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Influence of Carcinoma and Sarcoma on Neutrophil Gelatinase-Associated Lipocalin and Symmetric Dimethylarginine Concentrations in Dogs

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ABSTRACT

Background: It is unknown if tumors or concomitant renal disease influence neutrophil gelatinase–associated lipocalin (NGAL) and symmetric dimethylarginine (SDMA) concentrations in tumor-bearing dogs.

Objectives: Determine the effect of tumor presence, tumor type, and metastasis on concentrations of serum NGAL (sNGAL), SDMA, urinary NGAL (uNGAL), and uNGAL-to-creatinine ratio (uNGAL/Cr) in dogs with carcinoma or sarcoma without clinically relevant renal disease.

Animals: Twenty-one dogs with carcinoma, 18 with sarcoma, and 20 healthy age-controlled dogs.

Methods: Concentrations of sNGAL, SDMA, and uNGAL, and uNGAL/Cr ratio were measured from banked samples collected during a previous prospective study. Patient clinicopathological and histopathology records were reviewed, and those with renal azotemia or moderate to severe histopathological renal abnormalities were classified as having clinically relevant renal disease. Biomarker concentrations were compared between tumor-bearing dogs without clinically relevant renal disease and healthy age-controlled dogs. Additionally, comparisons were made between dogs with carcinoma and sarcoma, as well as between dogs with and without metastasis. Correlations between uNGAL and sNGAL concentrations, along with acute phase protein (APP) concentrations, were also analyzed.

Results: Tumor-bearing dogs without clinically relevant renal disease had increased uNGAL/Cr ($p < 0.001$), but not sNGAL, compared with healthy controls. Although median SDMA concentrations did not significantly differ between groups, increased concentrations were found in 32% of dogs with carcinoma and 20% of dogs with sarcoma. No differences were found between

Abbreviations: AKI, acute kidney injury; APP, acute phase protein; CKD, chronic kidney disease; GFR, glomerular filtration rate; HPF, high-powered field; IQR, interquartile range; IV, intravenous; MMP-9, matrix metalloproteinase-9; NGAL, neutrophil gelatinase–associated lipocalin; NSAIDS, nonsteroidal anti-inflammatory drugs; PRMT5, protein arginine methyltransferase 5; SAA, serum amyloid A; sCr, serum creatinine; sCRP, serum C-reactive protein; SDMA, symmetric dimethylarginine; sNGAL, serum neutrophil gelatinase–associated lipocalin; TNM, tumor, node, metastasis; uNGAL, urinary neutrophil gelatinase–associated lipocalin; uNGAL/Cr, urinary neutrophil gelatinase–associated lipocalin-to-creatinine ratio; USG, urine specific gravity; WBC, white blood cell.

dogs with carcinoma and those with sarcoma, or between dogs with metastasis and those without. Urinary and serum NGAL concentrations were moderately correlated, while weak to no correlations were observed with APPs.
Conclusion: Carcinomas and sarcomas, but not metastasis, influence uNGAL/Cr and SDMA concentrations in dogs.

1 | Introduction

Neutrophil gelatinase–associated lipocalin (NGAL) and symmetric dimethylarginine (SDMA) are used in veterinary medicine as sensitive biomarkers of acute kidney injury (AKI) and glomerular filtration rate (GFR), respectively [1–13]. Although extrarenal influences are known to decrease their specifity [3, 14–16], it is unclear if tumors directly influence biomarker concentrations.

Expressed by most cells in the body, circulating NGAL undergoes glomerular filtration and complete proximal renal tubular reabsorption [17, 18], resulting in negligible concentrations in the urine of healthy dogs [1–3, 19–21]. Upon renal injury, increased distal renal tubular basolateral expression of NGAL results in increased plasma concentrations [18]. Increased glomerular filtration and decreased proximal tubular reabsorption subsequently result in increased urinary concentrations [18]. Because serum NGAL (sNGAL) and urinary NGAL (uNGAL) concentrations increase before other conventional renal biomarkers, they serve as sensitive biomarkers for early AKI [1–10]. However, NGAL is not specific for AKI and concentrations have been shown to increase in dogs with chronic kidney disease (CKD) [6, 9, 22, 23], systemic inflammation [3, 16], pyuria [14, 15], carcinoma and lymphoma [22]. As an essential mediator of iron metabolism, NGAL has been shown to facilitate tumorigenesis [24]. Accordingly, increased concentrations have been described in various human cancers [17, 20, 21, 24] but in only a single veterinary study to date [22]. Moreover, the effect of inflammation, intricately associated with tumors [25, 26], on NGAL concentrations remains unknown.

Free glomerular filtration and lack of tubular modification facilitate SDMA's use as a surrogate marker of GFR [11–13]. Although considered to have minimal extrarenal influence, SDMA is increased in dogs with lymphoma [11, 27]. However, because of the lack of renal histopathology in previous studies, it is unknown if increased NGAL [22] and SDMA [27] concentrations reported in tumor-bearing dogs are caused by tumor presence or concomitant renal involvement. Comprehensive characterization of renal disease, including renal histopathology, and correlation to the inflammatory status of dogs with tumors is warranted to fully elucidate the influence of tumors on the specifity of NGAL and SDMA.

Despite SDMA [28] and NGAL [17, 29–36] concentrations demonstrating prognostic relevance in cancers of humans and enhancing the metastatic potential of tumors [24, 37], their association with metastatic disease has not been evaluated in dogs.

