonsignificant result ( $P > .05$ ) was considered to indicate reasonable model fit. The following continuous variables were assessed in all multivariable models: plasma creatinine concentration, age, SBP, urine specific gravity (USG), and NAG index. In Model 1, UP/C was included as a continuous variable as a measure of proteinuria and, in Model 2, UA/C replaced UP/C.

In addition, cats were classified at presentation by UP/C and plasma creatinine concentration using cut points of  $>0.2$  and  $>1.6$  mg/dL, respectively. These are not necessarily the optimal cut points for either UP/C or plasma creatinine concentration but they represent clinically useful criteria that have been defined previously by the International Renal Interest Society.<sup>27</sup>

Cats were categorized 1st by whether they had either a UP/C  $> 0.2$  or creatinine concentration  $> 1.6$  mg/dL and 2nd by whether they had a UP/C  $> 0.2$  and creatinine concentration  $> 1.6$  mg/dL.

These classifications were used to calculate observed prevalence, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (+LR), negative likelihood ratio (−LR), sensitivity, and specificity to detect the development of azotemia.

## Results

One hundred and eighteen cats had been followed or reached a study end point by 12 months; 15 were hypertensive at entry into the study and subsequently were treated with amlodipine besylate. The median age of cats ( $n = 115$ ) at entry into the study was 13.0 years [11.0, 15.0]. Age was unknown in 3 cats that had been obtained by their owners as adult strays. The predominant breeds were domestic shorthair ( $n = 83$ ), domestic longhair ( $n = 15$ ), and Persian ( $n = 12$ ) with the following breeds also represented: British Shorthair ( $n = 3$ ), Burmese ( $n = 2$ ), Russian Blue ( $n = 1$ ), Occicat ( $n = 1$ ), and Bengal ( $n = 1$ ). Within the population, there were 68 neutered female and 50 neutered male cats.

The most common clinical findings identified on physical examination were dental disease (60.0%; 71/118), palpable thyroid goiter (26.3%; 31/118), low-grade systolic murmur with no evidence of heart failure (11.9%; 14/118), and skin disease including flea infestation (9.3%; 11/118). At entry into the study 31.4% (37/118) of cats had USG  $\leq 1.035$  and 8 cats had irregular kidney contour on abdominal palpation. Of the 15 cats diagnosed with systemic hypertension at entry into the study, 9 (60%) had evidence of choroidopathy.

Within the 12 months after recruitment, 9.3% (11/118) of the cats died or were euthanized for the following reasons: abdominal mass ( $n = 3$ ), acute renal failure ( $n = 1$ ), sudden onset dyspnea ( $n = 2$ ), ulcerated dermal neoplasia ( $n = 2$ ), gastrointestinal lymphoma ( $n = 1$ ), and unknown cause ( $n = 2$ ). The median age of cats that died was 14.9 years [11.4, 17.0]. Often, a more specific diagnosis was not obtained and classification was based on information available from the clinical records. Eleven cats were LTF (9.3%) and 1 cat was excluded due to development of diabetes mellitus. Sixty-six cats remained nonazotemic at 12 months, of which 8 were treated hypertensive cats. Azotemia was diagnosed in 30.5% (29/95) of cats by 12 months, of which 5 were treated hypertensive cats. Hyperthyroidism was diagnosed in 8.5% (10/118) of the cats during the 1st 12 months, of which 2 were rendered euthyroid by treatment. Three previously normotensive cats were diagnosed and treated for systemic hypertension during the 1st 12 months, but these cats all remained nonazotemic.

