Journal of Veterinary Internal Medicine

Journal of Veterinary Internal Medicine Open Access
ACVIM American College of Veterinary Internal Medicine

STANDARD ARTICLE OPEN ACCESS
Small Animal Internal Medicine Nephrology/Urology

Longitudinal Study of Renal Health Screening in Apparently Healthy Aging Dogs

Sofie Marynissen[1] | Sylvie Daminet[1] | Evelyne Meyer[2] | Kristel Demeyere[2] | Emmelie Stock[3] | Joris Delanghe[4] | Luc Duchateau[2] | Dominique Paepe[1]

[1]Department of Small Animals, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium | [2]Department of Veterinary and Biosciences, Lab of Biochemistry, Ghent University, Merelbeke, Belgium | [3]Department of Morphology, Imaging, Orthopedics, Rehabilitation and Nutrition, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium | [4]Department of Clinical Chemistry, Microbiology and Immunology, Faculty of Medicine and Health Sciences, Ghent University, Ghent, Belgium

Correspondence: Sofie Marynissen (sofie.marynissen@ugent.be)

Received: 25 November 2024 | **Revised:** 18 April 2025 | **Accepted:** 18 April 2025

Keywords: albuminuria | cystatin C | proteinuria | renal biomarker | retinol-binding protein | SDMA

ABSTRACT

Background: Combined measurement of functional, glomerular, and tubular markers in aging dogs is essential to detect early renal disease.

Objectives: Prospective longitudinal study to describe renal function and assess which biomarkers are associated with the development of early renal disease or death.

Animals: One hundred and twenty-two apparently healthy senior and geriatric dogs.

Methods: Prospective longitudinal study. Renal function was evaluated at baseline (T0) and every 6-12 months over 2 years, using systolic blood pressure measurements (SBP) and validated serum (creatinine, symmetric dimethylarginine, cystatin C [sCysC]), and urinary (specific gravity [USG], protein:creatinine [UPC], albumin:creatinine, retinol-binding protein:creatinine [uRBPcr]) biomarkers. Glomerular filtration rate (GFR) was measured in a subgroup. Survival models were used to assess the predictive value of measured biomarkers at baseline for the onset of azotemic chronic kidney disease (CKD) or death, respectively.

Results: A total of 122 dogs were included; follow-up was available in 106 (T12) and 92 (T24); and GFR was estimated in 18 (T0), 11 (T12), and 10 (T24) dogs. Throughout the study, 15/122 (12%) dogs showed evidence of non-azotemic CKD, and in 11/106 (10%) dogs, azotemic CKD developed. Proteinuria was not associated with azotemic CKD, in contrast to muscle condition score, functional markers, and uRBPcr. Death was weakly associated with USG, UPC, and sCysC.

Conclusions and Clinical Importance: Over a 2-year period, 20% (26/122) of older dogs developed CKD, mostly persistent renal proteinuria (15/122). Muscle wasting and functional markers combined with uRBPcr had the best predictive value for the onset of azotemic CKD in these older, previously apparently healthy dogs.

1 | Introduction

Senior dogs are more likely to develop chronic illness [1]. As pet life expectancy is rising, senior care has become a greater priority to veterinary practices [1–4]. Early diagnosis of chronic conditions allows timely preventive measures to be taken, as well as the institution of adequate treatment to improve outcome [1–4]. In both human and veterinary medicine, early detection

Abbreviations: BP, borderline proteinuric; CKD, chronic kidney disease; GFR, glomerular filtration rate; *n*, number; NP, non-proteinuric; OP, overt proteinuric; sCr, serum creatinine; sCysC, serum cystatin C; SDMA, symmetric dimethylarginine; T, time point (months); uALBcr, urinary albumin:creatinine ratio; UPC, urinary protein:creatinine ratio; uRBPcr, urinary retinol-binding protein:creatinine ratio; USG, urine specific gravity.

of renal dysfunction is considered crucial to allow timely intervention and the institution of adequate treatment to improve outcome [5–7]. Existing senior care guidelines provide a good basis for routine health screening; however, which analyses are of most interest when investigating the renal health of older dogs over time is not well studied [8, 9]. Firstly, it is currently unknown whether the measurement of serum biomarkers, in addition to serum creatinine (sCr), such as serum symmetric dimethylarginine (SDMA) or serum Cystatin C (sCysC), is beneficial when screening older dogs [10, 11]. Secondly, whether urinary biomarkers can reveal early onset of renal injury and have the potential to localize the site of damage is unknown [12, 13]. Microalbuminuria, linked to glomerular leakage, is suggested to be an early indicator of kidney disease in dogs (G. F. Grauer, E. B. Oberhauser, R. J. Basaraba, et al., "Development of Microalbuminuria in Dogs With Heart Worm Disease. [abstract]," *Journal of Veterinary Internal Medicine* 16, (2002): 352 and G. E. Lees, W. A. Jensen, D. F. Simpson, and C. E. Kashtan, "Persistent Albuminuria Precedes the Onset of Overt Proteinuria in Male Dogs With X-Linked Hereditary Nephropathy. [abstract]," *Journal of Veterinary Internal Medicine* 16, (2002): 353) [14–16]. A recent study did not find a difference in urinary albumin:creatinine ratio (uALBcr) between dogs with IRIS Stage 1 and Stage 2 CKD [17]. Low molecular weight proteins, such as retinol-binding protein (RBP), appear in the urine because of decreased reabsorption by the renal tubules. Urinary RBP (uRBP) is significantly higher in dogs with early CKD compared to healthy controls and is considered an early indicator of proximal tubular injury [14, 18–20]. Thirdly, the clinical relevance of persistent borderline proteinuria (BP), present in a substantial number of healthy dogs, remains unclear [21–23]. Finally, in order to interpret (renal) health screening investigations, age-specific reference intervals (RIs) for biomarkers in elderly dogs are needed [3, 24, 25].

