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Urinary neutrophil gelatinase-associated lipocalin in dogs with stable or progressive kidney disease

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Background: Active kidney injury may play a role in chronic kidney disease (CKD) progression in dogs. Neutrophil gelatinase-associated lipocalin (NGAL), a novel tubular kidney injury biomarker, may help differentiate progressive CKD from stable CKD in dogs.

Objectives: To determine if urinary NGAL : creatinine ratio (UNCR) differentiates stable and progressive CKD in dogs. We hypothesized that UNCR would be higher in dogs with progressive CKD versus stable CKD.

Animals: Twenty-one healthy control dogs, 22 with prerenal azotemia, 19 with stable CKD, 30 with progressive CKD, and 27 with acute kidney injury (AKI).

Methods: Prospective study. Azotemic (serum creatinine concentration >1.6 mg/dL) dogs or nonazotemic AKI dogs were enrolled and classified into 4 groups: (1) prerenal azotemia, (2) stable CKD, (3) progressive CKD, and (4) AKI. Urinary NGAL was measured by ELISA and UNCR compared among groups. Urine protein : creatinine ratio (UPC) in dogs with stable and progressive CKD was compared to UNCR for differentiating CKD groups.

Results: UNCR was significantly higher in dogs with progressive CKD than stable CKD. UNCR of the prerenal azotemia group was significantly lower than that of the progressive CKD and AKI groups. No significant difference was found in UNCR between stable CKD and prerenal azotemia groups. ROC curve analysis of UNCR for differentiating progressive CKD from stable CKD resulted in an AUC of 0.816 (95% confidence interval [CI], 0.673-0.959), greater than that of UPC (0.696; 95% CI, 0.529-0.863).

Conclusions and Clinical Importance: Urinary NGAL could be helpful to predict the risk of progression in dogs with CKD.

KEYWORDS

AKI, CKD, lipocalin, NGAL, urinary biomarker

1 | INTRODUCTION

Traditionally, acute kidney injury (AKI) and chronic kidney disease (CKD) are viewed as 2 distinct forms of kidney disease.[1] Acute kidney injury typically results in a rapid decrease in kidney function that may be reversible, whereas CKD usually features a slower, irreversible loss of kidney function that develops over months or longer.[1] Maladaptation and failure of regenerative repair during and after active injury in AKI and CKD may lead to loss of nephrons and development or progression of CKD.[1] However, a subset of dogs with CKD has slow or no progression over extended periods of time (stable CKD), which may reflect either a low level of ongoing injury or a combination of ongoing injury with adaptive hyperfiltration, minimizing overall loss of glomerular filtration rate (GFR).[1-3] If sufficient adaptive hyperfiltration develops, a progressive decrease in kidney function may be difficult or impossible to recognize.[1-3]

Abbreviations: AKI, acute kidney injury; AUC, area under the curve; CI, confidence interval; CKD, chronic kidney disease; GFR, glomerular filtration rate; MAT, microscopic agglutination test; NGAL, neutrophil gelatinase-associated lipocalin; ROC, receiver operator characteristic; UNCR, urinary NGAL to creatinine ratio; uNGAL, urinary neutrophil gelatinase-associated lipocalin; USG, urine specific gravity.

To date, the timely identification of dogs with higher risk of CKD progression has been limited because of a lack of early diagnostic predictors. Such ability to discriminate progressive from stable CKD patients would allow clinicians to focus on treatments that slow down loss of nephrons and kidney function. Conventional kidney markers (eg, serum creatinine and symmetric dimethylarginine concentrations) can detect progressive loss of kidney function by serial monitoring. The limitation of this approach is that recognition occurs after loss of function has already occurred.

Tubular injury biomarkers detect ongoing active renal injury in CKD and therefore may predict the likelihood of progression of CKD before conventional functional markers. Among these is neutrophil gelatinase-associated lipocalin (NGAL), a 25-kD protein covalently bound to matrix metalloprotein-9 in neutrophils.[4] It is synthesized in low concentrations in renal tubular, intestinal, hepatic, and pulmonary tissue, but its synthesis is substantially upregulated with tissue injury.[4,5] Circulating NGAL is filtered by the glomerulus, reabsorbed in the proximal tubule, and secreted by the thick ascending limb of the loop of Henle.[4,6] The urinary NGAL (uNGAL) concentration is very low under normal physiologic conditions. In active renal tubular injury, NGAL synthesis is increased, reabsorption is decreased, and secretion is increased, resulting in increased uNGAL concentrations.[6] It has been recognized as 1 of the earliest and most strongly induced proteins in both ischemic and nephrotoxic animal models of AKI.[5] Several studies in human and veterinary medicine indicate that serum NGAL and uNGAL concentrations are increased in AKI earlier than is serum creatinine concentration.

We hypothesized that dogs with progressive CKD would have urinary NGAL to creatinine ratio (UNCR) higher than dogs with stable CKD. Our objective was to determine if UNCR can differentiate between stable and progressive CKD. Recognizing increased risk of progression could prompt enhanced diagnostic, monitoring, and therapeutic approaches with the goal of decreasing nephron injury and progression of CKD. A secondary aim was to compare UNCR in the CKD groups to dogs with prerenal azotemia and AKI, and with healthy dogs.