Our primary objective was to evaluate concentrations of sNGAL, SDMA, uNGAL, and uNGAL-to-creatinine (uNGAL/Cr) in tumor-bearing dogs without clinically relevant renal disease. Our secondary objectives were to evaluate the effect of tumor type (i.e., carcinoma and sarcoma) and metastasis on these biomarkers, as well as the correlation between sNGAL and uNGAL concentrations and inflammation, using serum C-reactive protein (sCRP) and serum amyloid A (SAA) concentrations. We hypothesized that all NGAL, but not SDMA, concentrations would be increased in tumor-bearing dogs compared with controls, and that biomarker concentrations would not differ significantly between dogs with carcinoma and those with sarcoma, nor between dogs with and without metastasis. Finally, we hypothesized a positive correlation between sNGAL and uNGAL concentrations and inflammatory markers.

2 | Materials and Methods

2.1 | Study Design

Concentrations of sNGAL, SDMA, uNGAL, and uNGAL/Cr were measured from banked urine and serum samples obtained during a previous prospective study [25]. Prior owner informed consent was obtained for all dogs, with research ethics approval granted by the Research Ethics Committee of the Faculty of Veterinary Science (REC 105-18 and REC 049-23) and the Animal Ethics Committee of the University of Pretoria (V100-18).

2.2 | Study Population and Sample Processing

All dogs presented to the Onderstepoort Veterinary Academic Hospital from December 2018 to September 2020 and euthanized because of poor prognosis or financial constraints relating to a clinical and cytological diagnosis of a primary carcinoma or sarcoma were prospectively enrolled as the tumor-bearing population [25]. The prospectively-enrolled healthy age-controlled population were all client- or staff-owned dogs, >9 years of age, and deemed to be healthy based on clinical and medical history, physical examination findings, peripheral blood smear evaluation, hematology, biochemistry fecal flotation, abdominal ultrasound examination, and three-view thoracic radiographs [25].

All enrolled dogs had a complete history taken and underwent a physical examination, blood sampling, and urinalysis. Additionally, all tumor-bearing dogs underwent full necropsy evaluation with histopathology after owner-elected euthanasia [25]. Blood samples were collected from all dogs in serum and EDTA tubes (Beckton Dickinson Vacutainer Systems, UK) by vacuum-assisted venipuncture using a 21-gauge needle, and within 40 min before euthanasia in tumor-bearing dogs [25]. Serum biochemical batch analysis included creatinine (Jaffé reactionon Cobas Integra 400 Plus, Roche Diagnostics) and the acute phase proteins (APPs) CRP (Gentian, Moss, Norway) and SAA (Eiken, Japan) [25]. Urine was collected from all dogs sterilely by cystocentesis using a 22-guage needle and a 5-mL syringe, and within 50 min of euthanasia in tumor-bearing dogs [25]. Complete urinalysis was performed within 1 h of sample collection and included sediment evaluation [25]. All remaining

centrifuged serum and urine was aliquoted into cryovials and stored at −80°C within 4 h of collection [25]. Complete necropsies of tumor-bearing dogs were performed within 50 min of euthanasia [25]. Histopathological examination of the tumor, draining lymph node (when identified), and all parenchymatous organs, including kidney, was performed by a board-certified pathologist [25].

Dogs diagnosed histopathologically with carcinoma or sarcoma in the previous prospective study were considered for inclusion as the tumor-bearing study population in the study. All healthy, age-controlled dogs in the previous prospective study were included in the study. Dogs were excluded if they met any of the following exclusion criteria: (1) a history of clinical signs consistent with lower urinary tract disease, including any combination of stranguria, pollakiuria, dysuria, or hematuria [38]; (2) no or insufficient urine or serum collected; (3) an active urine sediment, defined as the presence of hematuria (> 5 red blood cells per high-powered field [hpf]), pyuria (> 5 white blood cell [WBC] per hpf), or bacteriuria (any bacteria seen) [38, 39]; (4) presence of renal tubular casts or glucosuria; (5) incomplete clinicopathological, urinalysis, or histopathology records; (6) prerenal azotemia, defined as a serum creatinine (sCr) concentration ≥ 125 μmol/L with a concurrent urine specific gravity (USG) ≥ 1.030 [40]; or (7) severe hemorrhage (intracavitary or diffuse tissue hemorrhage) on necropsy evaluation. The latter two exclusions accounted for the effect of renal ischemia on NGAL concentrations before changes in renal histopathology became evident (i.e., 12 h) [4].

2.3 | Biomarker Analysis

Serum NGAL and uNGAL concentrations were determined using a dog-specific sandwich ELISA (Bioporto, Gentofte, Denmark), previously validated for use in dogs [41]. Analysis was performed according to the manufacturer's instructions and as previously described [22]. A four-parameter logistic curve-fitting program (Deltasoft JV, Biometallics Inc.) was used to generate the standard curve and calculate biomarker concentrations. Concentrations of uNGAL were reported as absolute values and as uNGAL/Cr to account for variability in urine flow rates, assuming steady-state creatinine excretion [42]. Serum SDMA concentrations were determined using liquid chromatography-mass spectrometry as previously described [43]. Based on recently published age-specific reference intervals for dogs, an increased SDMA concentration in this population was considered to be > 15 μg/dL [44].

2.4 | Histopathological Interpretation

In addition to tumor types, histopathology reports of tumor-bearing dogs were reviewed for evidence of renal or metastatic disease. No distinction was made between regional lymph node and distant organ metastasis of the primary tumor for the purposes of the study, and because of the array of tumor subtypes, tumors were not classified according to the World Health Organization's tumor, node, metastasis (TNM) staging system. The severity of histopathologically evident renal disease was subjectively categorized by a board-certified pathologist as being mild, moderate, or severe.

