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Relationship between Plasma Fibroblast Growth Factor-23 Concentration and Survival Time in Cats with Chronic Kidney Disease

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Background: Fibroblast growth factor-23 (FGF-23) and parathyroid hormone (PTH) are commonly increased in cats with azotemic chronic kidney disease (CKD). Both are predictors of survival time in human patients, but these relationships have not previously been examined in the cat.

Objectives: To investigate the relationship between plasma FGF-23 and PTH concentrations at diagnosis of CKD in cats with survival time and with disease progression over 12 months.

Animals: 214 azotemic, client-owned cats (≥9 years).

Methods: *Retrospective study:* Biochemical and urinary variables at diagnosis of azotemic CKD, including plasma FGF-23 and PTH concentrations were assessed as predictors of survival time (all-cause mortality) using Cox regression, and as predictors of CKD progression over 12 months using logistic regression.

Results: In the final multivariable Cox regression model, survival was negatively associated with plasma creatinine ($P = .002$) and FGF-23 concentrations ($P = .014$), urine protein-to-creatinine ratio ($P < .001$) and age ($P < .001$). Survival was positively associated with PCV ($P = .004$). In the final multivariable logistic regression model, independent predictors of CKD progression included logFGF-23 and age. Neither plasma phosphate nor PTH was found to be an independent predictor of survival time or of CKD progression.

Conclusions and Clinical Importance: Plasma FGF-23 concentration is a novel prognostic indicator in cats with CKD, independent of other factors including plasma creatinine and phosphate concentrations. Further work is required to assess if FGF-23 contributes directly to CKD progression, but regardless these findings may make FGF-23 a useful biomarker for predicting poorer outcomes in cats with CKD.

Key words: Feline; Parathyroid hormone; Phosphate; Progression.

Chronic kidney disease (CKD) in cats is highly prevalent; 31% of cats over 15 years of age have azotemic CKD[1] and there is evidence of CKD in 60.4% of cats, with a prevalence of 80.9% in cats over 15 years of age.[2] CKD causes severe morbidity and mortality, with renal disorders being the most common cause of mortality in cats ≥5 years of age in the UK, accounting for 13.6% of deaths.[3]

The syndrome of the clinical, biochemical and imaging abnormalities that are correlated with CKD and renal osteodystrophy is termed "chronic kidney disease-mineral and bone disorders" (CKD-MBD).[4] Development of CKD-MBD is considered to be a consequence of reduced Glomerular Filtration Rate (GFR), and associated phosphate retention. Calcium-phosphate (Ca-P) derangements appear prevalent in cats with CKD; with the most common biochemical derangement previously documented to be hyperparathyroidism, occurring in 84% of azotemic cats.[5] Additionally, the phosphatonin fibroblast growth factor-23 (FGF-23) has been demonstrated to increase with advancing International Renal Interest Society (IRIS) stage of CKD and to be further increased in cats with higher plasma phosphate concentrations.[6]

FGF-23 is secreted primarily by osteocytes[7] and osteoblasts,[8,9] in response to hyperphosphatemia and increased plasma calcitriol concentrations.[10] FGF-23 acts in the kidney to inhibit calcitriol production by reducing mRNA expression of the vitamin D synthesis

Abbreviations:

BSH	British short-hair
Ca-P	calcium-phosphate
CI	confidence interval
CKD	chronic kidney disease
CKD-MBD	chronic kidney disease-mineral and bone disorders
FGF-23	fibroblast growth factor 23
GFR	glomerular filtration rate
HR	hazard ratio
IRIS	International Renal Interest Society
OR	odds ratio
PTH	parathyroid hormone
SBP	systolic blood pressure
T.calcium	total calcium
TT4	total thyroxine
UPC	urine protein-to-creatinine ratio
USG	urine specific gravity
UTI	urinary tract infection

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enzyme (25-hydroxyvitamin D-1α-hydroxylase) and to increase phosphaturia by down-regulating sodium-phosphorus type II co-transporters (NaPi-IIa and NaPi-IIc) in the proximal tubules.[11] It also acts in the parathyroid gland to decrease parathyroid hormone (PTH) production and secretion.[12]

Plasma FGF-23 concentrations in human patients are an independent predictor of progression of CKD[13] and are independently associated with case fatality in patients starting haemodialysis for CKD.[14] Additionally, very high levels of PTH are associated with increased risk of death in CKD patients on hemodialysis.[15] Plasma phosphate concentration is an independent predictor of survival time,[16,17] and progression[18] in cats with CKD, however, no previous studies have examined the relationship between plasma PTH or FGF-23 concentration and CKD progression or survival time in cats.

