

Standard Article

J Vet Intern Med 2017;31:457–464**Acute-Phase Proteins and Iron Status in Cats with Chronic Kidney Disease**

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Background: The role of inflammation in the development and progression of chronic kidney disease (CKD) in cats is not well characterized. Hepcidin is a recently discovered acute-phase protein (APP) that plays an important role in iron metabolism and contributes to the development of anemia in humans with CKD.

Objectives: To compare serum APP concentrations, iron status, and erythropoietin (EPO) concentrations in healthy cats and cats with naturally occurring CKD.

Animals: A total of 18 healthy control cats and 38 cats with CKD.

Methods: Prospective study. After complete physical examination and routine blood analysis, the following tests were performed: serum amyloid A (SAA), haptoglobin (HAP), EPO, serum iron and ferritin concentration as well as total iron-binding capacity (TIBC). Serum hepcidin-25 concentration was measured by ELISA kit designed for use in humans.

Results: Mean SAA and hepcidin concentrations were significantly higher and mean total iron and TIBC were significantly lower in the CKD group ($P < .05$). There was a significant positive correlation between serum creatinine concentration (CRT) and 2 of the APPs (SAA and hepcidin; $P < .05$). Increases in SAA and hepcidin were associated with decreases in TIBC and hematocrit in the CKD group. Fourteen (37%) of the cats with CKD were anemic, and these cats had significantly lower TIBC ($P < .05$), suggesting a functional iron deficiency. There was no association between survival time and APP, iron status, or EPO concentrations.

Conclusions: Our data suggest that CKD in cats is associated with systemic inflammation and altered iron metabolism. With further validation in cats, hepcidin assays may help better characterize these relationships.

Key words: anemia; erythropoietin; ferritin; hepcidin; serum amyloid A.

Chronic kidney disease (CKD) affects approximately 15–30% of geriatric cats,^{1–3} and the role of inflammation in this disease is not well characterized. In human patients, chronic inflammation and oxidative stress play key roles in the development and progression of CKD.⁴ Increased concentrations of positive acute-phase proteins (APPs), such as C-reactive protein (CRP), and inflammatory cytokines, such as interleukin (IL)-6, have been described in humans with CKD and are associated with poor outcome.^{5–7} Anemia also is considered a negative prognostic factor in humans with renal failure, and evidence suggests that the severity of anemia is directly related to the progression of renal disease.^{5,8,9}

An estimated 30–65% of cats with CKD will develop anemia as their renal disease progresses.^{1–3} Although the relationship between anemia and prognosis in cats with

Abbreviations:

AKI	acute kidney injury
APP	acute-phase protein
CBC	complete blood count
CKD	chronic kidney disease
CRP	C-reactive protein
CRT	serum creatinine concentration
EPO	erythropoietin
GFR	glomerular filtration rate
HAP	haptoglobin
Hct	hematocrit
Hgb	hemoglobin
IL	interleukin
PUFA	polyunsaturated fatty acids
rhEPO	recombinant human EPO
SAA	serum amyloid A
TIBC	total iron-binding capacity
TNF- α	tumor necrosis factor-alpha

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Submitted July 13, 2016; Revised November 1, 2016; Accepted December 28, 2016.

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DOI: 10.1111/jvim.14661

CKD has not been well-characterized, anemia generally is thought to be associated with decreased immunity, progression of disease as a result of hypoxic injury to remaining renal tissue, poor body condition, and decreased quality of life.^{1,8,10–13} The development of anemia in patients with CKD is multifactorial, but is attributed principally to decreased production of erythropoietin (EPO) secondary to loss of functional renal mass. Decreased red blood cell lifespan secondary to accumulation of uremic toxins, gastrointestinal blood loss, and nutritional imbalances caused by decreased appetite also are thought to contribute.^{1,2,10,14,15} Data from human medicine indicate that alterations in iron metabolism caused by chronic inflammation, chronic GI blood loss, or both also may contribute to development of anemia in CKD.^{16,17}

Hepcidin is a recently discovered APP that is a key regulator of iron metabolism. Several cytokine-regulated interactions likely contribute to the development of anemia of inflammatory disease (e.g, decreased EPO production, modification of interactions between EPO and progenitor cells), and hepcidin appears to be a principal mediator of this process.^{13,18} Hepcidin downregulates expression of the membrane iron transport protein ferroportin, thereby limiting intestinal iron absorption and sequestering iron within cells of the reticuloendothelial system (RES). This causes functional iron deficiency, wherein iron is not available to developing erythroid cells, potentially leading to anemia.^{16,18–20} In addition to inflammation, changes in glomerular filtration rate (GFR) may affect the serum concentration of hepcidin, because plasma hepcidin is freely filtered through the glomerulus into the urine.^{17,21} Recent findings suggest that increased hepcidin in human patients with CKD may contribute to iron-limited erythropoiesis, which has important implications for the management of CKD.^{17,22}

The objectives of our study were to compare serum concentrations of hepcidin and other inflammatory markers, iron status, and serum EPO concentrations between healthy cats and cats with CKD. We hypothesized that inflammatory markers would be increased in cats with CKD, as compared to healthy controls, and that inflammation would be associated with iron dysregulation, anemia, and decreased patient survival time.