Within 12 months, 2 cats (1 normotensive, 1 treated hypertensive) were started on long-term benazepril treatment for cardiovascular disease. Other conditions treated in cats that remained nonazotemic at 12 months included conjunctivitis ( $n = 3$ ), miliary dermatitis ( $n = 3$ ), urinary tract infection ( $n = 2$ ), feline lower urinary tract disease ( $n = 1$ ), upper respiratory tract infection ( $n = 1$ ) and chronic diarrhea, and vomiting of unknown etiology ( $n = 1$ ). One cat developed an apocrine sweat gland carcinoma on the lower mandible, 1 cat had a mammary mass

**Table 1.** Biochemical and urine variables assessed at entry to the prospective study and during longitudinal follow-up

| Clinical Variable        | Data at Entry to Study |       | Data Available at 12 Months |      |                      |      |
|--------------------------|------------------------|-------|-----------------------------|------|----------------------|------|
|                          |                        |       | Nonazotaemic                |      | Azotemic             |      |
|                          |                        | n=118 |                             | n=66 |                      | n=29 |
| Age (years)              | 13.0 [11.0, 15.0]      | 115   | 13.6 [12.1, 17.6]           | 66   | 15.2 [12.8, 16.9]    | 29   |
| Weight (kg)              | 4.20 [3.53, 4.92]      | 111   | 4.13 [3.50, 4.65]           | 66   | 3.81 [2.90, 4.90]    | 27   |
| PCV (%)                  | 37.0 [34.0, 40.0]      | 112   | 37 [34, 39]                 | 66   | 34 [31, 36]          | 29   |
| Urea (mg/dL)             | 29.7 [26.3, 35.3]      | 118   | 30.5 [26.3, 35.6]           | 63   | 44.3 [36.7, 52.9]    | 29   |
| Creatinine (mg/dL)       | 1.53 [1.29, 1.72]      | 118   | 1.53 [1.33, 1.76]           | 63   | 2.25 [2.08, 2.48]    | 29   |
| Potassium (mEq/L)        | 3.9 [3.7, 4.2]         | 117   | 3.90 [3.60, 4.10]           | 63   | 3.90 [3.60, 4.15]    | 29   |
| Phosphorus (mg/dL)       | 4.05 [3.65, 4.49]      | 117   | 4.06 [3.44, 4.71]           | 63   | 4.58 [3.87, 5.91]    | 29   |
| Total thyroxine (nmol/L) | 23.5 [19.0, 28.8]      | 113   | 21.5 [15.4, 28.4]           | 59   | —                    | —    |
| USG                      | 1.047 [1.030, 1.058]   | 118   | 1.037 [1.029, 1.060]        | 52   | 1.020 [1.016, 1.030] | 22   |
| UP/C                     | 0.16 [0.12, 0.22]      | 113   | 0.14 [0.11, 0.19]           | 48   | 0.17 [0.09, 0.38]    | 20   |
| UA/C                     | 15.5 [8.0, 39.3]       | 109   | 14.8 [7.24, 34.1]           | 47   | 39.3 [13.0, 77.9]    | 20   |
| NAG index                | 1.07 [0.40, 1.78]      | 85    | —                           | —    | —                    | —    |
| SBP (mmHg)               | 139.8 [125.5, 163.3]   | 118   | 136.0 [124.0, 150.6]        | 66   | 149.2 [133.6, 174.2] | 29   |

Biochemical data were not available for 3 cats that were borderline or hyperthyroid at the 12-month visit.

n, number of cats for which data are available; SBP, systolic blood pressure; UA/C, urine albumin to creatinine ratio; UP/C, urine protein to creatinine ratio; USG, urine specific gravity.

removed, and 1 cat had an oral mass removed, which was inflammatory on histopathological examination. Three cats had dental procedures performed during the first 12-month period and 1 cat was treated 7 weeks before its 12-month examination with long-acting methylprednisolone for severe stomatitis and gingivitis.

Biochemical and urinalysis variables for cats at entry into the study and at 12 months were divided according to renal status and are presented in Table 1. Hypertensive cats (HT) had significantly higher UA/C (HT, 29.3 [15.0, 75.2]; NT, 14.0 [7.4, 37.4],  $P = .04$ ), and plasma urea concentration (HT, 36.0 mg/dL [33.0, 41.0]; NT 29.1 mg/dL [26.0, 33.9],  $P = .002$ ) than normotensive (NT) cats at entry into the study. Hypertensive cats also had significantly lower plasma potassium concentration (HT, 3.6 mEq/L [3.4, 3.9]; NT, 4.0 mEq/L [3.7, 4.2],  $P =$

.001) and USG (HT, 1.030 [1.022, 1.052]; NT, 1.050 [1.034, 1.058],  $P = .017$ ) than normotensive cats at entry into the study.