Our main objective was to describe renal function in senior and geriatric dogs at baseline and during follow-up using a panel of functional, glomerular, and tubular biomarkers. Secondly, we aimed to investigate whether various (renal) biomarkers assessed at baseline are predictive of the development of azotemic CKD, death, or both in older dogs. A third aim was to obtain age-specific RIs for the renal biomarkers sCr, sCysC, uALBcr, and uRBPcr.

2 | Materials and Methods

2.1 | Study Cohort and Study Design

The study was completed between July 2019 and June 2023. All dogs were privately owned, owners signed an informed consent, and the study was approved by the local ethical committee (EC 2019/39).

Senior or geriatric dogs, as defined based on a previously published human/pet Analogy Chart [26], were prospectively recruited through leaflets distributed to veterinarians, veterinarians in training and social media. The study protocol was based on a published health study [3]. To be included, dogs had to be "healthy for the owner," meaning that in the owner's opinion, the dog did not have any problem necessitating veterinary care. Dogs with known and ongoing metabolic diseases were excluded. Preventive medication (e.g., anti ecto-/endoparasitic drugs, vaccination) was allowed until 2 weeks before health assessments, other treatment was not allowed within 2 months preceding inclusion. Food was withheld overnight. Dogs underwent complete health screening (blood pressure (SBP) measurement using Doppler ultrasonography according to ACVIM guidelines [27], fundoscopy, extensive physical examination, blood-, and urinalysis) every 6–12 months. During the 2-year follow-up, medicinal or surgical therapy was allowed. Hypertensive dogs (SBP ≥ 160 mmHg) with signs of target organ damage were treated accordingly. At each time point, blood was collected from the jugular vein (21G needle) and urine was taken through ultrasonography-guided cystocentesis (22G needle) or free catch. Complete blood count, biochemistry profile, and electrolytes (Procyte One Hematology and Catalyst Dx Chemistry Analyzer, IDEXX laboratories Inc., USA) were performed. Urinalysis consisted of measurement of urine specific gravity (USG) with a manual refractometer (master refractometer, ATAGO Co. Ltd), urine protein:creatinine ratio (UPC; protein: colorimetric reaction with pyrogallol red molybdate, Beckman Coulter; creatinine: modified Jaffe assay, Beckman Coulter), urinary dipstick (iChem velocity stick, Beckman Coulter), and sediment analysis. Urinary sediment, prepared by centrifuging 5–10 mL of urine at 447 g for 3 min at 2°C (Jouan B4i, Thermo Scientific), was considered active in case of bacteriuria or > 3 casts or > 5 red blood cells, white blood cells, or epithelial cells per high power field [40× objective], respectively [28]. Aerobic bacterial culture of urine was performed at baseline only. Plasma, serum, and supernatant urine samples were stored at −80°C until further analysis.

The first time when proteinuria, that could not be explained by sediment analysis, was observed in a dog, the owner was asked to collect additional free catch urine samples from their dog after 1 and 2 months to assess the persistence of proteinuria. The owners were provided with a collection container (Dog-i-noir, Great Premiums Europe B.V.) to facilitate sampling. Owners were asked to bring the urine sample within 24 h to the clinic and to store the sample cooled (4°C–8°C) until transport. Repeated urinalysis was performed, except for bacterial culture. Dogs were classified based on UPC as non-proteinuric (NP; UPC < 0.2), BP (persistent UPC 0.2–< 0.5) or OP (persistent UPC ≥ 0.5). The term proteinuria/proteinuric will be used from this point to refer to dogs with UPC ≥ 0.2.

Dogs that showed USG ≤ 1.020, persistent proteinuria, or renal azotemia (sCr ≥ 1.8 mg/dL [≥ 159 μmol/L] or SDMA ≥ 16 μg/dL [10] in combination with USG < 1.030) were subsequently monitored every 6 months. Remaining dogs were examined every 12 months. Dogs with persistent, severe (UPC ≥ 2.0) renal proteinuria were offered additional testing before initiating treatment (Supporting Information S6).

Persistent renal OP based on ≥ 2 urine samples, without azotemia or other apparent reason for OP on physical, blood, and urinalysis, was defined as non-azotemic CKD [29]. Azotemic CKD was defined as sCr ≥ 1.8 mg/dL (≥ 159 μmol/L) or SDMA ≥ 18 μg/dL combined with USG < 1.030 [29].

Owners of dogs alive at the end of the study period were contacted in March 2024. In case of death, the date and cause of

death as reported by the owner were noted. In case of life, it was asked whether the dog developed azotemic CKD (blood- and urinalysis were requested).

2.2 | Biomarkers

At each visit, one serum aliquot was transported cooled (4°C) overnight for SDMA measurement (IDEXX BioAnalytics, Vet Med Labor GmBH, Kornwestheim, Germany). Frozen serum samples were transported at −20°C in four batches for sCysC measurement using a validated particle-enhanced nephelometric immuno-assay (PENIA) (Behring Nephelometer Prospec; Siemens Healthcare Diagnostics, Marburg, Germany) [23]. Aliquots for the other markers were defrosted on-site at room temperature before analysis. Urinary ALB was determined with a previously validated dog ELISA (Immunology Consultants Laboratory, Newberg, USA) [14]. Urinary RBP was determined with a human ELISA using a reconstituted dog RBP calibrator. Because the use of the dog calibrator was novel, an in-house validation was performed and appeared satisfactory (Table S1) (Immunology Consultants Laboratory, Newberg, USA). The results of urinary biomarkers are expressed as a ratio to the urinary creatinine concentration (cr).