Materials and Methods

Cats that presented to the veterinary teaching hospital of the University of Montreal between February 2013 and January 2015 were eligible for inclusion in the study. The University of Montreal Committee for the Ethical Use of Animals approved the study protocol, in accordance with Canadian Council of Animal Welfare guidelines, and owner consent was obtained before enrollment.

Patient Selection

Based on history, physical examination, and clinicopathologic results, 2 groups were formed: a (healthy) control group and a CKD group. Cats that had serum creatinine concentration (CRT) >140 $\mu\text{mol/L}$ at 2 sampling times and urine specific gravity ≤ 1.035 were included in the CKD group. The majority of these cats had been diagnosed previously with CKD; samples used in the study were collected during reevaluation appointments. Cats in the CKD group then were divided into subgroups based on their International Renal Interest Society (IRIS) classification^a stage (stage II [CKD-2], stage III [CKD-3], or stage IV [CKD-4]). The control group was composed of clinically healthy cats with CRT $\leq 140 \mu\text{mol/L}$ and urine specific gravity >1.035. Patients were included only 1 time in the study and then followed during a variable period (6–36 months), depending on the time of enrollment. Medical records of all cats were reviewed for the following data: age, breed, weight, sex, presence of concurrent disease (for the CKD group), CRT and blood urea nitrogen (BUN) concentrations, hematocrit (Hct), urine specific gravity, and survival time. Anemia was defined as a Hct <28%.

Cats were excluded from the study if they had received any form of iron supplementation or a previous blood transfusion.

Concurrent diseases were recorded but did not constitute exclusion criteria for the CKD group, unless evidence of acute kidney injury (AKI), based on IRIS AKI classification^a, was present. Evidence of AKI included acute onset of clinical signs and azotemia in a previously healthy cat, urinalysis findings suggestive of acute tubular injury (e.g, glucosuria without concurrent hyperglycemia, urinary casts), imaging findings compatible with AKI such as enlarged kidneys or perirenal free fluid and the absence of chronic kidney changes, and resolution or marked improvement of azotemia within 30 days of discharge (in survivors). Cats with known or suspected acute-on-chronic kidney disease also were excluded. Cats with CRT $\leq 140 \mu\text{mol/L}$ and urine specific gravity ≤ 1.035 (consistent with IRIS stage I classification) were excluded from the study.

Blood Sampling and Laboratory Evaluation

Blood was collected by jugular venipuncture into redtop (plastic), EDTA, or heparinized microhematocrit capillary tubes for measurement of Hct. Whole blood (EDTA tubes) was used to perform a complete blood count (CBC).^b Serum was collected from redtop tubes immediately after centrifugation at $1300 \times g$ for 10 minutes at room temperature. Serum was aliquoted into 1.5-mL Eppendorf tubes and stored at -80°C until analyzed for the following: routine biochemistry panel; iron panel^c including total iron, ferritin, and total iron-binding capacity (TIBC); serum amyloid A (SAA); haptoglobin (HAP) and erythropoietin (EPO). Serum amyloid A, HAP, and EPO were measured by commercially available sandwich enzyme-linked immunosorbent assays (ELISA) kits^{d,e} validated for use in cats and hepcidin by a commercially available sandwich ELISA kit^f validated for use in humans. Each ELISA analysis was performed in duplicate in accordance with the manufacturer's instructions, and the mean was used for data analysis. All samples were tested within 12 months of collection.

Statistical Analysis

Normality of the data was assessed with the Anderson-Darling test. A log base 10 transformation was used for non-normally distributed variables. An unequal-variance t-test was used to compare age and body mass between the 2 groups of cats (CKD and healthy). The same test was used to compare the mean between the 2 groups of cats for each biochemical variable. To compare the mean among the 3 CKD classes, a Welch's analysis of variance (ANOVA) was used with CKD subgroup as a between-subject factor, taking into account unequal variances among subgroups. Tukey's post hoc tests were used to compare pairs of means after the ANOVA. A Pearson's correlation was used to examine the linear relationship between pairs of biochemical variables. A Cox's survival analysis was used to determine whether the concentration of various biochemical variables affected survival time. The number of days between the onset of the study and time of death were used as time of survival. Follow-up duration was considered a censored event. In an attempt to better characterize the effect of CKD on the variables measured in a clinical situation, a secondary analysis was performed in which patients with evidence of diseases other than CKD based on history, physical examination, and laboratory results were excluded. The level of statistical significance was set at $P < .05$. Statistical analyses were carried out with SAS statistical software.^f

Results

Sixty-three cats initially were included in the study: 43 in the CKD group and 20 in the healthy (control)

group. Seven cats were excluded: 5 in the CKD group (2 because of evidence of AKI; 3 because of incomplete data) and 2 in the healthy group (because of low USG). The remaining 56 cats (38 in the CKD group and 18 in the healthy group) were included in the final statistical analyses. All clinicopathologic variables were available for the 56 cats except for EPO which was available for only 31 cats (20 in the CKD group and 11 in the healthy group). Cats in the CKD group were significantly older and weighed less than those in the healthy group (mean 11.3 ± 4.5 years and 4 ± 1.42 kg in the CKD group vs 7 ± 3.3 years and 4.9 ± 1.5 kg in the healthy group [$P = .0002$ and $.046$, respectively]). Twenty-six cats had CKD-2, 7 cats had CKD-3, and 5 cats had CKD-4.