2.5 | Statistical Analysis

For statistical purposes, dogs with histopathological evidence of moderate to severe renal changes or renal azotemia, defined as a sCr concentration ≥ 125 μmol/L with concurrent USG < 1.030, were categorized as having clinically relevant renal disease [40]. Data were analyzed using SPSS 27 software (IBM SPSS Inc., Armonk, NY). Categorical data were compared between groups using CShi-squared tests. Quantitative data sets were tested for normality using the Shapiro–Wilks test and evaluation of histograms. Because no data satisfied the criteria for normality, nonparametric statistical methods were employed. The Mann–Whitney *U*-test was used for comparisons between two groups. Only tumor-bearing dogs without clinically relevant renal disease were statistically compared, unless specifically stated otherwise. Biomarker concentrations were compared between tumor-bearing dogs and healthy, age-controlled dogs, between dogs with carcinoma and those with sarcoma, and between tumor-bearing dogs with and without evidence of metastatic disease. Finally, all tumor-bearing dogs with and without clinically relevant renal disease were compared to each other. Correlations between uNGAL and sNGAL and APP concentrations were performed using Spearman's rank correlation coefficient. Statistical significance was set at $p < 0.05$.

3 | Results

3.1 | Study Population

A total of 59 dogs were included: 39 tumor-bearing dogs and 20 healthy, age-controlled dogs. Twenty-three tumor-bearing dogs were excluded from the 62 dogs included in the previous prospective study [25]. Fourteen dogs were excluded because of either no or insufficient volume of urine (seven dogs) or an active urine sediment (seven dogs) and nine dogs because of the potential for renal hypoperfusion (intracavitary hemorrhage [seven dogs], severe diffuse tissue hemorrhage [one dog], and prerenal azotemia [one dog]; Figure 1). The resulting tumor-bearing study population consisted of 16 breeds, including mixed breed ($n = 7$), Labrador retriever (5), boerboel (5), Jack Russell terrier (4), dachshund (4), American pit bull terrier (2), cocker spaniel (2), German shepherd (2), and one each of beagle, border collie, boxer, Irish terrier, Pomeranian, pug, Rottweiler and Scottish terrier. The healthy age-controlled population consisted of nine breeds, including mixed breed (8), Jack Russell terrier (4), dachshund (2), and one each of basset hound, bull terrier, German shepherd, miniature French poodle, Pekingese, and Staffordshire bull terrier. The tumor-bearing study population included 25 females (intact, 16; spayed, 9) and 14 males (intact, 5; neutered, 9). The healthy, aged-controlled population included 9 females (all spayed) and 11 males (intact, 2; neutered, 9). The median age of the tumor-bearing study population was 10.0 years interquartile range ([IQR], 8.0–13.2) compared to 10.3 years ([IQR], 9.3–12.0) for the healthy, age-controlled population. No