There was no significant difference in baseline biochemical variables for nonazotemic cats at entry into the study and at 12 months. Comparing biochemical and urinalysis data for cats that developed azotemia at entry into the study and at the point of development of azotemia, there were significant increases in plasma creatinine ( $P < .001$ ), urea ( $P = .001$ ), and phosphate ( $P = .012$ ) concentrations and significant decrease in USG ( $P = .002$ ), weight ( $P = .03$ ), and PCV ( $P = .045$ ). No change in UP/C or UA/C was identified. Comparison of biochemical and urinalysis data at entry into the study between those cats that remained nonazotemic and those that developed azotemia is presented in Table 2.

**Table 2.** Biochemical and urine variables at entry to the prospective study of cats that remained nonazotemic and those that developed azotemia at 12 months

|                     | Clinical Parameters at Entry to the Prospective Study |    |                       |    |          |
|---------------------|---|----|-----------------------|----|----------|
|                     | Nonazotemic at 12 months                              | n  | Azotemic at 12 months | n  | <i>P</i> |
| Urea (mg/dL)        | 28.6 [25.5, 32.5]                                     | 66 | 34.3 [30.0, 44.0]     | 29 | < .001   |
| Creatinine (mg/dL)  | 1.52 [1.30, 1.71]                                     | 66 | 1.73 [1.57, 1.89]     | 29 | .001     |
| UA/C                | 10.7 [6.6, 29.5]                                      | 60 | 34.1 [15.1, 72.0]     | 27 | .002     |
| UP/C                | 0.14 [0.11, 0.20]                                     | 62 | 0.19 [0.14, 0.39]     | 29 | .004     |
| Age (years)         | 12.6 [11.0, 14.4]                                     | 64 | 14.6 [12.2, 15.1]     | 29 | .023     |
| USG                 | 1.050 [1.032, 1.060]                                  | 66 | 1.042 [1.023, 1.051]  | 29 | .043     |
| NAG index           | 0.85 [0.35, 1.30]                                     | 49 | 1.40 [0.74, 2.39]     | 21 | .05      |
| SBP (mmHg)          | 135.6 [121.6, 158.0]                                  | 66 | 151.2 [131.2, 165.7]  | 29 | .053     |
| Phosphorus (mg/dL)  | 4.09 [3.72, 4.49]                                     | 66 | 3.80 [3.47, 4.37]     | 28 | .216     |
| Cholesterol (mg/dL) | 177.6 [144.0, 212.4]                                  | 66 | 166.0 [151.7, 188.4]  | 28 | .321     |
| PCV (%)             | 38 [35, 41]   | 62 | 37 [34, 40]           | 27 | .366     |
| Potassium (mEq/L)   | 3.90 [3.60, 4.20]                                     | 66 | 3.95 [3.52, 4.10]     | 28 | .917     |

n, number; NAG index, urine *N*-acetyl- $\beta$ -D-glucosaminidase to creatinine ratio; *P*, significance; SBP, systolic blood pressure; UA/C, urine albumin to creatinine ratio; UP/C, urine protein to creatinine ratio; USG, urine specific gravity.