Acute-Phase Proteins

Mean SAA concentration was significantly higher in the CKD group (8.1 ± 6.5 $\mu\text{g/mL}$), compared with healthy cats (4.3 ± 1.2 $\mu\text{g/mL}$; $P = .002$; Fig 1). There was a significant positive correlation between CRT and SAA concentrations ($P = .02$). Mean SAA concentration in CKD subgroups was 7.7 ± 6.4 $\mu\text{g/mL}$ (CKD-2), 5.1 ± 2.1 $\mu\text{g/mL}$ (CKD-3), and 14.1 ± 8.2 $\mu\text{g/mL}$ (CKD-4). There was no significant difference in SAA concentrations among IRIS CKD stages 2-4 ($P = 0.1$). Higher SAA concentration in the CKD group also was significantly associated with higher hepcidin concentration ($P = .02$) and lower Hct ($P = .0002$).

Mean HAP concentration in the CKD group was 4.5 ± 3.3 $\mu\text{g/mL}$, compared to 3.6 ± 1.6 $\mu\text{g/mL}$ in healthy cats, but the difference was not significant ($P = .9$). Higher HAP concentrations in the CKD group were significantly associated with higher ferritin ($P = .047$) and lower hepcidin ($P = .045$) concentrations.

Mean hepcidin concentration was significantly higher in the CKD group (5 ± 3.7 ng/mL) compared to the healthy group (3.4 ± 2.3 ng/mL ; $P = .046$; Fig 2). There was a significant positive correlation between CRT and hepcidin concentrations ($P = .0008$; Fig 3). Mean hepcidin concentrations in CKD subgroups were 4.2 ± 3.2 ng/mL (CKD-2), 6.2 ± 4.6 ng/mL (CKD-3), and 7.6 ± 3.8 ng/mL (CKD-4). There was no

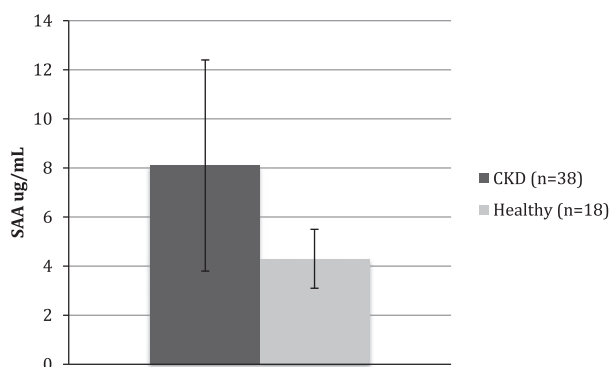


Fig 1. Serum amyloid A (SAA) concentration in healthy cats and cats with chronic kidney disease (mean \pm SD). $P = .002$.

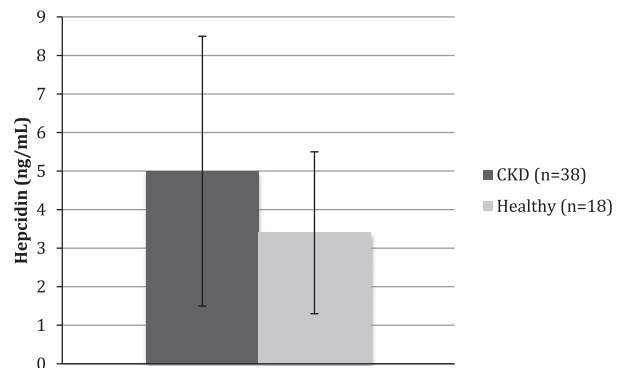


Fig 2. Serum hepcidin concentration in healthy cats and cats with chronic kidney disease (mean \pm SD). $P = .046$.

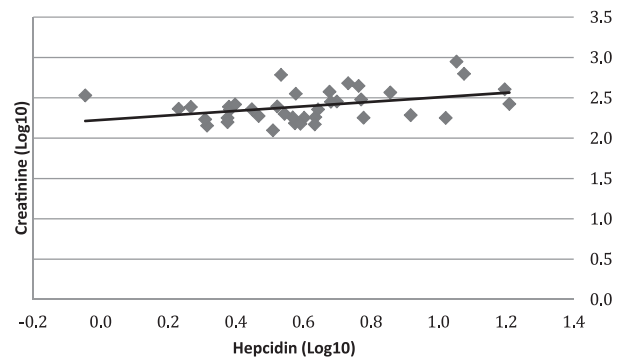


Fig 3. Correlation between serum creatinine and hepcidin concentrations in CKD group. $R = .43$, $P = .008$.

significant difference in hepcidin concentrations among the subgroups ($P = .12$). Higher hepcidin concentrations in the CKD group also were significantly associated with lower TIBC ($P = .02$), higher SAA concentration ($P = .02$), and lower Hct ($P = .02$).