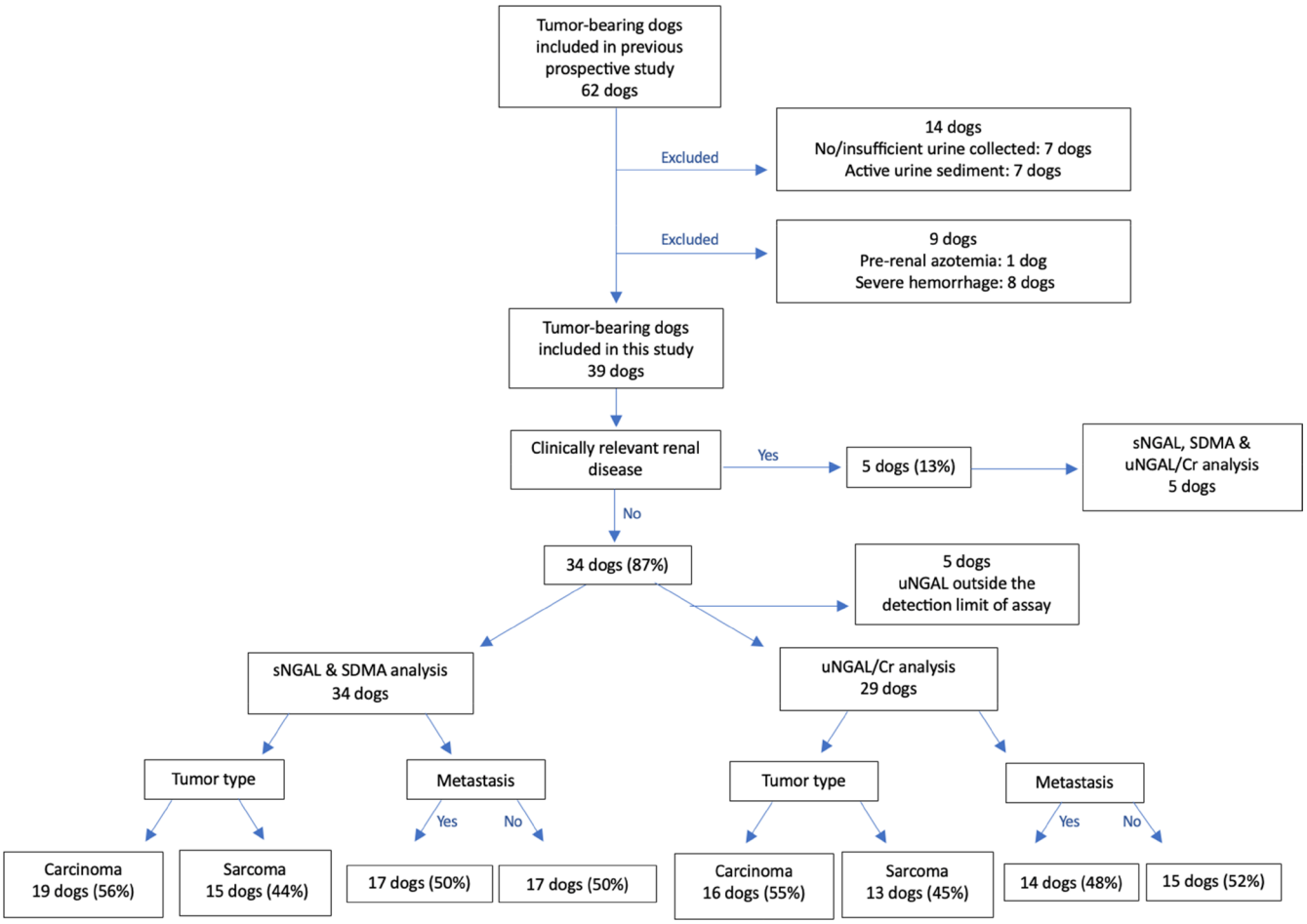

FIGURE 1 | Population characteristics, including tumor type and metastatic status, of the tumor-bearing study population from which sNGAL, SDMA and uNGAL/Cr were analyzed.

significant difference was found among breed categories, sex, or age between the study and control populations.

Of the 39 tumor-bearing dogs, 21 had carcinomas and 18 had sarcomas (Table S1). Metastatic disease was evident in 19/39 (49%) of all tumor-bearing dogs; 9/21 (43%) of dogs with carcinoma; and 10/18 (56%) of dogs with sarcoma. Five tumor-bearing study dogs (13%) were classified as having clinically relevant renal disease (Table S2), four exclusively with histopathological evidence of moderate to severe renal disease and 1 with concomitant renal azotemia. After exclusion of tumor-bearing dogs with clinically relevant renal disease, sNGAL and SDMA were statistically analyzed in the remaining 34 tumor-bearing dogs (Figure 1). Five tumor-bearing dogs were below (Table S3), and three control dogs were above, the quantification limit of the uNGAL assay, consequently a total of 29 tumor-bearing dogs (Figure 1) and 17 healthy age-controlled dogs were available for statistical analysis of uNGAL. Thirteen tumor-bearing dogs received medications before euthanasia, including a combination of pain control (8 dogs: nonsteroidal anti-inflammatory drugs [NSAIDs], 5; fentanyl, 1; gabapentin, 1; tramadol, 1; unspecified, 1); iv fluids (5 dogs: Ringer's lactate solution, 4; unspecified, 1); antibiotics (3 dogs: amoxicillin-clavulanic acid, 2; unspecified, 1); chemotherapy (1 dog: carboplatin 3 weeks before euthanasia); and miscellaneous medications (5 dogs: maropitant, 2; butorphanol, 1; cannabidiol, 1; omeprazole, 1; phenobarbitone, 1).

3.2 | Biomarkers

Serum creatinine, sNGAL, and SDMA concentrations did not differ significantly between tumor-bearing dogs without clinically relevant renal disease and healthy age-controlled dogs (Table 1). Compared with healthy age-controlled dogs, uNGAL concentrations and uNGAL/Cr were significantly increased in tumor-bearing dogs without clinically relevant renal disease (Table 1 and Figure 2). The sNGAL concentrations were moderately positively correlated with uNGAL/Cr ($r = 0.60$; $p = < 0.001$). Concentrations of SDMA above the age-appropriate reference interval for this population were identified in 6/19 dogs with carcinoma (32%), 3/15 (20%) of dogs with sarcoma without clinically relevant renal disease, and in 1/20 (5%) healthy age-controlled dogs (Table S4).

In tumor-bearing dogs without clinically relevant renal disease, sCr, sNGAL, uNGAL, uNGAL/Cr, and SDMA concentrations were not significantly different between dogs with carcinoma and those with sarcoma (Table 2), nor between dogs with and without metastasis (Table 3). In addition to

TABLE 1 | Comparison of renal biomarker and acute phase protein concentrations between tumor-bearing dogs without clinically relevant renal disease and healthy age-controlled dogs.

	Tumor-bearing dogs		Healthy age-controlled dogs		
Variable	***N***	**Median (IQR)**	***N***	**Median (IQR)**	***p***
sCr (μmol/L)	34	63.5 (48.8–78.5)	20	70.5 (61.0–76.5)	0.13
sNGAL (ng/mL)	34	21.5 (8.88–45.7)	20	10.8 (6.89–22.9)	0.13
uNGAL (ng/mL)	29	129 (21.9–292)	17	2.61 (1.40–4.92)	**< 0.001**
uNGAL/Cr (μg/g)	29	97.4 (25.3–302)	17	1.23 (0.60–2.27)	**< 0.001**
SDMA (μg/dL)	34	10.6 (8.9–14.5)	20	11.4 (10.4–12.8)	0.62
sCRP (mg/L)	34	65.8 (37.9–162)	20	< 10.0 (< 10.0– < 10.0)	**< 0.001**
SAA (mg/L)	34	40.1 (10.7–152)	20	< 2.00 (< 2.00– < 2.00)	**< 0.001**

Abbreviations: IQR, interquartile range; SAA, serum amyloid A; sCr, serum creatinine; sCRP, serum C-reactive protein; SDMA, symmetrical dimethylarginine; sNGAL, serum neutrophil gelatinase-associated lipocalin; uNGAL, urinary neutrophil gelatinase-associated lipocalin; uNGAL/Cr, urinary neutrophil gelatinase-associated lipocalin-to-creatinine.