Plasma exogenous creatinine clearance time (PECCT) test was performed annually in a subgroup of dogs with persistent proteinuria and in healthy appearing controls without proteinuria, using a previously reported protocol [24].

2.3 | Age-Specific Reference Interval

An age-specific 95% RI, including the 90% confidence interval (CI) for the reference limits, for sCr, sCysC, uALBcr, and uRBPcr was obtained using a previously reported protocol [24, 25]. A nonparametric method was used if the sample size was ≥ 120; if the sample size was ≥ 40 but < 120, a robust method was used [25]. For sCr and sCysC, a two-sided 95% RI was calculated; for uALBcr and uRBPcr, a right-sided 95% RI was determined. For sCr and sCysC, dogs with azotemic CKD were excluded. For uALBcr and uRBPcr, dogs with azotemic CKD, persistent OP, or active urinary sediment were excluded.

2.4 | Statistical Analysis

Statistical analysis was performed using the statistical software package R (R module of SAS version 9.3, SAS Institute, NC, USA). The normal distribution assumption for the biomarkers was not rejected based on the Shapiro–Wilks test. Mixed models were used to assess changes over time of the biomarkers. The predictive value of the biomarkers assessed at baseline to onset of azotemic CKD or death (of all cause) was assessed using the Cox proportional hazards model to cope with right censoring. Age groups (senior versus geriatric) and UPC groups at baseline (NP, BP, and OP) were compared for onset of azotemic CKD or death (of all cause) using the log-rank test, and the results were visualized by Kaplan–Meier plots. Spearman rank correlation coefficients were calculated at baseline for all assessed variables. The level of significance was set at 5%.

3 | Results

3.1 | Study Cohort

At baseline, 122 healthy appearing older dogs were included and their descriptive data are shown in Table 1. Sixty-seven and 55 of these dogs were considered senior and geriatric, respectively [26]. The study group comprised 14 mixed breed dogs, 10 Border Collies, nine Belgian Shepherds, eight Golden Retrievers, six Chihuahua's, six Labrador Retrievers, six Dachshunds, five Shetland Sheepdogs, five Jack Russell Terriers, four Cavalier King Charles Spaniels, four Shih Tzu's, four English Cocker Spaniels, four Rottweilers, and ≤ 3 dogs of 28 other breeds.

At baseline, none of the dogs showed renal azotemia based on sCr, but 4% (5/121) showed renal azotemia based on SDMA (median value 16 [range 16–17] µg/dL). Two of these five dogs showed renal azotemia based on SDMA at least once again during follow-up; however, not consistently over time. Both dogs developed azotemic CKD at the end of the study (743 and 748 days, respectively). In the remaining three dogs, SDMA normalized.

Fifteen (/122, 12%) (nine senior and six geriatric) dogs showed persistent OP at baseline. Of these dogs, three showed severe dental disease, but OP persisted after dental treatment. No other apparent causes for the observed OP were found based on physical, blood-, and urinalysis, and therefore the criteria for non-azotemic CKD was met. Ten (/15, 67%) and three (/15, 20%) showed a persistent UPC > 1.0 or ≥ 2.0, respectively. The three dogs with severe OP (Table S2) underwent additional work-up within 6 months: this revealed an anal gland sac adenocarcinoma WHO Stage 3a without the presence of hypercalcemia, for which radiation therapy and toceranib phosphate were initiated in one dog. In the two remaining dogs, no obvious extrarenal cause for the severe OP was found. Two of the non-azotemic CKD dogs at baseline developed azotemic CKD after 490 and 821 days of follow-up, respectively.

Another 17/122 dogs (14%, eight senior and nine geriatric) showed persistent BP at baseline, of which two developed azotemic CKD (at 374 and 743 days, respectively) during the study; one extra dog developed azotemic CKD after the end of the study (1545 days).

Glomerular filtration rate measurements were available in 18 dogs at baseline, respectively in eight NP, four BP, and six OP dogs, all within previously reported weight-based RIs (H. P. Lefebvre, E. Jeunesse, D. Concordet, et al., "Assessment of Glomerular Filtration Rate Using Plasma Exogenous Creatinine Clearance Test: Preliminary Results in a Healthy Canine Population [Abstract]," *Journal of Veterinary Internal* Medicine, (2004): 415). Of 5/18 dogs, only a baseline GFR value was obtained.

3.2 | Follow-up

Follow-up was available in 106 (T12) and 92 (T24) dogs (Table 1). The main reason for loss to follow-up was death of

TABLE 1 | Descriptive data (mean, standard deviation) of 122 older dogs included in the study. Data are presented at baseline (T0) and during follow-up.