The 2 cats that were excluded because of AKI had among the most increased concentrations of APPs (respectively >50 and 51.9 $\mu\text{g/mL}$ for SAA; 13.8 and 2.6 $\mu\text{g/mL}$ for HAP; and 11.9 and 11.7 ng/mL for hepcidin).

Iron Status

Mean serum iron concentration was significantly lower in the CKD group: 85.5 ± 32.9 $\mu\text{g/mL}$, compared with 100.7 ± 21.7 $\mu\text{g/mL}$ in healthy cats ($P = .01$; Fig 4). Nine cats (27%) in the CKD group were hypoferremic (reference interval [RI]: 62 – 162 $\mu\text{g/mL}$; $7/26$ with CKD-2, $1/7$ with CKD-3, and $2/5$ with CKD-4) and 1 was hyperferremic (178 $\mu\text{g/mL}$; CKD-2 group). None of the healthy cats had serum iron concentration outside of the RI. There was a significant positive correlation between serum iron concentration and Hct in the CKD group ($P = .03$).

Mean TIBC in the CKD group (297.7 ± 66.3 $\mu\text{g/mL}$) was significantly lower than that of healthy cats (341.1 ± 52 $\mu\text{g/mL}$; $P = .01$). Eleven cats (29%) in the CKD group had low TIBC (RI, 250 – 470 $\mu\text{g/mL}$; $5/26$ cats with CKD-2, $2/7$ with CKD-3, and $4/5$ with CKD-

4) and none had high TIBC. None of the healthy cats had TIBC results outside of the RI. In the CKD group, TIBC was negatively correlated with CRT ($P = .0008$) and hepcidin ($P = .02$) concentrations and positively correlated with Hct ($P = .003$).

Mean ferritin concentration in the CKD group was 247.9 ± 96 ng/mL compared to 261.4 ± 91.5 ng/mL in healthy cats, but the difference was not significant ($P = .62$). Four cats (11%) in the CKD group had high ferritin concentrations (RI, 82–395 ng/mL; 1/26 with CKD-2, 2/7 with CKD-3, and 1/5 with CKD-4), and none had low serum ferritin concentration. Two healthy cats had low ferritin concentrations (44 and 68 ng/mL). There was a significant positive correlation between serum ferritin and HAP concentrations in the CKD group ($P = .045$).

Among the 9 cats with low iron concentrations, 3 had decreased TIBC and normal ferritin concentrations and another 1 had decreased TIBC and increased ferritin concentration, all changes consistent with a functional iron deficiency. There were 3 other cats with increased ferritin concentrations, normal TIBC and low normal serum iron concentrations, also consistent with functional iron deficiency. The other 5 cats with low iron concentrations had normal ferritin concentrations and TIBC. None of the cats had decreased ferritin concentrations or increased TIBC, findings that could suggest true iron deficiency.

Effect of Anemia

Fourteen cats (37%) in the CKD group had low Hct (6/26 with CKD-2, 3/7 with CKD-3, and 5/5 with CKD-4). Mean Hct was significantly lower in the CKD group: $29.4 \pm 7.7\%$, compared to $41.2 \pm 3.7\%$ in

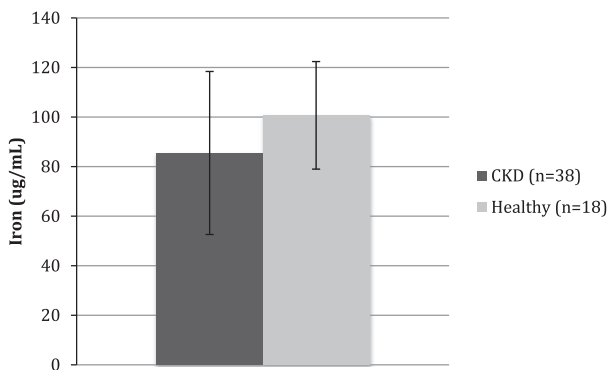


Fig 4. Mean serum iron concentration in healthy cats and cats with chronic kidney disease (mean \pm SD). $P = .01$.

healthy cats ($P < .0001$) (Table 1). None of the healthy cats was anemic. There was a significant negative correlation between CRT and Hct ($P < .0001$) in the CKD group, and cats with CKD-4 had significantly lower Hct compared with cats with CKD-3 and CKD-2 ($P = .01$). In the CKD group, anemic cats had significantly higher CRT ($P < .0001$), higher SAA concentrations ($P = .003$), and lower TIBC ($P = .02$), as compared with nonanemic cats (Table 2).

Erythropoietin

Of the 31 cats in which EPO concentrations were measured, there was no significant difference between mean EPO concentrations in the CKD group (0.090 ± 0.05 IU) compared with healthy cats (0.092 ± 0.05 IU; $P = .69$). There was no significant difference in EPO concentrations among CKD IRIS stages 2-4 ($P = .63$). However, anemic cats in the CKD group had significantly lower EPO concentrations relative to nonanemic CKD cats and healthy cats ($P = .007$).