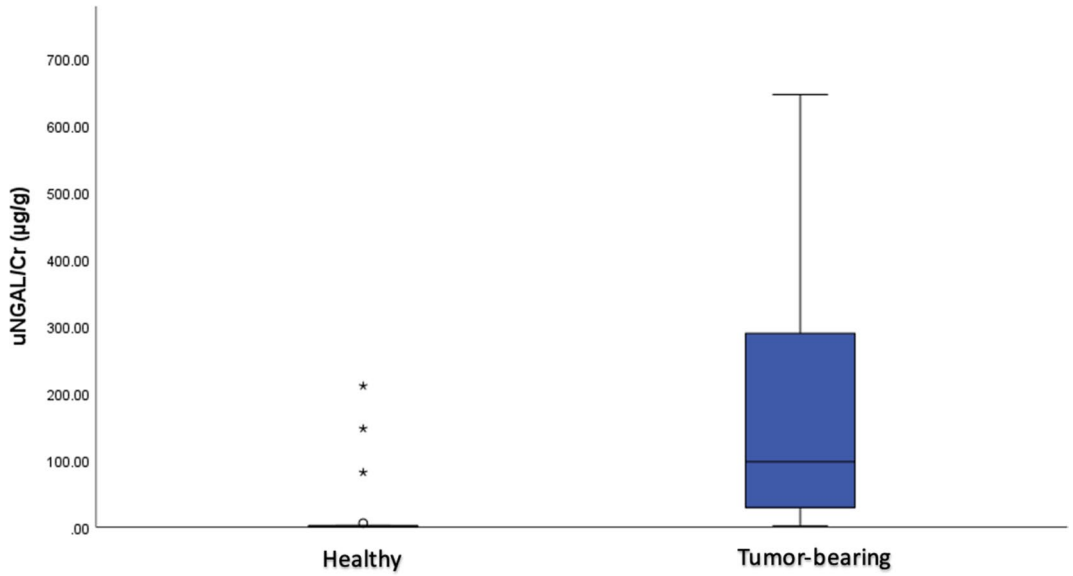

FIGURE 2 | Box and whisker plots of uNGAL/Cr in healthy age-controlled dogs and tumor-bearing dogs without clinically relevant renal disease. Stars = outliers. Three outliers removed from graphical representation of tumor-bearing population with values of 837, 1933, and 3226 μg/g.

sCr, only SDMA differed significantly between tumor-bearing dogs with and without clinically relevant renal disease (Table 4). The APP concentrations were significantly higher in tumor-bearing dogs without clinically relevant renal disease, compared with healthy age-controlled dogs (Table 1). Weak to no significant correlations were identified between APP concentrations and uNGAL/Cr or sNGAL concentrations, respectively (Table 5).

4 | Discussion

Dogs with carcinoma or sarcoma were found to have significantly increased urinary concentrations of NGAL, as compared with healthy age-controlled dogs. In the absence of clinically relevant clinicopathological and histopathological renal disease, a renal source for these increased concentrations was considered improbable. Tumor-associated systemic inflammation, although significantly higher (Table 1) in tumor-bearing dogs compared with control dogs, was only poorly correlated with uNGAL/Cr. Therefore, it could be considered a contributing factor rather than a primary source of increased uNGAL/Cr in dogs with carcinoma and sarcoma [25]. Despite tumor presence not influencing median SDMA concentrations in this tumor-bearing population, it did appear to influence concentrations in a considerable proportion of individual dogs with carcinoma and sarcoma. sNGAL and SDMA concentrations and uNGAL/Cr were not associated with tumor type or presence of metastatic disease in our study population.

The increase in uNGAL/Cr identified in dogs with carcinoma and sarcoma in our study was not unexpected. Increases in uNGAL/Cr previously have been reported in a small number of dogs with carcinoma and lymphoma [22], as well as in humans with brain, breast, and ovarian cancer [35, 36, 45]. The findings of our study, in a larger cohort of dogs with carcinoma, therefore

TABLE 2 | Comparison of renal biomarker and acute phase protein concentrations between dogs with carcinoma and those with sarcoma, without clinically relevant renal disease.

Variable	Dogs with carcinoma		Dogs with sarcoma		*p*
	N	Median (IQR)	*N*	Median (IQR)	
sCr (μmol/L)	19	65.0 (52.0–80)	15	62.0 (45.0–78.0)	0.45
sNGAL (ng/mL)	19	27.4 (8.34–38.7)	15	19.1 (10.0–70.1)	1.0
uNGAL (ng/mL)	16	103 (20.9–279)	13	142 (22.3–307)	0.78
uNGAL/Cr (μg/g)	16	92.8 (39.3–285)	13	134 (14.6–577)	0.98
SDMA (μg/dL)	19	10.7 (9.00–17.6)	15	10.4 (8.9–13.7)	0.58
sCRP (mg/L)	19	62.4 (42.8–160)	15	78.7 (30.9–169)	0.92
SAA (mg/L)	19	36.0 (8.67–291)	15	41.2 (11.4–103)	0.97

Abbreviations: IQR, interquartile range; SAA, serum amyloid A; sCr, serum creatinine; sCRP, serum C-reactive protein; SDMA, symmetrical dimethylarginine; sNGAL, serum neutrophil gelatinase-associated lipocalin; uNGAL, urinary neutrophil gelatinase-associated lipocalin; uNGAL/Cr, urinary neutrophil gelatinase-associated lipocalin-to-creatinine.

TABLE 3 | Comparison of renal biomarker and acute phase protein concentrations between tumor-bearing dogs without clinically relevant renal disease, with and without metastasis.