Variable	T0	T12	T24
Number	122	106	92
Age (SD) (years)	9.7 (±2.2)	10.6 (±2.2)	11.1 (±2.0)
Weight (SD) (kg)	18.5 (±1.2)	18.7 (±1.2)	18.3 (±1.2)
Sex (*n*) M/MN/F/FN	21/30/28/43	21/22/25/38	16/23/14/39
BCS			
Optimal (*n*) (4–6/9)	107 (88%)	90 (85%)	81 (88%)
Below optimum (*n*) (1–3/9)	2 (2%)	3 (3%)	2 (2%)
Above optimum (*n*) (7–9/9)	13 (10%)	13 (12%)	9 (10%)
MCS			
Normal (*n*) (1/4)	116 (95%)	91 (86%)	70 (77%)
Decreased (*n*) (2–4/4)	6 (5%)	15 (14%)	21 (23%)
SBP (SD) (mmHg)	148.9 (±2.4)	155.8 (±2.7)	156.1 (±2.8)
≥160 mmHg (*n*)	*n* = 31/118 (28%)	*n* = 37/93 (40%)	*n* = 33/84 (39%)
≥180 mmHg (*n*)	*n* = 9/118 (8%)	*n* = 16/93 (17%)	*n* = 15/84 (18%)
sCr (SD) (mg/dL)	1.02 (±0.03)	1.04 (±0.03)	1.02 (±0.03)
USG (SD)	1.033 (±0.001)	1.032 (±0.001)	1.034 (±0.001)
UPC (SD)	0.3 (±0.1)	0.5 (±0.1)	0.4 (±0.1)
NP	74 (61%)	65 (61%)	50 (54%)
BP	31 (25%)	22 (21%)	23 (25%)
OP	17 (14%)	19 (18%)	19 (21%)
SDMA (SD) (μg/dL)	11.2 (±0.4)[a]	12.1 (±0.4)[ab]	13.1 (±0.4)[b]
sCysC (SD) (mg/L)	0.28 (±0.01)[a]	0.31 (±0.01)[b]	0.29 (±0.01)[ab]
uALBcr (SD) (mg/g)	243.68 (±199.92)	829.02 (±217.52)	248.14 (±247.45)
uRBPcr (SD) (mg/g)	0.54 (±0.42)	1.24 (±0.45)	0.40 (±0.50)

Note: Statistical differences over time are shown with letters in superscript. Time points having a common letter do not differ significantly from each other.
Abbreviations: BCS, WSAVA body condition score; BP, borderline proteinuria (UPC ≥ 0.2–< 0.5); F, female; FN, female neutered; M, male; MCS, WSAVA muscle condition score; MN, male neutered; N, number; NP, no proteinuria (UPC < 0.2); OP, overt proteinuria (UPC ≥ 0.5); SBP, systolic blood pressure (Doppler method); sCr, serum creatinine; sCysC, serum cystatin C; SD, standard deviation; SDMA, symmetric dimethylarginine; T, time point (months); uALBcr, urinary albumin:creatinine ratio; UPC, urinary protein:creatinine ratio; uRBPcr, urinary retinol-binding protein:creatinine ratio. USG, urine specific gravity.

variable cause (28/120; 23%), only two dogs dropped out early during the study. Data of dogs with 6-monthly follow-up are provided in Table S3.

In the large majority, that is, 13/15 dogs, with baseline persistent OP, this persisted during follow-up (Figure 1). One dog was lost to follow-up after baseline; in the remaining dog, OP evolved to BP. In 6/13 dogs, the OP progressed to persistent severe OP, and owners of five dogs agreed to additional work-up and treatment (Table S2). In the majority of these five dogs, further diagnostics did not reveal an obvious extrarenal cause for the proteinuria; one dog was pregnant but continued to show severe OP after littering and weaning. Two more dogs developed non-azotemic CKD by T12; 11 other dogs showed OP at a single time point during follow-up, four of which showed severe OP, but this could be largely explained by either a disease (pyometra, *n* = 1) or drug therapy (glucocorticosteroids, *n* = 2). Six dogs with persistent severe OP were started on anti-proteinuric treatment after a median time of 252 (range, 121–429) days. Median starting and final dose of benazepril was 0.4 (0.2–0.5) and 1 (0.4–1.6) mg/kg/day, respectively. In three dogs, the switch to telmisartan (1 mg/kg/day) was required due to inadequate response (i.e., < 50% reduction of UPC, *n* = 2) or gastrointestinal adverse effects (*n* = 1). None of the dogs obtained complete resolution of OP. Median follow-up time under treatment was 435 (30–554) days.

In 11 dogs, azotemic CKD developed by T12 (four) and T24 (seven) (median time 733 [range 357–821] days), respectively. So, over a 2-year period, in total, 26/122 (20%) dogs showed signs of CKD, in the majority related to persistent renal proteinuria (15/122). Of the four dogs that developed azotemic CKD by T12, three had a SDMA concentration at the upper limit with

adequately concentrated urine at baseline; in the other dog, SDMA progressively increased by T6. Of the seven dogs that developed azotemic CKD by T24, three showed a progressive increase of both sCr and SDMA within normal limits over time. After the end of the health screening study (median 1579 [1194–1623] days), four other dogs developed azotemic CKD. Of the dogs with persistent BP and OP at baseline, 3/17 and 2/15, respectively, developed azotemic CKD within a median time of 795 days (range 116–1545) days. Median time to death was 728 days (range 116–1576 days) and 705 days (234–1601 days) for dogs with persistent BP and OP at baseline, respectively. Neither UPC value nor UPC group at baseline was predictive for azotemic CKD or death (Tables 2 and 3, Figure 2). Overall, the most common cause of death was neoplasia (23/61, 38%). In five dogs (/61, 8%), progressive renal disease was the sole cause of death or euthanasia; in another two dogs, underlying CKD played a role in the decision toward euthanasia.