Effect of Concomitant Diseases

There were 13 cats (34%) in the CKD group with evidence of concomitant disease, including 6 cats with urolithiasis (ureteroliths previously treated using SC ureteral bypass or ureteral stenting [4], nonobstructive nephroliths [2]); 3 cats with cardiac disease (hypertrophic cardiomyopathy [2], pericardial effusion [1]); 3 with neoplasia (colonic carcinoma [1], soft tissue sarcoma [1], cutaneous lymphoma [1]); 2 with inflammatory bowel disease; 1 with asthma; and 1 with hyperthyroidism. Three cats had ≥ 2 concurrent diseases. A secondary analysis was performed after excluding patients with evidence of concomitant disease from the CKD group. Patients with CKD and no evidence of concomitant disease still had higher hepcidin concentrations ($P = .047$), and lower TIBC ($P = .002$), compared to healthy cats. However, the differences in iron ($P = .06$) and SAA ($P = .06$) concentrations were no longer significant.

Survival Analysis

Follow-up information was available for all 56 cats and ranged from 6 months to 2 years. No relationship could be identified between survival time and CRT, EPO concentration, iron parameters, APP (including hepcidin) concentrations, or the presence of concomitant disease.

Table 1. Comparison of selected hematologic and serum values between healthy and CKD cats (mean \pm SD).

	Hct (%)	Creatinine ($\mu\text{mol/L}$)	SAA ($\mu\text{g/mL}$)	HAP ($\mu\text{g/mL}$)	Iron ($\mu\text{g/mL}$)	TIBC ($\mu\text{g/mL}$)	Hepcidin (ng/mL)	Ferritin (ng/mL)	EPO (IU)
CKD	29.4 (7.7)	280 (158)	8.1 (6.5)	4.5 (3.3)	85.5 (32.9)	297.7 (66.3)	5.0 (3.7)	247.9 (96)	0.090 (0.05)
Healthy	41.2 (3.7)	117 (22)	4.3 (1.2)	3.6 (1.6)	100.7 (21.7)	341.1 (52.0)	3.4 (2.3)	261.4 (91.5)	0.092 (0.05)
<i>P</i> -value	<.0001	<.0001	.002	.9	.01	.01	.046	.62	.69

Numbers in bold represents values of statistical significance using a $P < .05$.

Table 2. Comparison between anemic and nonanemic cats in the CKD group (mean \pm SD).

CKD group	Hct (%)	Creatinine (μ mol/L)	SAA (μ g/mL)	HAP (μ g/mL)	Iron (μ g/mL)	TIBC (μ g/mL)	Hepcidin (ng/mL)	Ferritin (ng/mL)	EPO (IU)
Anemic	21.9 (5.7)	374 (215)	11.2 (6.8)	5.0 (4.0)	86.3 (40.4)	261.1 (77.3)	5.8 (3.4)	249.9 (76.8)	0.06 (0.02)
Nonanemic	33.7 (4.6)	225 (75)	6.3 (5.6)	4.2 (2.9)	84.9 (28.5)	319.0 (49.0)	4.6 (3.8)	246.8 (107.2)	0.10 (0.05)
<i>P</i> -value	<.0001	.02	.003	.4	.8	.02	.2	.9	.008

Numbers in bold represents values of statistical significance using a $P < .05$.

Discussion

The aim of our study was to elucidate the role of inflammation in cats with CKD and characterize its relationship with iron metabolism, the development of anemia, and survival time. We found that cats with CKD had significantly increased concentrations of 2 different APPs: SAA and hepcidin. Serum amyloid A, a major APP in cats was significantly higher in cats with CKD, relative to healthy controls. Although increased SAA concentrations have been reported previously in cats with various renal diseases,^{23–25} the populations studied were not limited to patients with CKD. The presence of a positive correlation between CRT and APP concentrations suggests that more advanced CKD may be associated with more severe inflammation. The association between inflammation and CKD is well known in human medicine and is gaining increasing attention as a major cause of morbidity and mortality in end-stage renal disease.⁶ Uremia is recognized as an inflammatory state, with some human patients showing considerable increases in biomarkers of inflammation and immune activation. Many patients with azotemia have significant increases in pro-inflammatory cytokines, such as IL-6 and IL-12, when compared with healthy controls.²⁶ Increased concentrations of pro-inflammatory cytokines in CKD are associated with poor outcomes, and IL-6 is a predictor of mortality in hemodialysed patients.²⁷ Further research in veterinary medicine is needed to determine whether APPs have similar prognostic value, but no association between APPs and survival time was found in our study.

Hepcidin concentrations in cats with CKD were significantly higher when compared with those of healthy cats. Hepcidin was proposed to be the long-anticipated iron regulatory hormone, and overproduction of hepcidin during infection and inflammation was considered to be the cause of anemia of inflammatory disease.^{28,29} This hypothesis has been corroborated repeatedly. Moreover, concentration of serum hepcidin was found to be increased in pediatric and adult human patients with CKD, as a consequence of decreased GFR and increased inflammatory cytokines.¹⁷ In our study, we found that increased hepcidin concentration in cats with CKD was significantly associated with an increase in CRT ($P = .008$) and SAA concentrations ($P = .02$). Previous studies in humans determined that hepcidin is positively correlated with C-reactive protein (CRP), a major APP in this species.¹⁷ Synthesis of hepcidin, a positive APP, is upregulated by inflammation, and this relationship appears to be mediated by IL-6.³⁰ Because our data

support that CKD is an inflammatory state, this positive correlation is expected. We also found that increased SAA concentrations in the CKD group were significantly associated with decreased Hct ($P = .0002$). It therefore appears that, as in humans, decreased renal clearance and increased inflammation in cats with CKD may contribute to increased serum hepcidin concentrations.