Variable	Dogs with metastasis		Dogs without metastasis		*p*
	N	Median (IQR)	*N*	Median (IQR)	
sCr (μmol/L)	17	63.0 (48.5–71.5)	17	71.0 (48.5–84.5)	0.23
sNGAL (ng/mL)	17	29.2 (14.1–29.2)	17	12.9 (6.45–52.1)	0.16
uNGAL (ng/mL)	14	135 (28.2–319)	15	77.0 (20.2–291)	0.43
uNGAL/Cr (μg/g)	14	211 (33.7–566)	15	86.7 (7.62–289)	0.29
SDMA (μg/dL)	17	10.7 (8.95–15.1)	17	10.4 (8.85–14.1)	0.81
sCRP (mg/L)	17	78.7 (35.6–151)	17	63.3 (31.0–204)	0.81
SAA (mg/L)	17	38.9 (7.56–113)	17	44.0 (12.6–299)	0.79

Abbreviations: IQR, interquartile range; SAA, serum amyloid A; sCr, serum creatinine; sCRP, serum C-reactive protein; SDMA, symmetrical dimethylarginine; sNGAL, serum neutrophil gelatinase-associated lipocalin; uNGAL, urinary neutrophil gelatinase-associated lipocalin; uNGAL/Cr, urinary neutrophil gelatinase-associated lipocalin-to-creatinine.

TABLE 4 | Comparison of renal biomarker and acute phase protein concentrations between tumor-bearing dogs with and without clinically relevant renal disease.

Variable	Tumor-bearing dogs with clinically relevant renal disease		Tumor-bearing dogs without clinically relevant renal disease		*p*
	N	Median (IQR)	*N*	Median (IQR)	
sCr (μmol/L)	5	108 (72.0–122)	34	64.0 (49.0–80.0)	**0.01**
sNGAL (ng/mL)	5	22.4 (15.0–40.9)	34	22.5 (9.06–66.1)	0.75
uNGAL (ng/mL)	5	211 (59.9–500)	29	134 (22.3–291)	0.54
uNGAL/Cr (μg/g)	5	199 (61.9–611)	29	92.8 (27.0–296)	0.51
SDMA (μg/dL)	5	19.4 (11.6–27.1)	34	10.7 (8.90–14.3)	**0.05**
sCRP (mg/L)	5	49.2 (13.1–158)	34	68.2 (40.2–169)	0.61
SAA (mg/L)	5	9.67 (2.0–131)	34	41.2 (11.4–242)	0.28

Abbreviations: IQR, interquartile range; SAA, serum amyloid A; sCr, serum creatinine; sCRP, serum C-reactive protein; SDMA, symmetrical dimethylarginine; sNGAL, serum neutrophil gelatinase-associated lipocalin; uNGAL, urinary neutrophil gelatinase-associated lipocalin; uNGAL/Cr, urinary neutrophil gelatinase-associated lipocalin-to-creatinine.

TABLE 5 | Correlations between acute phase proteins and sNGAL or uNGAL/Cr in tumor-bearing dogs without clinically relevant renal disease.

Variable	sCRP		SAA	
	rho	*p*	rho	*p*
sNGAL (ng/mL)	0.17	0.35	0.19	0.29
uNGAL/Cr (μg/g)	0.42	**0.02**	0.43	0.**02**

Abbreviations: SAA, serum amyloid A; sCRP, serum C-reactive protein; sNGAL, serum neutrophil gelatinase-associated lipocalin; uNGAL/Cr, urinary neutrophil gelatinase-associated lipocalin-to-creatinine.

corroborates previous findings and also describes similar results in dogs with sarcoma. Dysregulated cellular metabolism, including dysregulation of iron metabolism, is a hallmark of tumorigenesis [21, 24]. Other hallmarks of tumor progression, namely, uncontrolled proliferation, resistance to apoptosis, and enhanced metastatic ability, are dependent on iron [24]. The ability of NGAL to supply the increased iron demand of proliferating tumor cells facilitates tumor progression and explains its upregulation by tumors [17, 21, 24].

The most common cause of increased uNGAL/Cr in dogs is renal injury [1–9, 22, 23, 41, 46]. Renal injury causes increased

basolateral expression of NGAL by distal renal tubular cells, resulting in increased plasma concentrations and subsequent glomerular filtration [18]. Damage to proximal renal tubular cells consequently results in increased uNGAL concentrations due to decreased resorptive capacity [18]. As a result, both plasma and urinary concentrations increase rapidly in the presence of renal injury, and well before conventional renal biomarkers, such as sCr and SDMA [1–4, 6–8, 10, 41]. Characterized as a sensitive biomarker of renal injury in dogs, it is unknown if increased uNGAL/Cr previously reported in dogs with carcinoma and lymphoma was in fact associated with renal disease in this aged population (median age, 10.5 and 6.5 years, respectively) [22]. The lack of renal histopathology and lenient clinicopathological exclusion criteria (sCr > 150 mmol/L) in a previous study potentially may have resulted in the inadvertent inclusion of carcinoma and lymphoma dogs with clinicopathologically inapparent but histologically evident renal injury or International Renal Interest Society (IRIS) Stage 2 CKD [22]. The inclusion of renal histopathology and stringent clinicopathological criteria of renal disease in our study supports the contention that a renal source of increased uNGAL/Cr in dogs with carcinoma and sarcoma is improbable. Interestingly, the uNGAL/Cr results in tumor-bearing dogs with renal disease also were not significantly different from tumor-bearing dogs without renal disease and were similar to those previously reported in dogs with AKI and CKD [1–3, 10, 23]. Therefore, tumor presence, rather than renal disease, appears to influence uNGAL/Cr in dogs with carcinoma and sarcoma, and has a direct effect on the renal specificity of uNGAL/Cr in dogs with tumors.