2 | MATERIALS AND METHODS

2.1 | Case dogs and group categorization

Client-owned dogs presented to the Veterinary Medical Center of the University of Minnesota between August 2014 and April 2016 with a serum creatinine concentration >1.6 mg/dL were eligible for group assignment. Nonazotemic dogs with documented AKI at the time of presentation also were eligible for enrollment. Urinary tract infection was excluded based on urinalysis findings and negative urine culture. Urethral obstruction was excluded based on imaging (survey abdominal radiography, abdominal ultrasound examination, or retrograde urethrogram). The study was approved by the University of Minnesota Institutional Animal Care and Use Committee.

Case dogs were assigned into 1 of the following groups: (1) dogs with prerenal azotemia, (2) dogs with stable CKD, (3) dogs with progressive CKD, and (4) dogs with AKI after reviewing history, physical examination findings, CBC, serum biochemistry profile, urinalysis, urine culture, and abdominal imaging (radiographs or ultrasound examination),

as well as clinical course including response to fluid therapy and historical and follow-up serum creatinine concentrations, *Leptospira* titers, and necropsy findings when available.

Group categorization was performed by 3 clinicians (Y. M. Kim, J. L. Granick, and D. J. Polzin), and dogs that could not be clearly assigned into 1 of the groups because of incomplete medical records or disagreement among the reviewers were excluded.

Dogs with prerenal azotemia (Group 1) had a urine specific gravity (USG) ≥1.030 at presentation or complete resolution of azotemia within 24 hours of IV fluid therapy. Dogs with hypoadrenocorticism that had a USG <1.025 despite prerenal azotemia also were assigned to this group only if their serum creatinine concentration normalized within 24 hours of presentation after initiation of treatment of hypoadrenocorticism. No history or evidence of CKD, including historical azotemia with inappropriately concentrated urine and radiographic or ultrasonographic evidence of CKD, was identified in this group. Radiographic or ultrasonographic evidence of CKD was defined as ≥2 of the following: small-sized kidneys, irregular renal margins, increased renal cortical echogenicity, presence of renal cysts, and decreased renal corticomedullary distinction.

Dogs with stable CKD (Group 2) had USG <1.025, documented history of azotemic CKD over 3 months, and stable serum creatinine concentrations with no overt signs of uremia such as vomiting, anorexia, weakness, or uremic ulcers. Azotemia was defined as serum creatinine concentration >1.6 mg/dL (reference range, 0.6-1.6 mg/dL). Stable serum creatinine concentration was defined as 1 of the following: (i) an increase in serum creatinine concentration of ≤0.1 mg/dL within 3 months before presentation, (ii) an increase in serum creatinine concentration of ≤0.2 mg/dL over the 3- to 6-month period before presentation, (iii) an increase in serum creatinine concentration of ≤0.3 mg/dL over the 6- to 9-month period before presentation, or (iv) an increase in serum creatinine concentration of ≤0.4 mg/dL over the 9- to 12-month period before presentation (Figure 1).

Dogs with progressive CKD (Group 3) had USG <1.025 and documented history of azotemic CKD over 3 months with progressive increases in serum creatinine concentrations defined as 1 of the following: (i) an increase in serum creatinine concentration of ≥0.1 mg/dL
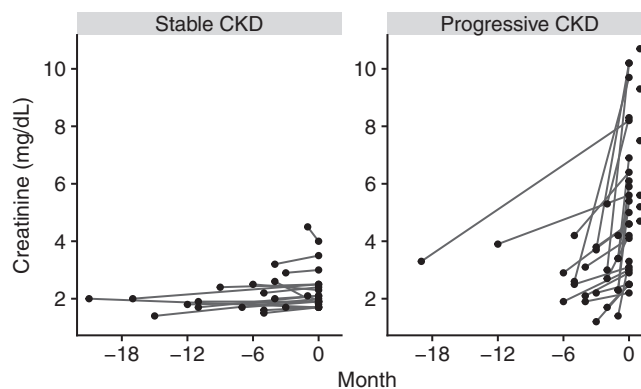

FIGURE 1 Creatinine rate change over time in the stable and progressive chronic kidney disease (CKD) groups. The dots without previous creatinine values in the progressive CKD represent dogs diagnosed with "acute on chronic" who did not have a previous baseline creatinine

within 3 months before presentation, (ii) an increase in serum creatinine concentration of ≥0.2 mg/dL over the 3- to 6-month period before presentation, (iii) an increase in serum creatinine concentration of ≥0.3 mg/dL over the 6- to 9-month period before presentation, or (iv) an increase in serum creatinine concentration of ≥0.4 mg/dL over the 9- to 12-month period before presentation (Figure 1). Dogs presented for an acute uremic episode also were assigned to this group if they had documented CKD previously or necropsy findings consistent with CKD and if their serum creatinine concentration failed to return to baseline or, if euthanized, necropsy failed to identify other clinically relevant comorbidities that would have contributed to azotemia.

Dogs with AKI (Group 4) had USG <1.025, serum creatinine concentration >1.6 mg/dL with no known history of renal azotemia (azotemic AKI) or an increase in serum creatinine concentration of ≥0.3 mg/dL within the nonazotemic range during a 48-hour interval (nonazotemic AKI) according to current International Renal Interest Society (IRIS) recommendations for grading of AKI, lack of clinical signs of CKD before the preceding 2 weeks, and ≥2 of the following: (i) absence of diagnostic imaging findings consistent with CKD, (ii) failure of azotemia to resolve within 24 hours of IV fluid therapy, (iii) known exposure to a nephrotoxin, (iv) a diagnosis of leptospirosis based on positive blood or urine polymerase chain reaction or microscopic agglutination titer (MAT) (a 4-fold titer increase in paired titers, a single MAT ≥1 : 800 or in dogs not vaccinated for leptospirosis within the previous 12 months, or a single MAT ≥1 : 3200 in dogs vaccinated for leptospirosis within 1 year), (v) development of oliguria or anuria despite adequate fluid therapy, (vi) a ≥50% decrease in serum creatinine concentration within a 2- to 6-week follow-up, or (vii) necropsy findings consistent with AKI.