Increased hepcidin likely alters iron homeostasis, contributing to the development of anemia. In the future, development of a hepcidin antagonist could be an important therapeutic tool in the treatment of CKD-associated anemia. Specifically, lowering hepcidin concentrations or antagonizing its actions might decrease the negative effects of inflammation on erythropoiesis by increasing iron availability through mobilization of iron stores and increased enteral iron absorption.²²

Another finding of our study is that CKD in cats is associated with changes in iron status. We found that cats with CKD had significantly decreased serum iron concentrations and TIBC, compared with healthy cats. Decreased serum iron concentration in cats with CKD may be associated with chronic gastrointestinal hemorrhage (secondary to uremia-induced thrombocytopeny), decreased intestinal absorption or intake, or may represent functional iron deficiency caused by hepcidin-induced sequestration of iron in storage pools.^{1,6,31} Because TIBC is an indirect measure of transferrin concentration and transferrin is a negative APP, a decrease in TIBC is expected in an inflammatory state.³¹ Renal protein loss should be considered as another potential cause for decreased TIBC in the CKD group. However, the urine protein: creatinine ratio of these patients was not systematically measured, preventing us from establishing the effect of renal loss. Interestingly, our results are corroborated by another recent study that evaluated iron status in cats with CKD, also observing significantly decreased TIBC in cats with CKD compared to a healthy group. These findings were felt to be most consistent with a functional, rather than absolute, iron deficiency, supporting the presence of an inflammatory state in cats with CKD.³²

Differentiation between true and functional iron deficiency can be difficult, and the use of iron supplementation must be considered judiciously, because inappropriate use could have deleterious effects associated with iron overload.^{1,17,31} True iron deficiency generally is characterized by decreased serum iron and ferritin concentrations and increased TIBC, whereas in cases of functional iron deficiency, ferritin concentrations generally are normal or above the reference interval and serum iron concentrations and TIBC generally

are decreased.^{1,31} In our study, there was no significant difference in ferritin concentrations between healthy cats and cats with CKD, but the significantly decreased serum iron concentration combined with low TIBC observed in the CKD group is most consistent with functional iron deficiency. This conclusion also is supported by the absence of hypoferritinemia in CKD cats. However, because ferritin is also an APP, hypoferritinemia may be masked by inflammation, underlying the importance of global assessment of iron and other clinicopathologic indices to determine true iron status. No cats had iron profiles suggestive of true iron deficiency, but 14 cats (37%) in the CKD group had decreased TIBC, increased ferritin, or both, and 4 of these cats had low serum iron concentrations, consistent with functional iron deficiency. A significant negative correlation between CRT concentration and TIBC ($P = .0008$) also was identified, suggesting that more advanced CKD may be associated with decreased iron availability. However, true iron deficiency also has been described in patients with CKD³³, highlighting the importance of assessing iron status in individual patients with CKD to determine whether iron supplementation is appropriate.

In our study, anemic cats with CKD had significantly higher SAA concentrations ($P = .003$) and lower TIBC ($P = .02$) compared to nonanemic CKD cats. Increased APP concentration and decreased TIBC in anemic cats with CKD in our study suggest that inflammation may contribute to iron sequestration and decreased hct. Anemia may be one of the many links between increased pro-inflammatory cytokines and poor outcomes in patients with renal disease.⁶ Effective treatment of inflammation targeted at decreasing anemia might therefore be an effective strategy for improving outcomes in cats with CKD.

Limited data are available regarding EPO concentrations in cats with CKD. In our study, anemic cats with CKD had significantly lower EPO concentrations when compared with nonanemic cats with CKD and healthy cats ($P = .02$). This finding supports the conclusion that EPO deficiency contributes to anemia in cats with CKD, as has been described in other species.^{34,35} Additionally, EPO concentration was not significantly associated with APP but a strong negative correlation between EPO and HAP was observed ($P = .07$). This finding may have resulted from a type II error, because EPO was assessed in only half of the cats in the CKD group.

Erythropoiesis-stimulating agents (ESAs) have revolutionized the treatment of anemia in humans with CKD. However, some patients with uremia are resistant to recombinant human EPO (rHuEPO), possibly because of immune activation, causing increased release of pro-inflammatory cytokines by the bone marrow.²⁶ In fact, human patients with CKD who had higher CRP concentrations were found to require higher doses of rHuEPO to manage their anemia.³⁶ Recent findings also suggest that hepcidin assessment in human patients with CKD could be useful in predicting and monitoring responsiveness to ESA therapy.³⁷ It is not known

whether the cats with low EPO concentrations in our study would have required higher dosages of EPO supplementation, but the relationships among EPO concentration, inflammation, and iron status in cats with CKD warrant further investigation.