**Table 3.** Multivariable logistic regression analysis evaluating variables associated with development of azotemia

| Variable       | <i>B</i> | SE    | <i>P</i> | OR    | 95% CI |       |
|----------------|----------|-------|----------|-------|--------|-------|
|                |          |       |          |       | Lower  | Upper |
| <b>Model 1</b> |          |       |          |       |        |       |
| Creatinine     | 0.059    | 0.021 | .004     | 1.061 | 1.019  | 1.105 |
| UP/C           | 1.254    | 0.441 | .004     | 3.505 | 1.479  | 8.304 |
| Constant       | -11.437  | 3.333 | .001     | 0.000 | —      | —     |
| <b>Model 2</b> |          |       |          |       |        |       |
| Creatinine     | 0.048    | 0.018 | .008     | 1.049 | 1.012  | 1.087 |
| UA/C           | 0.026    | 0.012 | .03      | 1.027 | 1.003  | 1.052 |
| Constant       | -8.405   | 2.671 | .002     | 0.000 | —      | —     |

Model 1 contained UP/C as a measure of proteinuria while Model 2 contained UA/C. Sixty-nine cases were included in Model 1 and 68 cases in model 2.

*B*, regression coefficient; CI, confidence interval of the odds ratio; Constant, baseline model with no parameters entered into the model; OR, odds ratio; *P*, *P* value; SE, standard error of the regression coefficient; UA/C, urine albumin to creatinine ratio; UP/C, urine protein to creatinine ratio.

### Multivariable Logistic Regression Analysis

The following variables were found to have  $P < .2$  within the univariable analysis and were therefore included in the multivariable analysis: age odds ratio (OR) 1.165, 95% confidence interval (CI) [0.985, 1.375] ( $n = 93$ ); USG OR 0.00 CI [0.00, 0.017] ( $n = 95$ ); SBP OR 1.010 CI [0.997, 1.014] ( $n = 95$ ); creatinine OR 1.040 CI [1.015, 1.066] ( $n = 95$ ); UP/C OR 1.366 CI [0.971, 1.921] ( $n = 91$ ); UA/C 1.018 CI [1.004, 1.031] ( $n = 87$ ); NAG index OR 1.299 CI [0.950, 1.776] ( $n = 70$ ).

NAG index was positively correlated with both UP/C ( $r = 0.411$ ;  $P < .001$ ) and UA/C ( $r = 0.278$ ;  $P = .018$ ). However, correlation coefficients were weak ( $< 0.7$ ) and therefore NAG index was included for evaluation in the multivariable models with other measures of proteinuria.<sup>28</sup> In Models 1 and 2, plasma creatinine concentration with either UP/C or UA/C, respectively, were the only variables significantly associated with development of azotemia (Table 3). The Hosmer-Lemeshow goodness-of-fit criteria was satisfied for both models (Model 1,  $P = .316$ ; Model 2,  $P = .286$ ). The observed prevalence, PPV, NPV, +LR, -LR, sensitivity, and specificity for the use of UP/C and plasma creatinine

concentration as indicators of the development of azotemia are presented in Table 4.

### Discussion

This is the 1st study designed to prospectively evaluate and follow longitudinally a group of nonazotemic geriatric cats and to identify biochemical and urinalysis variables at entry into the study that were indicative of the development of azotemia. In the multivariable logistic regression analysis, only plasma creatinine concentration together with a measure of proteinuria (either UP/C or UA/C) were found to be significantly associated with development of azotemia at 12 months.

In the future, the ability to diagnose cats with mild renal impairment before the onset of azotemia may justify the more routine use of invasive investigative procedures such as renal biopsy to determine the underlying primary disease process damaging the kidney. This may facilitate more specific disease-oriented intervention with the potential to either halt or slow progression to clinically relevant disease.

Thirty percent of cats recruited had developed azotemia by 12 months. In human adults, the prevalence of developing CKD is reported to be 1.5–9.4%, although this is dependent on both inclusion criteria and the classification schemes used for the diagnosis of CKD, particularly because some staging schemes for humans can include an estimated GFR within the physiological reference range.<sup>14,15,29</sup> Clinical variables that have been associated with development of CKD in human adults include age, estimated GFR, hypertension, diabetes mellitus, proteinuria, obesity, smoking status, and increased triglyceride concentration with decreased high-density lipoproteins.<sup>13–17,30,31</sup>

At entry into the study, 12.7% of cats were hypertensive. No previous studies have evaluated the prevalence of systemic hypertension in the nonazotemic feline population, but as may be expected it was lower than the prevalence of hypertension in cats with previously diagnosed CKD (19.4%).<sup>23</sup> Some of these cats may have had white-coat hypertension,<sup>32</sup> but hypertensive choroidopathy was identified in 60% of cats as primary evidence of target organ damage. The remaining cats were evaluated on multiple occasions and showed persistence of increased SBP before antihypertensive treatment was started.