2.2 | Healthy control dogs

Twenty healthy dogs presented for routine examination to the Primary Care Service at the Veterinary Medical Center or owned by staff members were recruited. Dogs were considered to be healthy and free of prerenal or renal azotemia based on history, physical examination findings, CBC, serum biochemistry profile, urinalysis, and urine culture.

2.3 | Sample collection

Remaining urine supernatant was obtained after urinalysis and used for uNGAL and urine protein to creatinine ratio (UPC) quantification. Urine was included if it was collected from dogs by voiding or cystocentesis at presentation (all groups) or within 36 hours of presentation for hospitalized dogs (Groups 3 and 4). In validation studies, uNGAL was stable in urine stored at 4°C up to 7 days and at −80°C up to 11 months and repeated freeze-thaw procedures did not affect NGAL measurement.[7,8] For dogs with prerenal azotemia or stable CKD, urine was accepted only if it was collected before fluid therapy. All urine samples were stored at −80°C pending NGAL determination. Complete blood count, serum biochemistry profile, urinalysis, and follow-up serum biochemistry were performed at the laboratory of the Veterinary Medical Center of the University of Minnesota. Urine culture and susceptibility testing were performed at an outside reference laboratory (Marshfield Labs, Marshfield, Wisconsin).

2.4 | NGAL ELISA and UNCR

One urine sample, collected at presentation or within up to 36 hours of presentation, was used for a single uNGAL measurement and UNCR calculation for all dogs. Urinary NGAL concentrations were measured by a commercially available sandwich ELISA kit (BioPorto, Denmark) according to the manufacturer's instructions, and optic density was measured at 450 nm using an ELISA plate reader (FluorChemHD2; Alpha Innotech, San Leandro, CA). A standard curve for NGAL was created using 8 dilutions (ranging from 0 to 400 pg/mL) of canine NGAL reference standard provided with the assay. The limits of the NGAL ELISA were 0-400 pg/mL. All samples in which NGAL measurements were above the upper limit of detection were diluted and run in duplicate. The highest dilution required to was 1 : 1200. The urinary NGAL concentrations (pg/mL) were calculated from a 4-parameter nonlinear standard curve created using curve fitting software (Optima; BMG Labtech, Cary, NC).

Urine creatinine concentrations (mg/dL) were measured in the laboratory of the Veterinary Medical Center using standard techniques by an automatic analyzer. The UNCR (in pg/mg) were calculated as (urine NGAL)/(urine creatinine).

2.5 | Proteinuria

Proteinuria was defined as a UPC > 0.5 when UPC data were available or urine protein ≥1+ on the sulfosalicylic acid turbidity test when UPC data were not available. All dogs with proteinuria had inactive sediment (no hematuria, pyuria, or bacteriuria). All dogs with positive urine cultures were excluded from the study.

2.6 | Urine protein : creatinine ratio

Although not an initial aim of the study, after analysis of UNCR between CKD groups, we elected to evaluate UPC in dogs with stable and progressive CKD to determine if this information was helpful in differentiating between these 2 groups. Urine protein concentrations were measured in a single urine sample in the laboratory of the Veterinary Medical Center using standard techniques by an automatic analyzer (AU480; Beckman Coulter, Brea, CA). The UPC ratios were calculated as (urine protein)/(urine creatinine).

2.7 | Statistical analysis

To test for differences between clinical groups, pairwise Wilcoxon rank sum tests were used for continuous variables (creatinine, UNCR, UPC) and pairwise proportion tests were used for binary variables (proteinuria). When >2 groups were compared, P-values were corrected for multiple comparisons using the Bonferroni-Holm adjustment. Additionally, to test for an association of serum creatinine concentration and UPC with UNCR, Pearson's correlation was used (with UNCR and UPC on the log scale), both for all dogs together and for each clinical group separately. To explore the power of UNCR and UPC for predicting stable or progressive CKD status, receiver operator characteristic (ROC) curves were plotted, and the area under the curve (AUC) reported, along with

sensitivity and specificity at the threshold that maximized the sum of sensitivity and specificity (Youden's index). Additionally, these data were subjected to multiple logistic regression, and the standardized odds ratios and confidence interval (CI) reported. Finally, to test for differences in signalment, the pairwise Wilcoxon test was used for age, and a chi-squared test was used for sex.

3 | RESULTS

One hundred nineteen dogs were included in the study: 21 healthy control dogs, 22 dogs with prerenal azotemia, 19 dogs with stable

CKD, 30 dogs with progressive CKD, and 27 dogs with AKI. Of these 119 dogs, 51 were spayed females, 4 were intact females, 57 were castrated males, and 7 were intact males. No statistically significant differences in sex or age (Figure 2) were found among the groups. Of 22 dogs with prerenal azotemia, 5 dogs were diagnosed with hypoadrenocorticism, 4 dogs had neoplastic disease, 4 dogs had gastrointestinal disease (2 mechanical obstruction and 2 hemorrhagic gastroenteritis), 2 dogs had pulmonary hypertension, and 2 dogs had trauma as the cause of azotemia. The underlying origin of prerenal azotemia was not conclusively identified in 5 of the 22 dogs.