Our study had several limitations. First, the hepcidin ELISA assay used was developed for use in humans. Although the assay has not been formally evaluated in cats, cross-reactivity with hepcidin in mice, rats, pigs, and dogs have been observed (personal communication with DRG^g). By specialized software^h, we determined that human and feline hepcidin share a relatively high (80%) homology (higher than that shared between humans and mice or humans and rats). Also, the results obtained in our CKD patients as compared to healthy controls are compatible with the known biological behavior of hepcidin in humans, giving additional credence to the relationships suggested.¹⁷ Interestingly, the 2 cats excluded from our study because of suspected pyelonephritis had the most increased concentrations of APP, including hepcidin. Increase in APP have previously been identified in various infectious diseases^{23,25}, and we believe that this information further supports that hepcidin concentrations are influenced by inflammation in cats.

Also, we chose to assess SAA and HAP to characterize the inflammatory response, because they represent 1 major (SAA) and 1 minor (HAP) APP in cats. However, other APPs, such as alpha-1-glycoprotein, could have been measured and may have provided complementary data.

Additional limitations of our study include the small number of cats, especially in the more advanced CKD IRIS stages, which likely decreased our ability to detect relevant trends in these groups. Additionally, we aimed to evaluate a population of cats with naturally occurring CKD and, because CKD often affects geriatric patients, many of our cats had concurrent disease. An attempt was made to eliminate the effect of this factor by removing patients with evidence of concomitant disease from the CKD group during the secondary analysis. Although the differences were no longer significant once these patients were removed from analysis, P -values for both SAA and iron were .06. This lack of statistical difference could be explained by a type II error, because the number of cats in this group was low ($n = 13$). However, the possibility that changes in SAA and iron were influenced by concomitant diseases cannot be completely ruled out and, given the older age of the CKD population relative to the control group, a possible effect of age on these variables cannot be ruled out.

As both are APPs, the inverse correlation between hepcidin and HAP in the CKD group was unexpected. Factors other than the disease state may have influenced the HAP results, because patient signalment (in particular, sex) has been identified as a confounding factor in interpretation of HAP in cats.²⁵ In addition, for 6 of 38 (15%) cats in the CKD group, a CBC was not available and Hct was determined directly by microhematocrit capillary tubes, which could have resulted in misclassification in the anemic groups.

Finally, the etiology of CKD was not determined for individual patients, because we did not perform histopathological analysis of kidney tissue, and imaging studies were not standardized. Because the definitive test to differentiate AKI from CKD is renal histopathology, some cats may have been falsely classified.

In conclusion, our data suggest that CKD in cats is associated with systemic inflammation and altered iron metabolism. The changes observed in iron stores are most consistent with functional iron deficiency, which raises the question of whether iron supplementation is appropriate for cats with CKD. We propose that iron status be assessed on an individual basis in cats with CKD to determine the necessity for iron supplementation. Moreover, because inflammation appears to play an important role in cats with CKD, further research regarding new therapeutic approaches targeting inflammation appears warranted. With further validation, hepcidin may become an important biomarker of iron status in cats with CKD.

Acknowledgments

We thank the “Association des Médecins Vétérinaires du Québec (AMVQ)” and the “Fond de Santé des Animaux de Compagnie (FSAC)” for their financial support.

Conflict of Interest Declaration: Authors declare no conflict of interest.

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

Footnotes

^a <http://www.iris-kidney.com>

^b Advia 120, Siemens, Tarrytown, NJ

^c Kansas State Veterinary Diagnostic laboratory. Kansas State University College of Veterinary Medicine. Manhattan, KS

^d SAA ELISA kit/Haptoglobin ELISA kit “Tridelta Development Limited”

^e Erythropoietin ELISA kit. Cusabio

^f SAS statistical software v9.4 (Cary, N.C.)