To explore the impact of CKD-MBD in cats the aim of this study was to examine if plasma FGF-23 or PTH concentration at diagnosis of azotemic CKD were independent predictors of progression of the disease over the following 12 months, and of survival time.

Materials and Methods

Case Selection

Records from 2 London-based first opinion practices between 7th January 1992 and 1st September 2011 were reviewed and cats ≥9 years of age with renal azotemia were identified. The majority of these cases had been included in a previous study.[18] The date of diagnosis of renal azotemia was defined as the date that a plasma creatinine concentration >2.0 mg/dL and a concurrent urine specific gravity (USG) <1.035 were documented. The Ethics and Welfare Committee of the Royal Veterinary College had approved the study protocol and informed consent was obtained from all owners. Biochemical analyses and measurement of plasma total thyroxine were performed at an external laboratory on the date samples were obtained.[a] Urine samples collected by cystocentesis and previously stored at −80°C were used for batch analysis of urine protein-to-creatinine ratio (UPC) at an external laboratory.[a] UPC measurements from samples with urinary tract infections (identified by the presence of active urine sediment on microscopy and confirmed by bacterial culture[b]) were not included in any analyses.

Cases required residual stored frozen EDTA plasma samples to be available for measurement of FGF–23 and PTH. Cases were excluded if they were already being fed a renal diet at diagnosis as this can result in a reduction in plasma FGF-23 and PTH concentrations.[19] Cases that demonstrated USG > 1.035 following diagnosis were excluded and those with concurrent hyperthyroidism, or that developed hyperthyroidism during follow-up. Cats were excluded if they had evidence of other concurrent disease, except systemic hypertension, or were taking medications that alter calcium or phosphate absorption or metabolism. All owners were advised to feed a commercially available renal diet after diagnosis of CKD. Other medications were prescribed as necessary on an individual case basis. No attempt was made to account for management strategies in the analyses.

Measurement of PTH and FGF-23

EDTA plasma samples previously stored at −80°C were used for batch analysis of PTH and FGF-23. The samples had been stored in 0.5 mL aliquots that had not undergone any freeze-thaw cycles in the majority of cases. In a small number of cases aliquots used to measure FGF-23 had undergone1 freeze-thaw cycle, which has previously been shown not to significantly affect feline FGF-23 concentrations.[6] PTH concentrations were measured using a validated total intact PTH immunoradiometric assay,[c] as previously described.[20] Samples with a PTH concentration below the lower limit of detection (<5.2 pg/mL)[20] were assigned a value of 2.6 pg/mL. Intact plasma FGF-23 concentrations were measured using a validated ELISA[d] as previously described.[6]

Statistical Analysis

Survival times using all-cause mortality were calculated from the date of diagnosis of azotemic CKD to the date of death or euthanasia. Date of death was assigned as the 15th of the month if the exact date was unknown. Cases still alive on 1st January 2013 or lost to follow-up were censored. Survival times were compared between groups using a Mann-Whitney *U*-test.

Progression of CKD was defined as an increase of >25% in plasma creatinine during the 12 months after the date of diagnosis of azotemic CKD. Cases were classified as stable if they were followed-up for a minimum of 12 months, without an increase of >25% in plasma creatinine concentration. Cases followed-up for <12 months were censored unless they demonstrated CKD progression. Renal follow-up was defined as the time from date of diagnosis of azotemic CKD to the date of the last available plasma creatinine concentration. Blood and urine samples were routinely taken approximately every 16 weeks, or sooner if it was deemed clinically necessary.