Throughout the study, 135/610 (22%) of SBP measurements were ≥160 mmHg (Table 1). In none of the dogs, fundoscopy revealed signs of ocular damage caused by hypertension. Thirty-one (31/118, 28%) dogs were hypertensive at baseline, which only persisted at each time point in 11/118 (9%). In one dog, severe (≥180 mmHg) persistent hypertension was noted. Of these 31 hypertensive dogs at baseline, six showed persistent OP. In 3/6 (50%), situational hypertension was confirmed; in the remaining dogs, this could not be demonstrated but was clinically suspected [30]. No treatment was started specific for hypertension in our cohort. One baseline hypertensive dog developed azotemic CKD after 426 days of follow-up. Survival analysis did not reveal a significant effect of SBP at baseline on the development of azotemic CKD or death (Table 3).

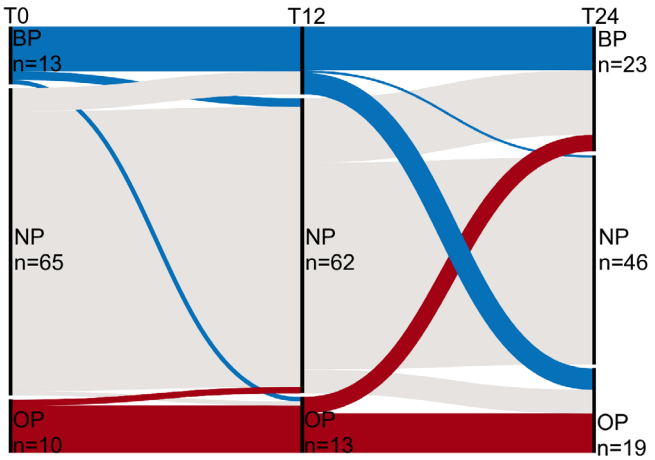

FIGURE 1 | Alluvial diagram to visualize the general flow of dogs between UPC groups (represented as vertical nodes) over time (baseline, 12 and 24 months). The thickness of each flow-line is determined by its value. Dogs were assigned to the BP or OP group based on a persistent UPC value between 0.2 and 0.5 or ≥0.5, respectively. Only dogs ($n=88$) of which UPC was assessed at each time point are included in this figure. BP, borderline proteinuria; *n*, number; NP, no proteinuria; OP, overt proteinuria.

3.3 | Biomarkers: Correlations, Trends Over Time, and Prediction of Azotemic CKD and Death

Results of various biomarkers assessed throughout the study are provided in Tables 1 and S3. Significant biomarkers correlations found at baseline are shown in Table S4.

A significant ($p<0.001$) increase was observed for sCysC of 0.031 mg/L (standard error, SE = 0.008) at T12, and for SDMA of 1.881 μg/dL (SE = 0.452) at T24 compared with baseline, respectively. No other significant changes over time were observed (Table 1). The evolution of renal biomarkers over time for the 14 dogs that developed azotemic CKD after inclusion is shown graphically in Figure 3. In five of these dogs, repeated PECCT measurements were available, revealing a decreasing trend over time (Figure 4). Although functional renal biomarkers (sCr, sCysC and SDMA) showed overall an increasing trend, this was less consistent; for the urinary renal biomarkers, no clear change could be observed.

Tables 2 and 3 provide results of survival analysis for the development of azotemic CKD as well as for death, for various (renal) biomarkers. Age was not significantly associated with the development of azotemic CKD, but it was with death. For all three functional renal biomarkers (sCr, SDMA, sCysC) assessed at baseline, a significant association with the development of azotemic CKD was observed (Table 3). Moreover, sCysC at baseline was predictive for death. Although the baseline USG value was not predictive for azotemic CKD, it was associated with death. For the remaining urinary biomarkers, only baseline uRBP was associated with both azotemic CKD and death. Of note, the rate for the development of azotemic CKD increased almost six-fold for each unit increase in MCS.

3.4 | Age-Specific Reference Interval

Obtained age-specific 95% RIs for sCr, sCysC, uALBcr, and uRBPcr are shown in Table 4. Seventy-six (/122, 62%) dogs

TABLE 2 | Time-to-event analysis for the development of azotemic CKD or death in 122 older, healthy appearing dogs using categorical (renal) biomarkers measured at baseline.

Covariate	Comparison	CKD HR (95% CI)	*p*	Death HR (95% CI)	*p*
UPC group	NP BP	2.0 (0.5–7.8)	0.5	1.4 (0.7–2.8)	0.4
	NP OP	1.4 (0.3–6.6)		1.5 (0.7–3.3)	
Age group	Senior geriatric	2.2 (0.75–6.27)	0.2	3.8 (2.18–6.68)	<0.001

Abbreviations: BP, borderline proteinuric; CI, 95% confidence interval; CKD, chronic kidney disease; HR, hazard ratio; NP, non-proteinuric; OP, overt proteinuric; UPC, urinary protein:creatinine ratio.

TABLE 3 | Time-to-event analysis for the development of azotemic CKD or death in 122 older, healthy appearing dogs using various continuous (renal) biomarkers measured at baseline.