Four of 19 dogs with stable CKD were presumptively diagnosed with renal dysplasia based on early-age onset azotemia and ultrasonographic
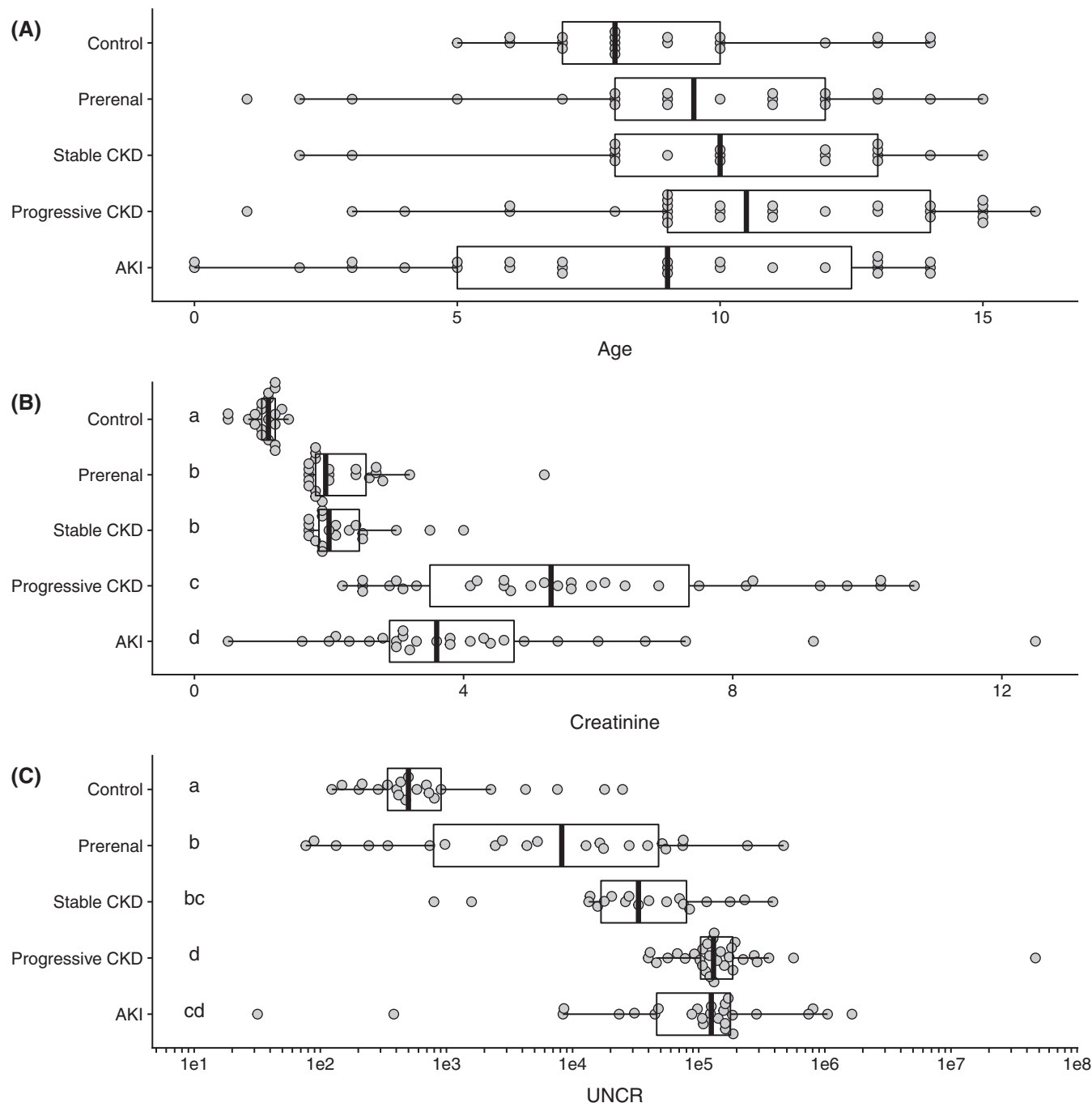