Statistics were performed using SPSS.[e] Results are reported as median [25th, 75th percentiles] and significance was determined as $P < .05$. Available data for the following variables obtained at the time of CKD diagnosis were included in analyses: plasma creatinine, phosphate, total calcium, PTH, FGF-23, potassium, total protein, globulin, albumin and cholesterol concentrations, packed cell volume (PCV), USG, UPC, age, presence of a urinary tract infection (UTI) and a diagnosis of hypertension (systolic blood pressure [SBP] >160 mmHg with evidence of concurrent hypertensive retinopathy or SBP > 170 mmHg on 2 separate occasions).

Variables associated with survival time were examined using Cox regression models. Variables were recoded into categorical variables if they did not meet the assumption of proportional hazards, assessed by inspection of Kaplan-Meier curves. Categorical variables were assigned based on biologically relevant cutpoints where possible or otherwise by terciles. Variables that were significantly associated with survival time at the 10% level were included in a backwards conditional multivariable Cox regression model.

Variables associated with CKD progression were examined using binary logistic regression models. FGF-23 and PTH were logarithmically transformed because of their highly skewed distributions. USG values were subjected to removal of the one before the decimal point and multiplied by 1,000 to allow interpretation of odds ratio (OR). UPC values were multiplied by 10 to allow interpretation of OR. To allow the inclusion of variables of particular interest, variables significant at the 20% level in the univariable analyses were carried into a backwards conditional multivariable binary logistic regression model to determine independent predictors of progression.

No attempt was made to impute missing data or to assess interactions because of the large number of variables in the models.

Results

Survival Analysis

A total of 722 cats with a diagnosis of azotemic CKD were identified. Cases were excluded for the fol-

lowing reasons: a diagnosis of hyperthyroidism (n = 233), no sample available for measurement of FGF-23 and PTH (n = 272), no urine sample taken on date of diagnosis (n = 2) and a diagnosis of nephrotic syndrome with atypical disease pathology (n = 1). This left 214 cats available for inclusion in the survival analysis. The majority (n = 180) were nonpedigree domestic cats, but there were 13 Persian or Persian crosses, 10 Burmese or Burmese crosses, 3 Tiffany, 2 British shorthair, 2 Chinchilla and one of each of the following: American shorthair, Ocicat, Siamese and Russian Blue cross. Ninety-nine cats were female (one entire, 98 neutered) and 115 were male (2 entire, 113 neutered). UPC measurements were performed in 174 cases, the remaining cases either had a UTI or no urine sample was available in storage.

The median age at diagnosis was 14.4 [11.0, 16.0] years. Seven cats were acquired as adults and their age was unknown. One hundred and sixty cats (74.8%) had died or were euthanized at or before the end of the follow-up period (1st January 2013) with a median survival time of 327 [109, 646] days. Fifty-four cats were either still alive at the study end point (33) or were lost to follow-up (21) and were censored from the analysis. The median follow-up time for censored cases was 658 [282, 919] days. Follow-up time was significantly longer in the censored cases compared to cases that died or were euthanized ($P < .001$).

Variables associated with survival time at the 10% level ($P < .1$) in univariable Cox regression analyses are shown in Table 1 and were included in the multivariable model. PCV was considered as a continuous variable as it met the assumption of proportional hazards. For FGF-23 and phosphate 4 categories were used for the analysis based on biologically relevant cutpoints, for other variables 3 biologically relevant cutpoints or terciles (for age, albumin and USG) were used. A Kaplan-Meier curve of survival using plasma FGF-23 concentration at diagnosis of CKD is shown in Figure 1. In the final multivariable model (Table 2), survival was negatively associated with plasma creatinine ($P = .002$) and FGF-23 concentrations ($P = .014$), UPC ($P < .001$) and age ($P < .001$). Survival was positively associated with PCV ($P = .004$).

Progression Analysis

Of the 214 cases available for inclusion in this study, 148 (69.2%) cases were classified as IRIS stage 2 at

Table 1. Univariable Cox regression analysis of factors associated with survival (all-cause mortality) at the 10% level ($P < .1$) at diagnosis of azotemic chronic kidney disease.