Covariate	Azotemic CKD HR (CI); *p*	Death HR (CI): *p*
BCS	0.7 (0.4–1.4); $p=0.4$	1.0 (0.8–1.3); $p=0.8$
MCS	5.9 (1.4–24.7); $p=0.004$*	2.0 (0.8–5.0); $p=0.1$
SBP (mmHg)	0.98 (0.96–1.01); $p=0.3$	1.0 (0.99–1.02); $p=0.6$
sCr (mg/dL)	1.04 (1.01–1.06); $p=0.003$*	0.99 (0.98–1.01), $p=0.8$
SDMA (μg/dL)	1.58 (1.26–1.99); $p<0.001$*	1.04 (0.95–1.14); $p=0.4$
sCysC (dg/L)	1.14 (1.06–1.22); $p<0.001$*	1.07 (1.03–1.11); $p<0.001$*
USG	1.00 (0.96–1.04); $p=0.9$	0.97 (0.95–0.99); $p=0.003$*
UPC	1.7 (0.7–4.3); $p=0.3$	1.5 (1.0–2.4); $p=0.06$
uALBcr (mg/g)	1.00 (0.99–1.00); $p=0.5$	1.00 (0.99–1.00); $p=0.5$
uRBPcr (mg/g)	1.40 (1.12–1.75); $p<0.01$*	1.18 (1.03–1.35); $p=0.01$*

Note: Serum cystatin C concentrations were converted to dg/L to allow straightforward interpretation. For each 0.01 unit increase in sCysC, the relative risk for development of azotemic CKD and death was 1.14 and 1.07, respectively.
Abbreviations: BCS, WSAVA body condition score; CI, 95% confidence interval; CKD, chronic kidney disease; HR, hazard ratio; MCS, WSAVA muscle condition score; SBP, systolic blood pressure; sCr, serum creatinine; sCysC, serum cystatin C; SDMA, serum dimethylarginine; uALBcr, urinary albumin:creatinine ratio; UPC, urinary protein:creatinine ratio; uRBPcr, urinary retinol-binding protein:creatinine ratio; USG, urine specific gravity.
*Significant result ($p<0.05$).

did not show active urinary sediment or persistent BP/OP and were included to establish the RIs for both urinary biomarkers. Histograms to illustrate the distribution of sCr, sCysC, uALBcr, and uRBPcr are shown in Figures S1–S4.

4 | Discussion

Based on the data from this prospective and longitudinal study, we here show that older dogs are prone to the development of non-azotemic CKD, emphasizing the importance of health screening protocols, including urinalysis. Functional renal biomarkers have the best predictive value for the onset of azotemic CKD, next to uRBPcr and MCS, being unexpected yet not investigated in older dogs. Proteinuria is not associated with the development of azotemic CKD or death in older dogs, remaining to be confirmed in other cohorts. Age-specific RIs are of importance to correctly interpret data obtained from health screening.

At baseline, 12% of healthy appearing older dogs showed non-azotemic CKD and 9% developed azotemic CKD within 2 years. Both were unexpectedly high because CKD is considered a rare condition in dogs, with prevalences ranging from 0.5%–1.5% [31], 0.4% [32] up to 3.7% [33]. These three studies focus on azotemic CKD; however, not on old dogs, but all mention an increasing prevalence with aging [31–33]. Our findings for azotemic CKD are in line with one report that up to 10% of dogs > 15 years of age will develop CKD [34]. It supports the importance of regular health screenings in older dogs, as dogs diagnosed with non-azotemic CKD at baseline appeared healthy to their owners. Screening for early renal disease should include both urinalysis and serum functional biomarkers. Indeed, when neglecting urine, a substantial part of older dogs with early CKD is missed, preventing timely preventive measures from being taken. Of the 11 dogs diagnosed with azotemic CKD, seven had a sCr < 1.8 mg/dL and two dogs had a SDMA < 18 μg/dL; only in 2/11 dogs were both biomarkers concordant at the time of diagnosis. The percentage of dogs with increased SDMA before an increase in sCr when developing azotemic CKD was observed to be 60%, which is lower compared with the almost 90% reported in one study [35]. This apparent contradiction can at least partly be related to a different cut-off value used for SDMA in both studies, that is, 18 μg/dL compared to 14 μg/dL, as well as the requirement of suboptimally concentrated urine to obtain a diagnosis of azotemic CKD [29]. Also, a different cut-off value was used for sCr (i.e., 1.8 mg/dL compared with 1.4 mg/dL) in our study. When using a cut-off of 1.4 mg/dL, in one dog sCr would have been increased before SDMA; in one other dog, both markers would have been increased concordantly instead of sCr after SDMA. It is recognized that functional biomarkers should be interpreted as adjunct markers of each other for interpretation of GFR [10]. We here confirmed this, as all three functional biomarkers (sCr, SDMA, and sCysC) correlated well with each other and showed a good predictive value for the onset of azotemic CKD, with sCysC and SDMA showing a higher relative risk per unit increase compared with sCr.

A predictive value could not be shown for the selected urinary renal biomarkers with the exception of uRBPcr. This observation is unexpected, as urinary biomarkers are considered to detect early onset of renal injury or disease [12, 17, 19]. A potential limitation of urinary biomarkers is the lack of renal specificity, as shown for urinary albumin [12, 17]. In contrast, uRBP is considered a specific tubular damage marker, which could explain the predictive value found [19]. Urinary RBP is an indicator of proximal tubular injury (or stress) in dogs with early onset of CKD, even before the development of azotemia [18]. A predominance of tubular proteins was observed in CKD Stage 1 urine, which was significantly higher compared to glomerular proteins ($p<0.05$) [18]. Based on previous and our findings, uRBPcr combined with functional biomarkers is preferred over uALBcr as a health screening tool to identify which dogs will develop azotemic CKD. A limitation is that uRBP assays are currently not routinely available in clinical practice.