**Table 4.** Observed prevalence, sensitivity and specificity, positive and negative predictive values for classification of cats combining plasma creatinine concentration with UP/C

| Model   | Observed Prevalence (%) | PPV (%) | NPV (%) | Sensitivity (%) | Specificity (%) | LR [CI]              |                      |
|---|-------------------------|---------|---------|-----------------|-----------------|----------------------|----------------------|
|   |                         |         |         |                 |                 | +LR [CI]             | -LR [CI]             |
| UP/C > 0.2 or plasma creatinine > 1.6 mg/dL or both | 31.9                    | 43.6    | 85.3    | 82.8            | 46.8            | 1.555 [1.168, 2.071] | 0.369 [0.159, 0.854] |
| UP/C > 0.2 and plasma creatinine > 1.6 mg/dL        | 31.9                    | 58.8    | 74.3    | 34.5            | 79.7            | 3.054 [1.293, 7.213] | 0.739 [0.559, 0.976] |

CI, 95% confidence interval; -LR, negative likelihood ratio; +LR, positive likelihood ratio; NPV, negative predictive value; Observed prevalence, observed prevalence of cats developing azotemia; PPV, positive predictive value; UP/C, urine protein to creatinine ratio.

Hypertensive cats had significantly lower USG and higher UA/C than did normotensive cats. The decreased USG may imply either early loss of renal concentrating ability or hypertension-induced diuresis. In human hypertensive patients, albuminuria is considered to be a marker of endothelial dysfunction and may be a more sensitive marker than proteinuria.<sup>33,34</sup> In addition, urine albumin excretion is significantly associated with the risk of developing hypertension and has been shown to be predictive of cardiovascular and noncardiovascular mortality.<sup>35–37</sup> The increased UA/C may indicate that hypertensive cats have concurrent glomerular hypertension and impaired tubular reabsorptive capacity. Hypertensive cats also had significantly lower plasma potassium concentration. This finding has previously been demonstrated in azotemic cats with systemic hypertension and may be the result of hyperaldosteronism.<sup>n,23,38–40</sup>

In the current study, azotemia was defined as a plasma creatinine concentration higher than the upper limit of the laboratory reference range. Plasma creatinine concentration is an insensitive indicator of GFR, and the use of this cut off is an arbitrary diagnostic criterion. In human adults, decreased estimated GFR has been significantly associated with the development of CKD.<sup>14,15</sup> Despite being within the laboratory reference range at entry into the study, cats that developed azotemia had significantly higher plasma urea and creatinine concentrations than did those that remained nonazotemic at 12 months. Even when plasma creatinine concentration is within the laboratory reference range, a relatively high plasma creatinine concentration still may reflect a lower GFR and as such, without direct measurement of GFR, it is impossible to be certain that these cats had no evidence of renal impairment at entry into the study. Experimental studies performing subtotal nephrectomy procedures in rats have indicated that after an initial renal insult, compensatory mechanisms are initiated to maintain effective GFR including renal arteriolar vasodilatation and hypertrophy of remaining nephrons.<sup>41,42</sup> These adaptive mechanisms also have been identified in the cat after subtotal nephrectomy and although initially beneficial, may ultimately be detrimental to the kidney and lead to progression of CKD.<sup>43</sup> In cats that develop azotemia, such compensatory mechanisms can be hypothesized to play a role in the ultimate decrease in GFR and onset of overt azotemia.