Variable	n	Survival Time by Cutpoints (Days) Median [25th, 75th Percentiles]	*P* Value	HR	95% CI for HR
Creatinine (≤2.8 mg/dL)	148	490 [208, 836]	<.001		
>2.8–5.0	55	263 [42, 657]	.068	1.39	0.975–1.99
>5.0	11	20 [2, 108]	<.001	10.7	5.45–21.0
Phosphate (≤4.5 mg/dL)	99	462 [200, 812]	<.001		
4.5–5.0	26	708 [333, 976]	.16	0.679	0.394–1.17
5.0–6.0	37	343 [186, 875]	.55	1.14	0.737–1.77
>6.0	52	98 [18, 502]	<.001	1.99	1.36–2.92
USG (>1.020)	67	586 [207, 840]	.020		
≤1.016	79	318 [54, 628]	.005	1.75	1.18–2.60
1.017–1.020	68	365 [131, 848]	.092	1.42	0.994–2.13
UPC (<0.2)	95	544 [280, 905]	<.001		
0.2–0.4	51	246 [67, 705]	<.001	2.07	1.38–3.10
>0.4	28	126 [31, 464]	<.001	2.63	1.63–4.26
FGF23 (≤700 pg/mL)	100	577 [289, 895]	<.001		
>700–3,000	69	354 [168, 734]	.53	1.13	0.777–1.64
>3,000–10,000	24	277 [65, 632]	.003	2.08	1.29–3.36
>10,000	21	38 [10, 152]	<.001	7.40	4.35–12.6
PTH (≤17.6 pg/mL)	110	377 [158, 873]	.026		
>17.6–30	40	570 [172, 909]	.15	0.714	0.454–1.12
>30	64	261 [50, 601]	.076	1.38	0.968–1.96
PCV (%)	213	NA	<.001	0.911	0.885–0.937
Albumin (>3.21 g/dL)	71	546 [161, 880]	.010		
≤2.95	72	255 [70, 581]	.003	1.81	1.23–2.67
2.96–3.21	71	394 [211, 705]	.21	1.29	0.866–1.93
Age (≤12.7 years)	69	484 [138, 928]	<.001		
12.7–15.4	69	587 [231, 910]	.39	1.19	0.795–1.79
>15.4	69	236 [109, 506]	<.001	2.29	1.58–3.60
Normotensive cases	164	423 [126, 840]			
Diagnosis of hypertension	50	310 [181, 608]	.004	1.68	1.18–2.37

n, number of cats in group; HR, hazard ratio; CI, confidence interval; NA, not applicable.

For continuous variables, the hazard ratio represents the effect of a unit change in the predictor variable on the frequency of the outcome (death).

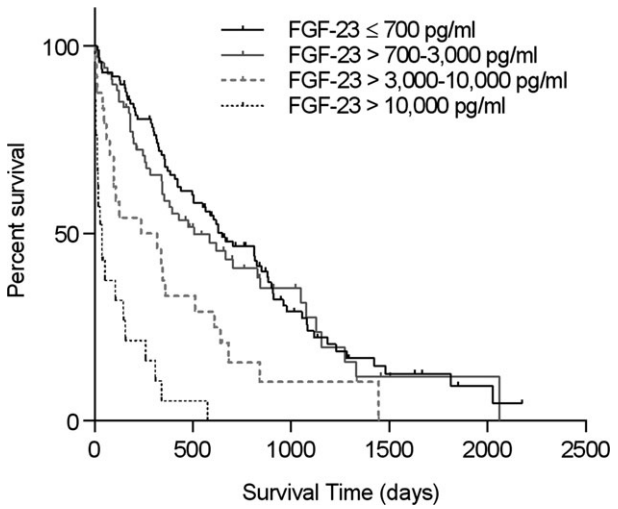

Fig 1. Kaplan-Meier curve for survival of cats (n = 214) based on plasma fibroblast growth factor-23 (FGF-23) concentration at diagnosis of chronic kidney disease. Cats with baseline plasma FGF-23 > 10,000 pg/mL ($P = .003$) and 3,000–10,000 pg/mL ($P < .001$), but not 700–3,000 pg/mL ($P = .53$) had significantly shorter survival times than cats with FGF-23 within the reference interval at baseline (≥700 pg/mL). Censored individuals are represented by ticks.