FIGURE 2   Age (A), serum creatinine concentration (B), and urine NGAL to creatinine ratio (UNCR) (C) by group. Each box includes interquartile range values and horizontal lines represent the median. [a-d]Medians in columns without a common superscript letter differ significantly (P < .05). Pairwise comparisons were made using the Wilcoxon rank sum test. AKI, acute kidney injury; CKD, chronic kidney disease
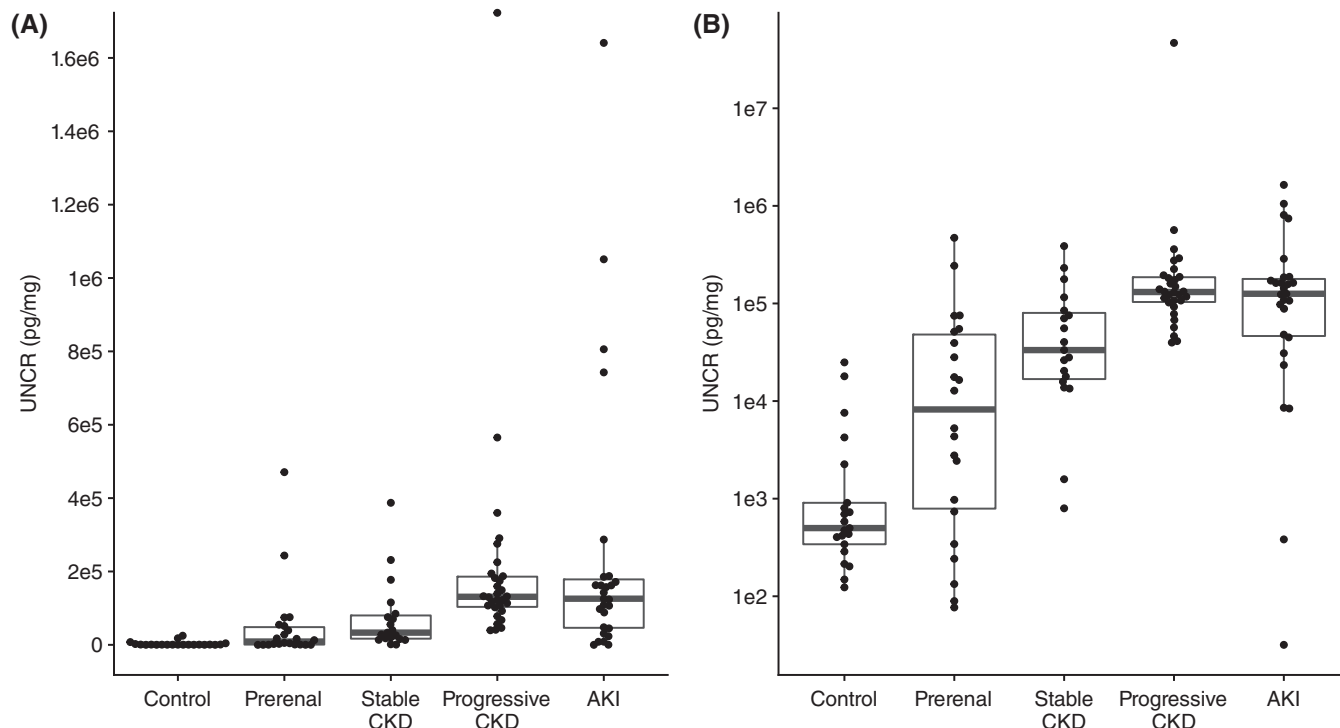
(A)

(B)

FIGURE 3   Urine neutrophil gelatinase-associated lipocalinL to creatinine ratio (UNCR) of dogs by group on the original scale (A) and the log scale (B). Both scales are shown to illustrate the wide range of high outliers (on the original scale) and the differences at lower levels (on the log scale). Each box includes interquartile range values and horizontal lines represent the median. Whiskers extend to at most 1.5 times the interquartile range. Outliers are depicted as circles. AKI, acute kidney injury; CKD, chronic kidney disease

appearance of the kidneys. One of 30 dogs with progressive CKD was presumptively diagnosed with renal dysplasia. Three of 30 dogs with progressive CKD had concurrent cardiac disease (chronic degenerative valvular disease).

Of 27 dogs with AKI, 9 dogs were diagnosed with leptospirosis, 2 dogs developed AKI after anesthesia, and 2 dogs presented for ingestion of nephrotoxins (1 ibuprofen and 1 raisins). A cause of AKI was not definitively determined for the remaining 14 dogs.

The median serum creatinine concentration was highest in dogs with progressive CKD (5.30 mg/dL), followed by dogs with AKI (3.60 mg/dL), dogs with stable CKD (2.00 mg/dL), dogs with prerenal azotemia (1.95 mg/dL), and healthy control dogs (1.10 mg/dL). Serum creatinine concentrations were significantly different among all groups except between the stable CKD and prerenal azotemia groups (Figure 2).

The UNCR results for all groups are presented in Figure 3. The median UNCR of healthy control dogs was 499 pg/mg (range, 123-24 913 pg/mg), which was significantly lower than that of all other study groups ($P < .05$ for prerenal azotemia, $P < .0001$ for the other

study groups). Dogs with progressive CKD had the highest median UNCR (131 061 pg/mg), which was significantly higher than that of the stable CKD (33 287 pg/mg, $P < .001$) and prerenal azotemia (9025 pg/mg,

TABLE 1   Number of dogs with proteinuria in dogs with stable or progressive chronic kidney disease

Proteinuria	Stable CKD (n = 19)	Progressive CKD (n = 30)
No (UPC 0.5 or lower)	11	7
Yes (UPC > 0.5)	8	23
% Positive	35.3	74.2