In our study cohort, three biomarkers (i.e., two functional: USG, sCysC and one urinary: uRBPcr) were significantly associated with death. For each unit increase in USG, the death rate decreased by 3%. In dogs with primary glomerulopathy and Cushing's syndrome, a low USG has also been associated with an increased risk to death [36]. Although the clinical relevance

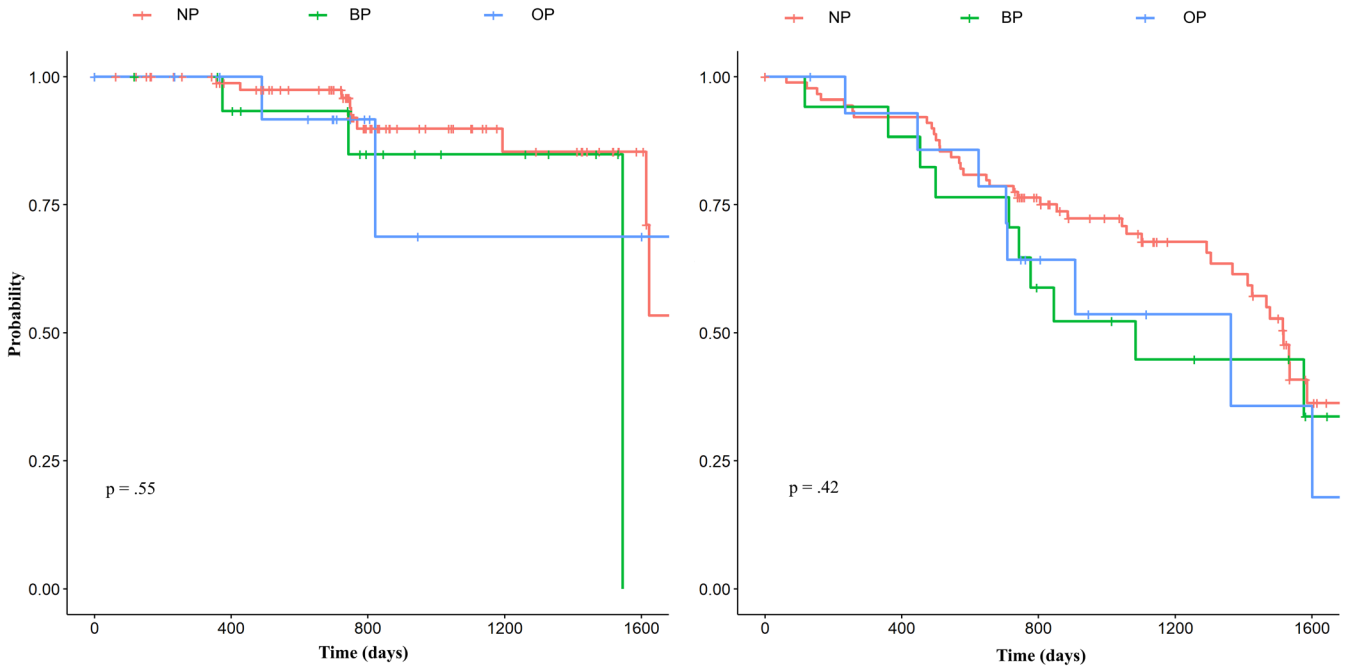

FIGURE 2 | Kaplan–Meier plots for development of azotemic chronic kidney disease (left, log-rank $p = 0.55$) and death (right, log-rank $p = 0.42$) in 122 healthy appearing, older dogs, with stratification according to urinary protein: Creatinine ratio (UPC) group at baseline: NP (non-proteinuric [UPC < 0.2, red]) versus BP (borderline proteinuric [UPC 0.2–< 0.5, green]) versus OP (overt proteinuric [UPC ≥ 0.5, blue]). Time expressed in days.

is probably low, our study also shows an association between USG and death in apparently healthy aging dogs. Serum CysC is associated with an increased death rate in elderly men [37]. The association between sCysC and cardiovascular or renal disease-related death is shown in dogs [38, 39]. The finding in apparently healthy older dogs is new. Although uRBP is an independent predictor for renal outcomes in people with a variety of renal-related disorders, it has not been directly associated with death [40–42]. As 11% (7/61) of deaths in our cohort were renal related, this could have contributed to the observed association. In dogs, the predictive value of uRBPcr on death was not previously shown, and we report this in the context of apparently healthy aging dogs.

Longitudinal follow-up of variables associated with renal health did not reveal obvious variations over time, making annual follow-up a justified interval. While the significant minimal difference found for SDMA and sCysC is in line with previously reported within-subject variation over time (16% and 8%, respectively) [43, 44], the absence of significant variation over time for uRBPcr is in contrast with a report where up to 33% variability was observed [44]. Similar to our study, batch analysis was performed, making inter-assay variability an unlikely explanation for the observed differences.

Despite the finding of a high incidence of non-azotemic CKD, BP or OP at baseline were not associated with the development of either azotemic CKD or death. The limited number ($n = 15$) of dogs developing azotemic CKD could have affected our results. In azotemic dogs, an UPC > 1.0 is associated with survival [45]. However, in non-azotemic dogs, only a much higher UPC (4.1) is associated with renal outcome [46]. In our study cohort, only a minority showed such severe OP. Also, anti-proteinuric measures were started in dogs with severe OP. It is possible that if we had not treated this latter group, a predictive value could have been demonstrated, as there is a survival benefit to treatment for renal proteinuria [47]. It was deemed unethical not to treat these dogs.

At baseline, hypertension was observed in 25% of dogs, being lower than the > 50% prevalence in a previous health screening study, in a similar geographic area and using the same methodology [3]. Despite precautions taken, situational hypertension was present in some of our hypertensive dogs [30]. At-home SBP measurements were, however, not feasible in all hypertensive dogs for each time point. Only in < 10% persistent hypertension was present, and no association between SBP at baseline and the development of CKD was observed. Our findings, therefore, suggest that situational hypertension is prevalent in dogs. Therefore, we propose more frequent SBP measurements in healthy dogs, even at a younger age, to habituate dogs to this procedure.