Inflammation also has been reported to significantly increase uNGAL/Cr in dogs with sepsis and inflammatory AKI, as compared with noninflammatory AKI [3, 16]. Therefore, a tumor-associated inflammatory state potentially could account, in part, for the increases in uNGAL/Cr seen in our study population. Local inflammation, ulceration, and necrosis of tumor tissue are believed to be responsible for tumor-associated inflammation, and is attributed to either rapid tumor growth outgrowing, or microthrombi curtailing, local blood supply [25]. However, only weak to no correlations were found between APP concentrations, known to be sensitive markers of systemic inflammation [47, 48], and uNGAL/Cr and sNGAL concentrations, respectively. These results indicate that systemic inflammation, although potentially contributory, is unlikely to be the only reason for increased uNGAL/Cr in tumor-bearing dogs.

Increased NGAL expression by tumorous tissue, although not directly investigated in our study, is a probable source of the observed increase in uNGAL/Cr. Although NGAL tissue expression is variable depending on tumor type, increased expression, as determined by immunohistochemistry or real time-polymerase chain reaction, is reported in most tumors in humans [17, 20, 24]. To date, NGAL tissue expression by immunohistochemical staining only has been investigated in mammary carcinomas in dogs [49]. Although found to be decreased [49], because only half of carcinomas in this study were of a mammary origin, extrapolation of these findings to our study population is not appropriate. Additional studies investigating tissue expression in other tumor types are warranted to better characterize the possible implication of tumor expression on NGAL concentrations in dogs with tumors.

Increased plasma concentrations of NGAL originating from a distant source, along with subsequent increased glomerular filtration, would result in increased urinary concentrations in the presence of decreased proximal renal tubular reabsorption [18]. Although a distant source could explain the increases in uNGAL/Cr, it would not explain the lower-than-expected sNGAL concentrations and the severely disproportionate increase in urinary concentrations (median > 79-fold) compared to serum concentrations (median > 2-fold).

Local production of NGAL within the lower urinary tract could explain the disproportionate increase in uNGAL/Cr, as well as the lack of an increase in serum concentrations. However, pyuria is the only local influence that has been shown to increase uNGAL/Cr [14, 15]. Therefore, the exclusion of pyuric patients in our study negates the potential local influence of pyuria on uNGAL/Cr in these dogs with carcinoma and sarcoma. The moderate correlation between uNGAL and sNGAL concentrations in our study population also corroborates the role of a distant source.

An increase in glomerular permeability could explain both the disproportionately increased uNGAL/Cr and the lower-than-expected serum concentrations. In dogs, three urinary forms of NGAL exist: a monomeric form (25 kDa), a dimeric form (50 kDa), and a NGAL/matrix metalloproteinase-9 (MMP-9) heterodimer dimer complex (130 kDa) [19]. Only the monomeric and dimeric forms would be considered small enough to undergo glomerular filtration [19]. However, despite being considered too large to be freely filtered, the NGAL/MMP-9 heterodimer dimer complex previously has been detected in the urine of dogs with mammary carcinoma [49]. The ability of NGAL to complex with MMP-9 is associated with its oncogenic role [24] and may potentially result in a complex that is too large to undergo proximal renal tubular reabsorption. Increased glomerular passage of the heterodimer complex, associated with increased glomerular permeability, combined with decreased proximal tubular reabsorption, could explain the disproportionate increase in uNGAL, as well as the lower-than-expected serum concentrations found in dogs with carcinoma and sarcoma in our study. Similar findings of increased uNGAL/Cr in the absence of increased serum concentrations have been reported in dogs with leishmaniasis and were shown to reflect histological glomerular lesions [46]. Moreover, correlations between the degree of proteinuria and glomerular lesions, in the absence of tubular lesions, in these dogs was suggestive of increased glomerular permeability [46]. Although not yet investigated in dogs with sarcoma, glomerular lesions have been found to be highly prevalent in dogs with mammary carcinoma [50]. A high prevalence of clinical proteinuria reported in these dogs, as for leishmania-infected dogs, is suggestive of potentially increased glomerular permeability in dogs with mammary carcinoma, as hypothesized for the dogs in our study [46, 50].

In contrast to the findings of our study, increases in uNGAL/Cr were accompanied by increases in sNGAL concentrations in a previous study of dogs with carcinoma and lymphoma [22]. A potential explanation for this finding could relate to variable

degrees of glomerular permeability dysfunction between study populations. Although uNGAL/Cr exceeding sNGAL concentrations in dogs with carcinoma found in a previous study supports the notion of increased glomerular permeability, the severity of this finding was potentially insufficient to cause an absolute decrease in serum concentrations [22]. In contrast, tumor-bearing dogs in our study ultimately were euthanized because of the cost of treatment or prognosis related to their primary tumors [25]. Therefore, if disease severity reflects glomerular permeability dysfunction, this possibility could account for the discrepancy reported in serum concentrations between studies. Additional studies incorporating complete characterization of glomerular lesions, glomerular permeability, and uNGAL isoforms using Western blot analysis, however, are warranted in dogs with carcinoma and sarcoma to investigate this hypothesis.

Systemic inflammation was found to be weakly correlated with uNGAL/Cr but not sNGAL concentrations in our study. Although additional studies are required, it has previously been proposed that increased APP concentrations associated with glomerular lesions in dogs with mammary carcinoma may indicate tumor-associated immune complex deposition, and conceivably could be responsible for the observed glomerular lesions in tumor-bearing dogs [50]. If glomerular lesions in tumor-bearing dogs do result in increased glomerular permeability, it may explain why APP concentrations were better correlated with uNGAL, rather than sNGAL, concentrations in our study.