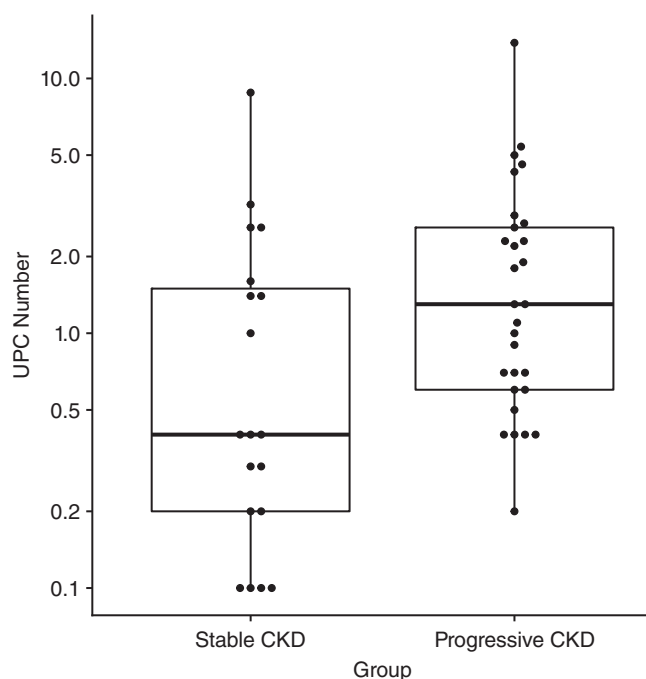Abbreviations: CKD, chronic kidney disease; UPC, urine protein : creatinine ratio.

FIGURE 4   Urine protein : creatinine ratio (UPC) in stable and progressive chronic kidney disease (CKD) groups. The UPC value was significantly different between stable and progressive CKD groups ($P = .02$). The median UPC value was 1.30 for the progressive group and 0.40 for the stable group. UPC, urine protein : creatinine ratio
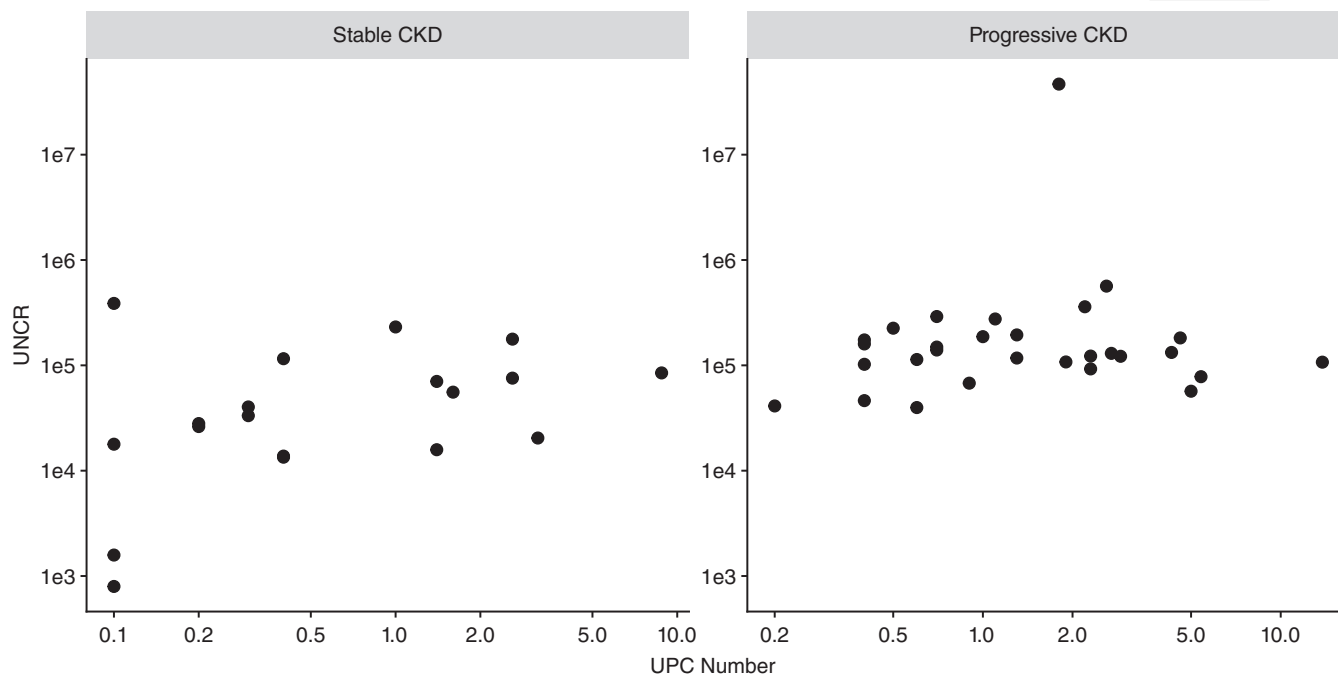
FIGURE 5 Relationship between urine protein : creatinine ratio (UPC) and urine neutrophil gelatinase-associated lipocalin to creatinine ratio (UNCR) in the stable and progressive chronic kidney disease (CKD) groups. The UPC was not significantly correlated with UNCR in the stable CKD group (r = 0.38) or progressive CKD group (r = 0.05)

$P < .0001$) groups; no significant difference in UNCR was found between the prerenal and stable CKD groups ($P = .07$). Although AKI had the second highest median UNCR (125 781 pg/mg), because of a wide range within that group, there was not a statistically significant difference with either the progressive CKD ($P = .56$) or stable CKD groups ($P = .07$), only the prerenal azotemia group ($P < .001$). Serum creatinine concentration was significantly correlated with UNCR when evaluating the entire cohort, ignoring the groups, ($r = 0.70$, $P < .0001$) and across the 2 CKD groups ($r = 0.50$, $P = .0003$). However, when the correlation between serum creatinine concentration and UNCR was analyzed in each group separately, statistically significant correlation was found in the control group ($r = -0.49$, $P = .02$) and the AKI group ($r = 0.53$, $P = .005$), but not in the prerenal ($r = 0.35$), stable CKD ($r = -0.13$), or progressive CKD ($r = 0.32$) groups.