Table 2. Multivariable Cox regression analysis of factors associated with survival time (all-cause mortality) in cats at diagnosis of azotemic chronic kidney disease (n = 167).

Variable	β	SE	*P* Value	HR	95% CI for HR
PCV (%)	−0.060	0.021	.004	0.942	0.904–0.981
Creatinine (≤2.8 mg/dL)			.002		
>2.8–5.0	0.378	0.231	.10	1.46	0.927–2.30
>5.0	1.71	0.478	<.001	5.50	2.16–14.0
UPC (<0.2)			<.001		
0.2–0.4	0.789	0.221	<.001	2.20	1.43–3.39
>0.4	0.965	0.325	.003	2.62	1.39–4.96
Age (≤12.7 years)			<.001		
12.7–15.4	0.702	0.271	.010	2.02	1.19–3.43
>15.4	1.53	0.271	<.001	4.63	2.72–7.88
FGF23 (≤700 pg/mL)			.014		
>700–3,000	0.042	0.233	.86	1.04	0.660–1.65
>3,000–10,000	0.668	0.333	.045	1.95	1.02–3.74
>10,000	1.36	0.458	.003	3.91	1.59–9.58

β, coefficient; SE, standard error; HR, hazard ratio; CI, confidence interval.

Variables significant at the 10% level in univariable analyses were included in this model to determine independent predictors of survival time (all-cause mortality). For continuous variables, the hazard ratio represents the effect of a unit change in the predictor variable on the frequency of the outcome (death).

diagnosis, 55 (25.7%) were stage 3, and 11 (5.1%) were stage 4. Of the stage 2 cases, 106 (71.6%) remained in stage 2 during renal follow-up, 39 (26.4%) reached stage 3, and 3 (2.0%) reached stage 4. Of the stage 3 cases, 48 (87.3%) remained in stage 3 during renal follow-up and 7 (12.7%) reached stage 4.

One hundred and nine cats (50.9%) did not demonstrate progression but were followed-up for less than 12 months and were therefore censored from the progression analysis. In the remaining 105 cats, 38 cases (36.2%) demonstrated CKD progression during the initial 12 month follow-up period and 67 cases (63.8%) did not demonstrate progression of CKD but were followed-up for a minimum of 12 months and were therefore classified as stable. Renal follow-up was 277 [144, 399] days for progressive cases, 742 [539, 1001] days for stable cases and 56 [0, 147] days for censored cases. Summary statistics for clinicopathological variables for progressive, stable and censored cases taken on the date of diagnosis of CKD are shown in Table 3.

Variables associated with CKD progression within 12 months at the 20% level ($P < .2$) in univariable logistic regression analyses are shown in Table 4. In the final multivariable model, independent predictors of CKD progression within 12 months of diagnosis included logFGF-23 and age (Table 5). An increase in age of 1 year was associated with an increase in the odds of progression of 21% and a 1 unit increase in logFGF-23 (or a 10-fold increase in plasma FGF-23 concentration) was associated with an increase in the odds of progression of 289%.

Discussion

This study examined if plasma FGF-23 or PTH concentration are associated with disease progression and survival time in cats with azotemic CKD. Plasma FGF-23 concentration, but not plasma PTH or phosphate concentrations was found to be independently associated with survival time using all-cause mortality and to be an independent predictor of progression of CKD over 12 months.

CKD-MBD is thought to cause damage to renal tissue and contribute to disease progression, although the mechanisms involved are not completely understood. Phosphate might have direct deleterious effects on the kidney by causing nephrocalcinosis,[21] and indirect effects by initiating CKD-MBD development. Plasma phosphate concentration is independently associated with risk of death in a meta-analysis of human CKD patients,[22] and to be an independent predictor of survival time[16,17] in cats with CKD. In the present study, plasma phosphate was associated with survival time in univariable analyses, but was not an independent predictor of survival time when FGF-23 was also included. In a previous study, plasma phosphate concentration at CKD diagnosis was an independent predictor of CKD progression in cats,[18] but this result was not replicated in the present study. This might be because of the differences in the severity of CKD in each study population, with a smaller proportion of IRIS stage 3 and 4 cases in the present study versus the previous study (25% [26/105] versus 38% [81/213]). Since phosphate retention increases as GFR declines, having a greater proportion of cases in the higher IRIS stages would

Table 3. Summary statistics for baseline clinicopathological variables for cats with stable or progressive chronic kidney disease over the following 12 months.