Similar to previous studies [27], SDMA concentrations did not differ significantly between tumor-bearing dogs and healthy age-controlled dogs in our study. However, increased SDMA concentrations, based on recently published reference intervals [44], were identified in approximately 32% of dogs with carcinoma and 20% of dogs with sarcoma. These findings are in contrast to the observation of only 5% and 10% previously reported in dogs with carcinoma and sarcoma, respectively [27]. Although the extent to which SDMA concentrations were increased in dogs with carcinoma and sarcoma in our study was not sufficient to influence population statistics, these findings, in the absence of histopathologically relevant renal disease, could have implications for the renal specificity of increased SDMA concentrations in individual dogs with carcinoma and sarcoma, as previously described for dogs with lymphoma [27].

A decrease in GFR is considered an improbable explanation for the increase in SDMA concentrations seen in a proportion of dogs in our study. This conclusion is based on normal sCr concentrations, which are considered comparable to SDMA in detecting decreased GFR when using population-based reference intervals [11, 51], and the lack of histopathological findings suggestive of diffuse neoplastic renal infiltration. Overexpression of protein arginine methyltransferase 5 (PRMT5), an enzyme responsible for the production of SDMA, by neoplastic tissue offers another potential explanation for the increase in concentrations seen in some tumor-bearing dogs. Increased expression of PRMT5 has been identified in lymphoma tissue in dogs [27, 52] and various tumors in humans [53], although it has not yet been evaluated in dogs with carcinoma or sarcoma. Lastly, because SDMA is released into circulation during proteolysis, increased protein catabolism, characteristic of cancer cachexia, potentially could explain the increases in SDMA seen in some dogs with tumors [11, 28, 54]. The prevalence of cachexia varies according to tumor type in humans [55], and the limited range of carcinomas (only of mammary origin) and sarcomas (only hemangiosarcoma) included in a previous study potentially may have resulted in underestimation of the reported prevalence of increased SDMA concentrations in dogs with carcinoma and sarcoma [27].

Despite the association between increased NGAL concentrations and metastatic status in colorectal [32] and gastric cancer in humans [56], as well as SDMA's prognostic relevance in chronic lymphoid leukemia patients [28], these biomarker concentrations were not associated with metastasis in dogs with carcinoma and sarcoma in our study. Also, because NGAL expression is not consistently associated with tumor size in humans [57–60], TNM stage may not be correlated with biomarker concentrations in dogs with carcinoma and sarcoma. However, evaluating this possibility was beyond the scope of our study, and additional studies incorporating TNM staging for specific tumors and prognostic study end points are needed to elucidate the true prognostic potential of these biomarkers in dogs with tumors.

Our study had some limitations. Despite the fact that all dogs included in our study underwent urine sediment examination, urine cultures were not performed. Urinary NGAL/Cr has been shown to be increased in dogs with urinary tract infection [15]. However, uNGAL/Cr also has been found to be increased in both infectious and noninfectious pyuria and correlated with WBC count in the urinary sediment [14, 15]. Therefore, inflammation rather than an infectious cause appears to be responsible for the observed increases in uNGAL/Cr reported in dogs with pyuria [14]. Thus, the exclusion of pyuric patients was considered sufficient to negate this potential local influence on uNGAL/Cr in the dogs in our study. Secondly, the retrospective nature of our study meant that 13% and 3% of tumor-bearing dogs received NSAIDs or chemotherapy treatment, respectively. Both treatments are considered to have potential indirect effects on biomarker concentrations based on their renal or antitumoral effects [61, 62]. However, the small proportion of treated dogs, the exclusion of those with evident kidney injury, and the variable antitumoral effect of NSAIDs at clinical doses limit the potential influence of medications on our results [62–64]. Thirdly, although the stability of uNGAL and SDMA in dogs has been reported, that of sNGAL has not [12, 41, 43, 65]. However, urine NGAL concentrations have been shown to be unaffected by multiple freeze–thaw cycles and storage at −80°C for up to 8 years, and presumably, sNGAL would have similar properties [41, 43, 65]. Fourthly, despite the lack of association between biomarker concentrations and metastatic status, TNM staging may have provided a more accurate assessment of the influence of disease progression. However, the applicability of TMN staging was limited in our study because of the array of tumor subtypes included and the small sample size per group. Lastly, histopathological findings such as global glomerulosclerosis, interstitial fibrosis, and tubular atrophy are common findings in older dogs, and not all tumor-bearing dogs with histological evidence of renal disease were excluded from the analysis [66]. Importantly, the severity, rather than the mere presence, of renal lesions should define clinically relevant renal disease [66].

5 | Conclusions

The presence of a primary carcinoma or sarcoma, but not metastasis, influences uNGAL/Cr and SDMA concentrations in dogs with carcinoma and sarcoma. Therefore, uNGAL should not be used, and SDMA should be used with caution as renal biomarkers in dogs with carcinoma and sarcoma. The disproportionate increase in uNGAL, compared to sNGAL, concentrations in this cohort of dogs with carcinoma and sarcoma is suggestive of a potential glomerulopathy and increased glomerular permeability secondary to tumor presence. Further comprehensive characterization of glomerular lesions and permeability in dogs with tumors is necessary.

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Disclosure

Authors declare no off-label use of antimicrobials.

Ethics Statement

Approved by the Research Ethics Committee of the Faculty of Veterinary Science (REC 105-18 and REC 049-23) and the Animal Ethics Committee of the University of Pretoria (V100-18). The authors declare that human ethics approval was not required.

Conflicts of Interest

The authors declare no conflicts of interest.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.