Proteinuria was present in 8/19 (42.1%) of the stable CKD group and 23/30 (76.7%) of the progressive CKD group (Table 1). After correction for multiple comparisons, this difference was not statistically significant ($P = .16$), but because of the large observed difference and small sample size, we cannot rule out that a meaningful difference truly exists. Proteinuria was present in 1/21 (4.8%) of the control group, 5/23 (21.7%) of the prerenal group, and 10/27 (37.0%) of the AKI group. After correcting for multiple comparisons, these were all significantly different than the progressive CKD group but not the stable CKD group.

The UPC ratio was significantly different between the stable and progressive CKD groups ($P = .02$; Figure 4); the median was 1.30 for the progressive group and 0.40 for the stable group. This data was only available for these 2 groups, and thus no correction for multiple comparisons was needed. The UPC ratio was significantly correlated with UNCR when evaluating across both CKD groups ($r = 0.32$, $P = .03$), but not when analyzing the stable CKD group ($r = 0.38$) or progressive CKD group ($r = 0.05$) separately (Figure 5).

Because UNCR and UPC were statistically different between the stable and progressive CKD groups, we proceeded to determine which 1 or combination of these was best for predicting progressive versus stable CKD. The ROC analysis of UNCR differentiating progressive CKD from stable CKD resulted in an AUC of 0.816 (95% CI, 0.673-0.959; Figure 6A). Using a UNCR threshold of 88 600 pg/mg, UNCR was able to distinguish between progressive and stable CKD with a sensitivity of 0.800 and specificity of 0.789. In contrast, the ROC using UPC as a predictor for progressive CKD had an AUC of 0.696 (95% CI, 0.529-0.863), resulting in a sensitivity of 0.828 but a specificity of 0.579 when the optimal threshold UPC of 0.45 was used (Figure 6B). In a multiple logistic regression model, using log(UNCR) and log(UPC) to predict progressive CKD, UNCR was significant ($P = .005$), but UPC was not ($P = .35$). For a 1 SD increase in log(UNCR), the odds of a dog having progressive CKD increase by
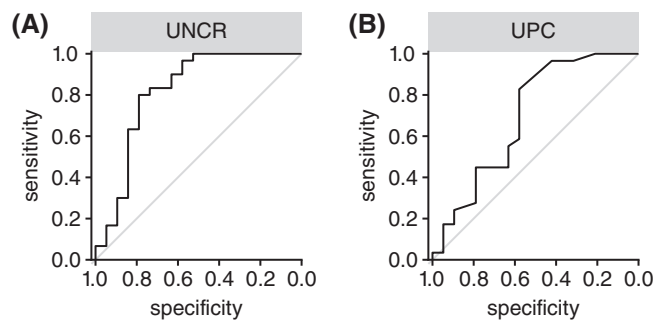

FIGURE 6 Receiver operator characteristic curve for differentiating dogs with progressive chronic kidney disease (CKD) from dogs with stable CKD based on a urinary neutrophil gelatinase-associated lipocalin to creatinine ratio (A) and urine protein : creatinine ratio (B). Area under the curve (AUC) of urine NGAL/creatinine ratio (UNCR) is 0.816; AUC of UPC is 0.696. UPC, urine protein : creatinine ratio

8.05 times (95% CI, 2.24-43.1) whereas a 1 SD increase in log(UPC) increased the odds of having progressive CKD by only 1.47 times (95% CI, 0.67-3.42). Thus, combining UPC with UNCR did not improve the ability of UNCR to differentiate dogs with progressive versus stable CKD.

4 | DISCUSSION

Our findings suggest that increased UNCR may serve as a useful marker of progression of CKD in dogs. Dogs with progressive CKD had significantly higher UNCR than did dogs with stable CKD. To our knowledge, ours is the first study to investigate differences in NGAL between dogs with progressive CKD and stable CKD. No statistically significant difference was found in UNCR between stable CKD and prerenal azotemia. Higher UNCR associated with progressive CKD is consistent with the presence of active kidney injury promoting progressive CKD. These findings suggest that UNCR may not only be a marker of AKI but may also warrant further investigation to determine if it can predict CKD progression.

The mechanisms and risk factors of initiation and progression of CKD in dogs remain incompletely defined and are likely multifactorial. Some dogs with CKD remain stable with very slowly declining kidney function and do not develop overt clinical signs or worsening azotemia for months to years after initial diagnosis,[3] whereas others have evidence of progression of CKD. Proteinuria, hypertension, hyperphosphatemia, activation of the renin-angiotensin-aldosterone system, hyperfiltration, hypoxia, and oxidative stress have been suggested as possible factors promoting CKD progression.[2] However, whether some of these factors promote progressive CKD or are rather markers of CKD progression is yet to be determined. Current management of CKD in dogs relies on monitoring and treating known risk factors and effects. Because progression of disease is inevitable in most dogs with CKD despite efforts to control these factors, earlier therapeutic intervention may improve the effectiveness of slowing CKD progression. In theory, NGAL may help in monitoring the effectiveness of treatments directed at decreasing ongoing kidney injuries.