Variable	Stable (n = 67)	Progressive (n = 38)	Short Follow-Up (n = 109)
Creatinine (mg/dL)	2.4 [2.2, 2.8]	2.5 [2.3, 2.9]	2.5 [2.3, 3.6]
Urea (mg/dL)	47.6 [40.0, 56]	50.7 [44.8, 66.1]	53.5 [42.6, 78.7]
USG	1.020 [1.016, 1.024]	1.017 [1.014, 1.026]	1.018 [1.016, 1.021]
UPC	0.14 [0.07, 0.21]	0.20 [0.14, 0.39]	0.23 [0.14, 0.36]
Phosphate (mg/dL)	4.4 [3.6, 5.1]	4.7 [3.8, 8.0]	5.1 [4.0, 6.7]
FGF-23 (pg/mL)	504.3 [340.3, 1291.2]	1243.2 [555.6, 2066.5]	1103.8 [414.1, 3977.4]
PTH (pg/mL)	13.8 [5.8, 26.6]	19.2 [7.0, 32.7]	17.8 [7.8, 45.9]
Potassium (mEq/L)	4.0 [3.7, 4.3]	4.1 [3.8. 4.2]	4.0 [3.7, 4.4]
T. Calcium (mg/dL)	10.1 [9.76, 10.5]	9.96 [9.6, 10.4]	10.1 [9.72, 10.52]
Total Protein (g/dL)	7.83 [7.47, 8.28]	7.91 [7.53, 8.45]	7.67 [7.25, 8.27]
Globulin (g/dL)	4.66 [4.31, 5.02]	4.75 [4.32, 5.54]	4.58 [4.18, 5.26]
Albumin (g/dL)	3.14 [2.97, 3.32]	3.09 [2.81, 3.25]	3.07 [2.81, 3.24]
Cholesterol (mg/dL)	205 [158, 251]	185 [162, 250]	197 [154, 236]
PCV (%)	35 [32, 37]	32 [29, 38]	32 [27, 36]
SBP (mmHg)	142 [127, 154]	154 [130, 174]	141 [126, 160]
% with hypertension	18	39	21
% with UTI	16	8	7
Age (years)	13 [11, 15]	15 [12, 16]	15 [11, 17]
Weight	4.13 [3.54, 4.88]	3.80 [3.10, 4.60]	3.4 [2.8, 4.2]
% male	58	58	50
Follow-up (days)	742 [539, 1001]	277 [144, 399]	56 [0, 147]
Year of diagnosis	2008 [2005, 2010]	2007 [2003, 2009]	2008 [2005, 2010]

Data presented as Median [25th, 75th percentile] or prevalence (%).

Table 4. Univariable binary logistic regression analysis of baseline variables associated with progression of chronic kidney disease (CKD) over 12 months at the 20% level ($P < .2$).

Variable	n	OR with 95% CI	*P* Value
Diagnosis of hypertension	105	2.989 [1.213, 7.365]	.017
LogFGF-23 (pg/mL)	105	2.657 [1.183, 5.967]	.018
USG × 1000	105	0.925 [0.858, 0.998]	.043
PCV (%)	105	0.925 [0.854, 1.002]	.055
UPC × 10	88	1.128 [0.982,1.296]	.088
Albumin (g/dL)	105	0.333 [0.082, 1.353]	.12
Age (years)	97	1.122 [0.969, 1.301]	.13
Phosphate (mg/dL)	105	1.207 [0.936, 1.556]	.15
Creatinine (mg/d/L)	105	1.54 [0.806, 2.93]	.19

The odds ratio indicates the increase in the odds of CKD progressing for each unit increase in the explanatory variable.

Table 5. Multivariable binary logistic regression analysis of baseline variables associated with progression of chronic kidney disease (CKD) over 12 months (n = 84).