No prerequisite was made for urine concentrating ability for entry to the study. Those cats that developed azotemia at 12 months had significantly lower USG at entry into the study than did those that remained nonazotemic. They also showed a significant decrease in USG between entry to the study and the point of development of azotemia. However, interpretation of urine concentrating ability using spot assessment can be misleading. Although decreased urine concentrating ability may be indicative of renal damage, there are also situations in which low USG may be considered a normal physiological response (eg, pressure induced diuresis). Experimental studies performed in cats have previously demonstrated that even after 50–80% nephrectomy, cats are able to maintain urine concentrating ability.<sup>5</sup> Four

cats in the current study developed azotemia and clinical signs consistent with a diagnosis of CKD but maintained urine concentrating ability (USG 1.064, 1.050, 1.041, 1.036). Three of these cats were younger (10, 11.5, and 13 years) than the median age of cats with azotemia. Radiographic studies were not performed but it is possible that in these younger cats the underlying cause for CKD may not be the same as in the population as a whole and may result from a different underlying pathology.<sup>44</sup>

Cats that developed azotemia were also significantly older than those that remained nonazotemic. The prevalence of CKD in cats has been reported to increase with advancing age and in human adults age is a factor significantly associated with the development of CKD.<sup>1,15</sup> Recently, a study by Lawler et al<sup>45</sup> suggested that feline CKD may be part of a normal aging phenomenon and a survival driven adaptive process. In that study, cats that died of renal causes displayed progressive tubular deletion and interstitial fibrosis and were significantly older than cats that died from nonrenal causes. In the current study, the age of cats at euthanasia was not significantly different from the age at the point of developing azotemia. This implies that if azotemic cats in our study were followed longitudinally they too would be older at the time of renal death than those euthanized.

In human patients, SBP is significantly associated with both the development and progression of CKD.<sup>13–15</sup> In the study by Syme and colleagues, SBP was not associated with survival in cats with CKD. However, cats in that study were all treated appropriately whenever systemic hypertension was diagnosed.<sup>21</sup> Similarly, long-term blood pressure control was not associated with all-cause mortality in a population of hypertensive cats.<sup>46</sup> In the current study, only 5/13 (38.5%) treated hypertensive cats developed azotemia within 12 months of presentation, suggesting that the prevalence was no higher than in normotensive cats. In the human literature, there is concern regarding the sole use of 2nd generation dihydropyridine calcium channel blockers due to their preferential vasodilatation of the afferent arteriole and risk of exacerbation of glomerular hypertension if systemic blood pressure is inadequately controlled.<sup>47</sup> However, in the current study, the group of treated hypertensive cats was small and no attempt was made to classify the adequacy of blood pressure control over time. Clearly, it would be unethical to have a group of untreated hypertensive cats because many hypertensive cats had evidence of target organ damage (particularly ocular) at entry into the study.

NAG has been shown to be a predictor of future renal failure in human patients entering an intensive care unit and was predictive of nonalbuminuric, nonazotemic human patients with type-II diabetes that went on to develop diabetic nephropathy over a 5-year period.<sup>48,49</sup> When renal function is normal, assessment of NAG index may represent protein processing in the proximal tubules rather than direct tubular damage. In this situation, the ability to differentiate NAG A, the isoenzyme continuously excreted from proximal tubular cells and up-regulated during increased protein processing, from NAG B, released during proximal tubular cell damage

may be of interest.<sup>50</sup> In the current study, NAG index was positively correlated with UP/C and was significantly higher in cats that developed azotemia than in those remaining nonazotemic. However, it was not retained within the multivariable model and this leads us to question whether NAG index has any major additional benefit to the measurement of proteinuria in predicting the development of azotemia.