^g DRG Diagnostics® Heparin-25 bioactive ELISA kit, 96T

^h CLC sequence-viewer v.6

References

- Chalhoub S, Langston CE, Eatroff A. Anemia of renal disease. *J Feline Med Surg* 2011;13:629–640.
- Cowgill LD. Pathophysiology and management of anemia in chronic progressive renal failure. *Semin Vet Med Surg (Small Anim)* 1992;7:175–182.
- Elliott J, Barber PJ. Feline chronic renal failure: Clinical findings in 80 cases diagnosed between 1992 and 1995. *J Small Anim Pract* 1998;39:78–85.
- Impellizzeri D, Esposito E, Attley J, et al. Targeting inflammation: New therapeutic approaches in chronic kidney disease (CKD). *Pharmacol Res* 2014;81C:91–102.
- Chervier C, Cadore JL, Rodriguez-Piñeiro MI, et al. Causes of anaemia other than acute blood loss and their clinical significance in dogs. *J Small Anim Pract* 2012;53:223–227.
- Stenvinkel P. The role of inflammation in the anaemia of end-stage renal disease. *Nephrol Dial Transplant* 2001;7:175–182.
- Stenvinkel P, Wanner C, Metzger T, et al. Inflammation and outcome in end-stage renal failure: Does female gender constitute a survival advantage? *Kidney Int* 2002;62:1791–1798.
- King JN, Tasker S, Gunn-Moore DA, et al. Prognostic factors in cats with chronic kidney disease. *J Vet Intern Med* 2007;21:906–916.
- Thorp ML, Johnson ES, Yang X, et al. Effect of anaemia on mortality, cardiovascular hospitalizations and end-stage renal disease among patients with chronic kidney disease. *Nephrol* 2009;14:240–246.
- Bartges JW. Chronic kidney disease in dogs and cats. *Vet Clin North Am Small Anim Pract* 2012;42:669–692.
- Brown SA. Oxidative stress and chronic kidney disease. *Vet Clin North Am Small Anim Pract* 2008;38:157–166.
- Hoerger TJ, Wittenborn JS, Segel JE, et al. A health policy model of CKD: 1. Model construction, assumptions, and validation of health consequences. *Am J Kidney Dis* 2010;55:452–462.
- Weiss G. Iron and immunity: A double-edged sword. *Europ J Clin Invest* 2002;32:70–78.
- Caride VJ. Occult gastrointestinal bleeding. *N Engl J Med* 1999;341:1477–1478.
- Polzin DJ. Chronic kidney disease in small animals. *Vet Clin North Am Small Anim Pract* 2011;41:15–30.
- Weiss G, Goodnough LT. Anemia of chronic disease. *N Engl J Med* 2005;352:1011–1023.
- Zaritsky J, Young B, Wang HJ, et al. Heparin-A potential novel biomarker for iron status in chronic kidney disease. *Clin J Am Soc Nephrol* 2009;4:1051–1056.
- Ganz T. Heparin, a key regulator of iron metabolism and mediator of anemia of inflammation. *Blood* 2003;102:783–788.
- Andrews NC. Disorders of iron metabolism. *N Engl J Med* 1999;341:1986–1995.
- Dec GW. Anemia and iron deficiency—new therapeutic targets in heart failure. *N Engl J Med* 2009;361:2475–2477.
- Grimes CN, Giori L, Fry MM. Role of hepcidin in iron metabolism and potential clinical applications. *Vet Clin North Am Small Anim Pract*. 2012;42:85–96.
- Young B, Zaritsky J. Heparin for clinicians. *Clin J Am Soc Nephrol* 2009;4:1384–1387.
- Sasaki K, Ma Z, Khatlani TS, et al. Evaluation of feline Serum Amyloid A (SAA) as an inflammatory marker. *J Vet Med Sci* 2003;65:545–548.
- Paltrinieri S. The feline acute phase reaction. *Vet J* 2008;177:26–35.
- Kann RKC, Seddon JM, Henning J, et al. Acute phase proteins in healthy and sick cats. *Res Vet Sci* 2012;93:649–654.
- Macdougall IC, Cooper AC. Erythropoietin resistance: The role of inflammation and pro-inflammatory cytokines. *Nephrol Dial Transplant* 2002;17:39–43.
- Bologa RM, Levine DM, Parker TS, et al. Interleukin-6 predicts hypoalbuminemia, hypocholesterolemia, and mortality in hemodialysis patients. *Am J Kidney Dis* 1998;32:107–114.
- Fleming RE, Sly WS. Heparin: A putative iron-regulatory hormone relevant to hereditary hemochromatosis and the anemia of chronic disease. *Proc Natl Acad Sci USA* 2001;98:8160–8162.
- Pigeon C, Ilyin G, Courselaud B, et al. A new mouse liver-specific gene, encoding a protein homologous to human antimicrobial peptide hepcidin, is overexpressed during iron overload. *J Biol Chem* 2001;276:7811–7819.
- Nicolas G, Chauvet C, Viatte L, et al. The gene encoding the iron regulatory peptide hepcidin is regulated by anemia, hypoxia, and inflammation. *J Clin Invest* 2002;110:1037–1044.
- Bohn AA. Diagnosis of disorders of iron metabolism in dogs and cats. *Vet Clin North Am Small Anim Pract* 2013;43:1319–1330–vii.

32. Gest J, Langston C, Eatroff A. Iron status of cats with chronic kidney disease. *J Vet Intern Med* 2015;29:1488–1493.
33. Nissenson AR, Strobos J. Iron deficiency in patients with renal failure. *Kidney Int Suppl* 1999;69:S18–S21.
34. Erslev AJ, Besarab A. Erythropoietin in the pathogenesis and treatment of the anemia of chronic renal failure. *Kidney Int* 1997;51:622–630.
35. Randolph JE, Scarlett J, Stokol T, et al. Clinical efficacy and safety of recombinant canine erythropoietin in dogs with anemia of chronic renal failure and dogs with recombinant human erythropoietin-induced red cell aplasia. *J Vet Intern Med* 2004;18:81–91.
36. Horl WH, Jacobs C, Macdougall IC, et al. European best practice guidelines 14-16 inadequate response to epoetin. *Nephrol Dial Transplant* 2000;15:43–50.
37. Morelle J, Labriola L, Jadoul M. Plasma hepcidin levels are elevated but responsive to erythropoietin therapy in renal disease. *Kidney Int* 2009;76:1116–6.