Variable	Odds Ratio with 95% CI	*P* Value
Log (FGF-23 pg/mL)	3.89 [1.27, 11.9]	.017
Age (years)	1.21 [1.001, 1.45]	.049

The odds ratio indicates the increase in the odds of CKD progressing for each unit increase in the explanatory variable.

have included more cats with hyperphosphatemia in the previous study. Comparison of plasma phosphate concentrations reveals that these values are very similar in both studies for the stable cases (4.4 [3.6, 5.1] versus 4.4 [3.6, 5.2]), but lower in this study for the progressive cases (4.7 [3.8, 8.0] versus 5.1 [4.0, 6.8]), which might account for why phosphate was not associated with progression in this study.

PTH was a predictor of survival time in univariable analyses in this study, but was not found to be an independent predictor of mortality in the multivariable model. In human patients, high levels of PTH are associated with an increased risk of death in CKD patients on hemodialysis;[15] however, this result has not been consistent in all studies and a meta-analysis did not demonstrate a consistent association between death and serum PTH concentration in CKD patients of varying stages.[22] This study examined the association between PTH and survival time or CKD progression in the cat. Plasma PTH is increased in nonazotemic cats that develop azotemia within 12 months, compared to cats that remain nonazotemic (plasma creatinine ≤1.6 mg/dL).[23] It is likely that PTH was increased in the cats that went on to develop azotemic CKD to help maintain normal plasma phosphate concentrations in the face of a reduced GFR. What could not be determined from this association study was whether PTH was contributing to CKD progression in these cats or acting as a biomarker for reduced renal function. PTH is considered to act as a uremic toxin because of its effects on bone remodeling and the heart and vasculature.[24] Whether increased PTH can directly contribute to CKD progression remains to be demonstrated, but the present study suggests that its use as a biomarker for more progressive CKD in the cat is limited. The only variable involved in Ca-P homeostasis that was independently

associated with survival time and CKD progression in this study was plasma FGF-23 concentration.

Plasma FGF-23 increases early in the course of CKD, before the development of overt hyperphosphatemia,[25] where it acts to increase the fractional excretion of phosphate in remaining nephrons. It has been shown to be increased in cats that go on to develop azotemia within 12 months, compared to those remaining nonazotemic.[26] Feline plasma FGF-23 concentration is therefore likely to better reflect total body phosphate retention during mild to moderate CKD than plasma phosphate concentration, which may explain firstly, why an association between FGF-23 and CKD progression was identified when an association between phosphate and CKD progression was not and secondly, why including FGF-23 in the survival analysis resulted in the exclusion of phosphate from the multivariable model.

A clear concentration-dependent relationship between plasma FGF-23 concentration and survival time was found in this study, where highly increased FGF-23 concentrations (>10,000 pg/mL) were associated with an almost 4-times increase in risk of death. Additionally, a 10-fold increase in FGF-23 concentration at diagnosis was associated with a 289% increase in the odds of feline CKD progression over the next 12 months. These effects were independent of plasma creatinine concentration, suggesting that derangements in Ca-P homeostasis are important contributors to morbidity and mortality in all stages of azotemic CKD.

FGF-23 predicts the progression of CKD in human patients,[13,27,28] and FGF-23 concentrations are also well established as being independently associated with case fatality in CKD patients at the start of hemodialysis,[14,29] and in earlier stages of CKD.[27,28] At present, just as has been debated for many years concerning PTH, it is unclear whether FGF-23 is a uremic toxin, or whether it is a surrogate marker for other causes of uremic toxicity. Recent evidence suggests that in humans, FGF-23 can induce left ventricular hypertrophy,[30] and is an independent predictor of myocardial infarction, stroke, and cardiovascular mortality in CKD patients.[27] However, although these findings may shed some light on how FGF-23 may act as a uremic toxin in human patients, cardiovascular incidents are not a commonly recognized cause of death or euthanasia in feline CKD patients. It has been demonstrated retrospectively that plasma FGF-23 concentrations can be reduced in feline CKD patients by feeding a phosphate restricted diet.[19] Further work is now required to explore why FGF-23 is associated with CKD progression in cats and if targeted reduction of FGF-23 can improve survival time.