The multivariable logistic regression analysis found only plasma creatinine concentration together with a measure of proteinuria (either UP/C or UA/C) to contribute toward a model associated with the development of azotemia. UA/C did not appear to offer any advantage over the measurement of UP/C. In fact, UP/C had a substantially higher OR in these models than either plasma creatinine or UA/C. UP/C has been significantly associated with decreased survival of cats with CKD and systemic hypertension.<sup>21,46</sup> The results of the current multivariable analysis suggest that even a low magnitude of proteinuria is associated with the development of azotemia. In experimental studies, the transport and breakdown of protein within the proximal tubules has been demonstrated to stimulate an inflammatory cascade that can result in progressive tubulointerstitial inflammation and tubule loss.<sup>51</sup> However, whether proteinuria contributes to the pathophysiology leading to the development of azotemia or is just a marker of decreased tubular uptake capacity cannot be ascertained from the present study.

All variables assessed in relation to the development of azotemia were evaluated at a single time points only and no attempt was made to assess the role of change in variable as a predictor of the development of azotemia. One of the requirements for entry into this prospective study was the clinician's ability to obtain a urine sample within the consultation period. Cystocentesis is more difficult to perform in cats that are overweight and have small bladders perhaps due to their superior urine concentrating ability. Our sample population may therefore be biased against healthy or overweight geriatric cats with good urine concentrating ability. In an ideal situation, such cats would have been hospitalized until their bladder was of a suitable size to perform cystocentesis or alternatively ultrasound-guided cystocentesis might have been used. A further limitation was that TT4 was not evaluated in all cats at the time of diagnosis of azotemia. Renal disease may lead to suppression of thyroxine concentrations and substantially complicate the diagnosis of hyperthyroidism. In a small number of cats, therefore, the diagnosis of hyperthyroidism may have been missed at the time of the diagnosis of azotemia.

This longitudinal study is, to our knowledge, the first to show prospectively the proportion of an at-risk population of normal healthy aging cats that become azotemic within 1 year of recruitment. Plasma creatinine concentration, UP/C, and UA/C were predictors of the development of azotemia in cats. UP/C is a relatively inexpensive commercially available laboratory test that requires only a small volume of urine. Monitoring UP/C in conjunction with plasma creatinine concentration should therefore be advocated as part of geriatric screening programs for cats.

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## Footnotes

- <sup>a</sup> Elliott J, Fletcher M, Syme HM. Idiopathic feline hypertension; epidemiological study. *J Vet Intern Med* 2003; 17 (3): 754 (abstract)
- <sup>b</sup> Jepson, RE, Vallance, C, Syme, HM, Elliott, J. Urinary *N*-acetyl- $\beta$ -D-glucosaminidase (NAG) index in cats with variable azotaemia and as a predictor of chronic kidney disease. *J Vet Intern Med* 2008; 22 (3): 730 (abstract)
- <sup>c</sup> Parks Electronic Doppler Model 811B, Perimed UK, Bury St Edmunds, UK
- <sup>d</sup> Tropicamide 1%, Midriacyl, Alcon, Hemel Hempstead, UK
- <sup>e</sup> Idexx Laboratories, Wetherby, Yorkshire, UK
- <sup>f</sup> Royal Veterinary College, Clinical Services Division Diagnostic Laboratory, North Mymms, Hertfordshire, UK
- <sup>g</sup> HI 9224 pH meter, Hanna Instruments, Leighton Buzzard, UK
- <sup>h</sup> Multistix Urine Chemistry Reagent Strips, Bayer Diagnostics, Newbury, Berks, UK
- <sup>i</sup> Mistral 3000, Sanyo-Gallenkamp, Leics, UK
- <sup>j</sup> *N*-acetyl- $\beta$ -glucosaminidase kit Cat no: 875406, Roche, Switzerland
- <sup>k</sup> Amlodipine 0.625–1.25 mg/cat/day, Istin, Pfizer, Sandwich, Kent, UK
- <sup>l</sup> GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego, CA
- <sup>m</sup> SPSS 15.0 for Windows, SPSS Inc, Chicago, IL
- <sup>n</sup> Syme HM, Markwell PJ, Elliott J. Aldosterone and plasma renin activity in cats with hypertension and/or chronic renal failure. *J Vet Intern Med* 2002; 16: 354 (abstract)
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