Dogs with CKD are reported to have increased risk of developing AKI, which may promote progression of CKD.[1] Similarly, ongoing active kidney injury may play a role in initiating and promoting progression of CKD by causing loss of nephrons. When surviving nephrons have the capacity to develop sufficient hyperfiltration, nephron loss may be partially or completely hidden. However, development of overt progressive CKD may appear to accelerate when active kidney injuries lead to fewer nephrons, but surviving nephrons are unable to initiate compensatory adaptations. Neutrophil gelatinase-associated lipocalin may be useful in identifying dogs with active kidney injury that is masked by compensatory mechanisms.

Urinary NGAL and UNCR have been shown to be sensitive markers for induced or naturally occurring AKI in dogs, and increases have been detected substantially earlier than those of serum creatinine concentration in dogs with AKI.[5,9-14] The UNCR also was significantly increased in dogs and cats with naturally occurring CKD compared to healthy controls or dogs with lower urinary tract disease.[15] In our study, we categorized dogs with CKD into 2 separate groups, progressive CKD and

stable CKD, and a significant difference in UNCR was found in those groups. Dogs with progressive CKD had higher UNCR compared to dogs with stable CKD. The ROC analysis of UNCR showed that UNCR can differentiate progressive CKD from stable CKD. Although the median serum creatinine concentration in the progressive CKD group was higher than that of the stable CKD group, serum creatinine concentration was not correlated with UNCR within CKD groups. Thus, serum creatinine concentration alone is not a predictor of active kidney insult. However, it is apparent that dogs at higher IRIS stages are likely to have higher NGAL concentration, but it is also true that higher IRIS stages are more likely to progress. Similarly, dogs with lower IRIS stages (1 and 2) tend to have lower NGAL concentration, and they are less likely to progress. Based on our findings, we believe that dogs with stable CKD are experiencing less active kidney injury. Active kidney injury, which is reflected by an increase in tubular injury biomarkers such as NGAL, may play an important role in CKD progression. A limitation of our study is the limited number of dogs with serum creatinine concentration between 2.5 and 4 mg/dL. Ultimately, a larger cohort of dogs with CKD across a spectrum of serum creatinine concentrations is needed to determine if UNCR can predict CKD progression at early IRIS stages.

Proteinuria is recognized as a robust risk factor for progression to end-stage CKD in humans as well as progression of kidney disease in dogs and cats.[3] Proteinuria may promote CKD progression by increased toxicity to the renal tubules and mesangium and induction of a proinflammatory cascade.[3] A recent study in humans showed that renal tubular injury biomarkers including NGAL did not improve prediction of progression to end-stage renal disease beyond serum creatinine concentration or albuminuria.[16] However, their prediction models focused on detection of patients with end-stage kidney disease who required renal dialysis, whereas our progressive CKD group included dogs with a wide range of established CKD and IRIS stages that advance inevitably over time but not necessarily to end stage. The median UPC of dogs in our study with progressive CKD was significantly higher than that of those with stable CKD. However, in a multiple logistic regression model using both UNCR and UPC to differentiate progressive CKD from stable CKD, UNCR was a significant predictor but UPC was not. Additionally, the AUC of UPC differentiating the 2 groups was lower than that of UNCR. These findings suggest that UNCR can provide useful information in predicting CKD progression beyond UPC. We also hypothesize that episodes of active kidney injury might be an important risk factor for CKD progression in dogs, and tubular injury biomarkers may be useful in subsets of dogs with CKD that remain relatively free of proteinuria.

Our study's findings suggest that a continuum may exist between AKI and CKD, wherein these conditions are similar in their origins and responses to kidney injury but dissimilar in their rate of decrease in kidney function. As expected, the lowest UNCR was seen in the normal control group, followed by the prerenal azotemia group and then stable CKD, with the highest UNCR observed in the AKI and progressive CKD groups. The UNCR in dogs with AKI did not significantly differ from that of dogs with progressive CKD. Surprisingly, no significant difference was found between the AKI group and the stable CKD group, whereas a previous study found a significant difference in UNCR between those groups.[5] The lack of statistical difference between the stable CKD group and the AKI group might be explained if

the AKI group dogs were sampled at a time different from when their peak active renal injury occurred. We designed our study to simulate the most common clinical settings in veterinary medicine to evaluate the utility of UNCR in such settings. Thus, at the time of sampling, almost all dogs with AKI in our study already had established azotemia with unknown durations of time from the onset of azotemia. In gentamicin-induced AKI, peak uNGAL occurred 19 days after initial renal insult (after a steep increase after day 7).[13] Therefore, we hypothesize that UNCR in our AKI group could have been underestimated by missing the optimal time for detecting the highest urinary NGAL in the midst of active ongoing renal injury.

One limitation of our study is that no clear definition for dogs with progressive CKD exists, and thus we grouped dogs based on changes in serum creatinine concentration over time, because doing so was the only way to assess whether the dogs' status was changing. Another related limitation was that the time between serum creatinine concentration measurements was not standardized in this pilot study using convenience sampling. Some dogs might have been misclassified because repeated GFR assessment was not available. Another limitation of our study is that a single UNCR was measured but higher UNCR results could occur with progression. These findings justify a study with multiple UNCR determinations over time to confirm the value of increased UNCR in predicting progression of CKD.

In conclusion, UNCR may be a promising predictor of progression in CKD. It may provide meaningful information regarding the clinical course of CKD. Knowledge that a patient with CKD is likely to progress should prompt the clinician to increase monitoring and adjust treatment as compared to dogs with stable CKD.

CONFLICTS OF INTEREST DECLARATION

Authors disclose no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

This study was approved by the University of Minnesota IACUC, Protocol ID: 1402-31392A.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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