In this study, age was an independent predictor of survival time and of progression of CKD, with an increase of 1 year in age at diagnosis of CKD associated with a 21% increase in the risk of progression over the next 12 months. An association between age and survival time is not a novel finding,[31] and makes sense biologically. Aging is associated with a decreased ability to repair and regenerate the kidney[32] and renal histopathologic changes are observed with increasing frequency in older cats.[33] Aged rodent kidneys develop significantly more interstitial fibrosis post-reperfusion than young kidneys, despite normal aged kidneys having no interstitial fibrosis.[34] Therefore, it is possible that any injury to the kidney in an older cat could lead to an exaggerated repair response and a more progressive form of CKD.

Median survival time for cats in this study was 367 [147, 813] days, which is similar to other reports in the literature.[16,31,35] Plasma creatinine concentration, UPC, and PCV were independent predictors of all-cause mortality in the present study. Plasma creatinine concentration was found to be associated with survival time in this, and in previous studies of cats with CKD,[17,31,36] and median survival times for each IRIS stage in this study are similar to those reported previously.[16,37] UPC has previously been shown to be an independent predictor of survival time in cats with CKD,[31] and in this study being classified as borderline proteinuric (UPC 0.2–0.4) or proteinuric (UPC > 0.4) under the IRIS proteinuria classification scheme[f] was associated with a greater than 2-times increase in risk of death. A previous study found UPC to be an independent predictor of progression of CKD;[18] however, UPC did not remain in the multivariable model in the present analysis. A placebo-controlled interventional study failed to demonstrate a significant increase in survival time when feline CKD patients with proteinuria were administered an angiotensin converting enzyme (ACE) inhibitor, despite a significant lowering of UPC with treatment.[38] The role of proteinuria in progression of CKD is therefore currently unclear.

PCV or hematocrit has previously been found to be significantly associated with survival time in univariable analyses[36] and found to be associated with death within 1 month for cats with CKD.[17] PCV is not an independent predictor of survival time, although it has been linked to CKD progression in stage 2 cats, but not when cats in all stages were considered together.[18] No attempt was made to examine the cats in IRIS stages 2 and 3 separately in the present study, because there were only 9 cases in IRIS stage 3 that demonstrated progression. Anemia of CKD is considered to be multifactorial, but in part a result of decreased erythropoietin production by the kidney.[39] Although few cases in this study were anemic, with a median PCV of 33% [30, 37], as PCV decreases there will be a reduction in the oxygen carrying capacity of the blood, which in the diseased kidney might result in hypoxia. One marker of hypoxia, urinary vascular endothelial growth factor, has been associated with shorter survival times and progression in feline CKD,[40] therefore it is possible that small changes in PCV might exacerbate CKD and shorten survival time through hypoxia.

There were a number of limitations in this study. Cases might not have had a blood sample taken at the time of death or euthanasia, therefore some of the cats might have progressed without it being documented. Additionally, plasma creatinine concentration was used as a marker for GFR, which is dependent on muscle mass; therefore changes in muscle mass might have

resulted in cats being identified as stable that actually had a substantial change in their GFR. Furthermore, all-cause mortality was used in the survival analysis, with a large number of cats being euthanized. There might have been circumstances in which owners elected for euthanasia for reasons other than severe retractable illness, which will have introduced some bias into the analysis.

In summary, increased FGF-23 at diagnosis of CKD is associated with an increased risk of progression of the disease over the next 12 months and associated with an increased risk of death. These findings might make it a useful biomarker for predicting poorer outcomes in cats with CKD. Further work is now required to elucidate the complex relationships between variables involved in Ca-P derangements in CKD and to establish if FGF-23 is a mediator, or simply a marker of CKD-MBD in cats.

Footnotes

[a] Idexx laboratories, Wetherby, UK
[b] Performed at the Royal Veterinary College Diagnostic Laboratory Services, Hatfield, UK
[c] Total intact PTH immunoradiometric assay – coated bead version, 3KG600, Scantibodies, Santee, CA
[d] FGF-23 ELISA Kit, Kainos Laboratories, Tokyo, Japan
[e] IBM SPSS Statistics 21, Armonk, NY
[f] www.iris-kidney.com

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Off-label Antimicrobial Declaration Authors declare no off-label use of antimicrobials.

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