In people, early to overt CKD is associated with a four-fold higher odds of having sarcopenia compared with a similar age group [48]. Sarcopenia is a syndrome characterized by a progressive decline in skeletal muscle mass, muscle strength, and physical performance [48]. Loss of muscle mass occurs in both human and veterinary patients with CKD, attributable to accelerated protein catabolism and decreased energy and protein intake [48–51]. Beyond neoplasia and congestive heart failure, CKD and aging are the most common causes of muscle and fat loss in dogs [50]. Sarcopenia, characterized by decreased MCS, is associated with decreased quality of life and increased death rate in people, dogs, and cats [48–51]. In our study, a significant high relative risk of 5.9 was found for MCS at baseline on the development of azotemic CKD, suggesting that muscle wasting is present before the presence of renal azotemia. This probably

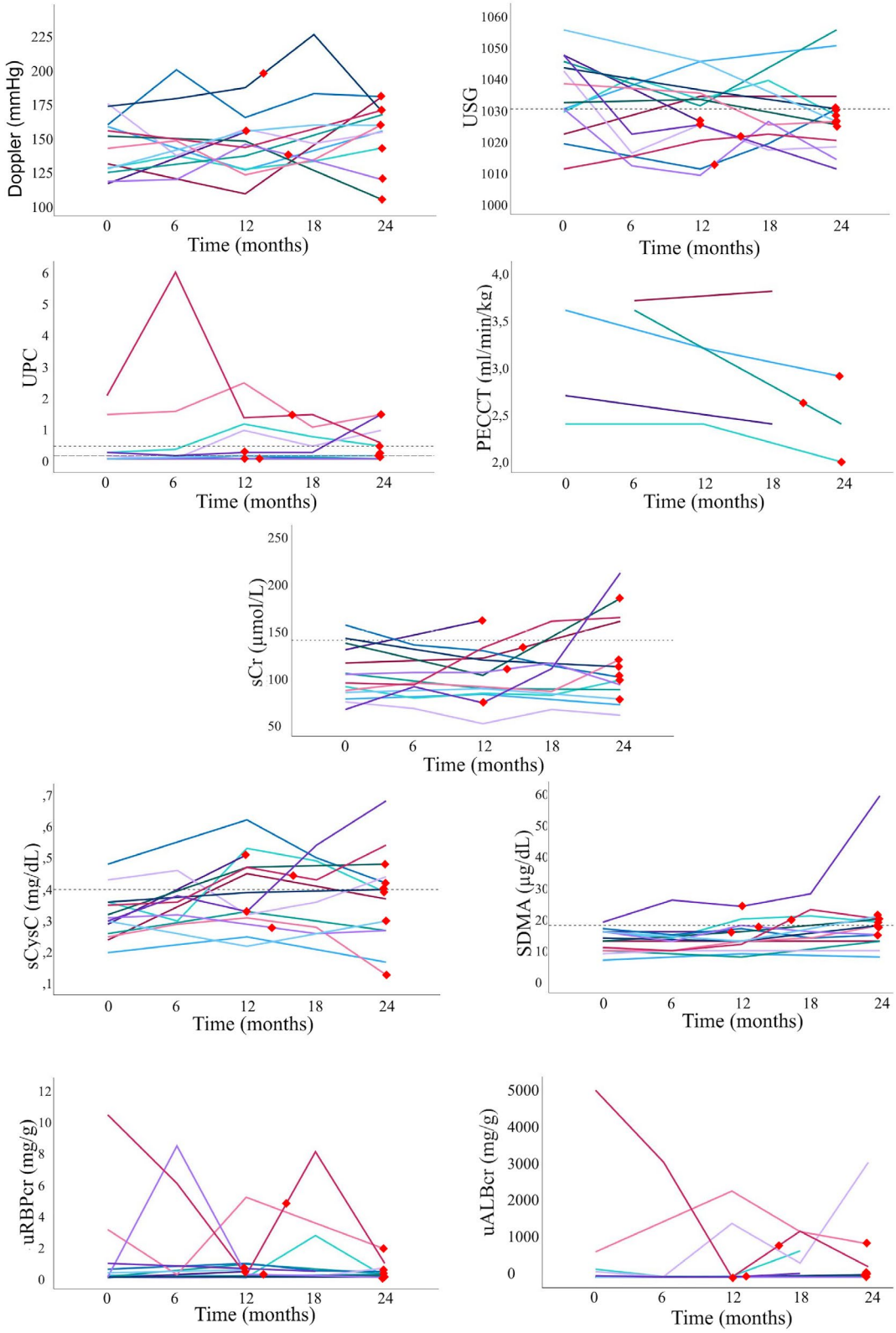

FIGURE 3 | Graphic representation, using line graphs, of the evolution of assessed renal biomarkers (SBP, USG, UPC, PECCT measurements, sCr, SDMA, sCysC, uALBcr, and uRBPcr, respectively) over time for 14 dogs that developed azotemic chronic kidney disease (CKD) during or after 2 year follow-up. Each dog was assigned a color. Dotted lines indicate the used cut-off values for time-to-event analysis. Red diamonds indicate the time of onset of azotemic CKD if reached by time point 24 months. PECCT, glomerular filtration rate value; sCr, serum creatinine; sCysC, serum cystatin C; SDMA, symmetric dimethyl arginine; uALBcr, urinary albumin:creatinine ratio; UPC, urine protein:creatinine ratio; uRBPcr, urinary retinol-binding protein:creatinine ratio; USG, urine specific gravity.

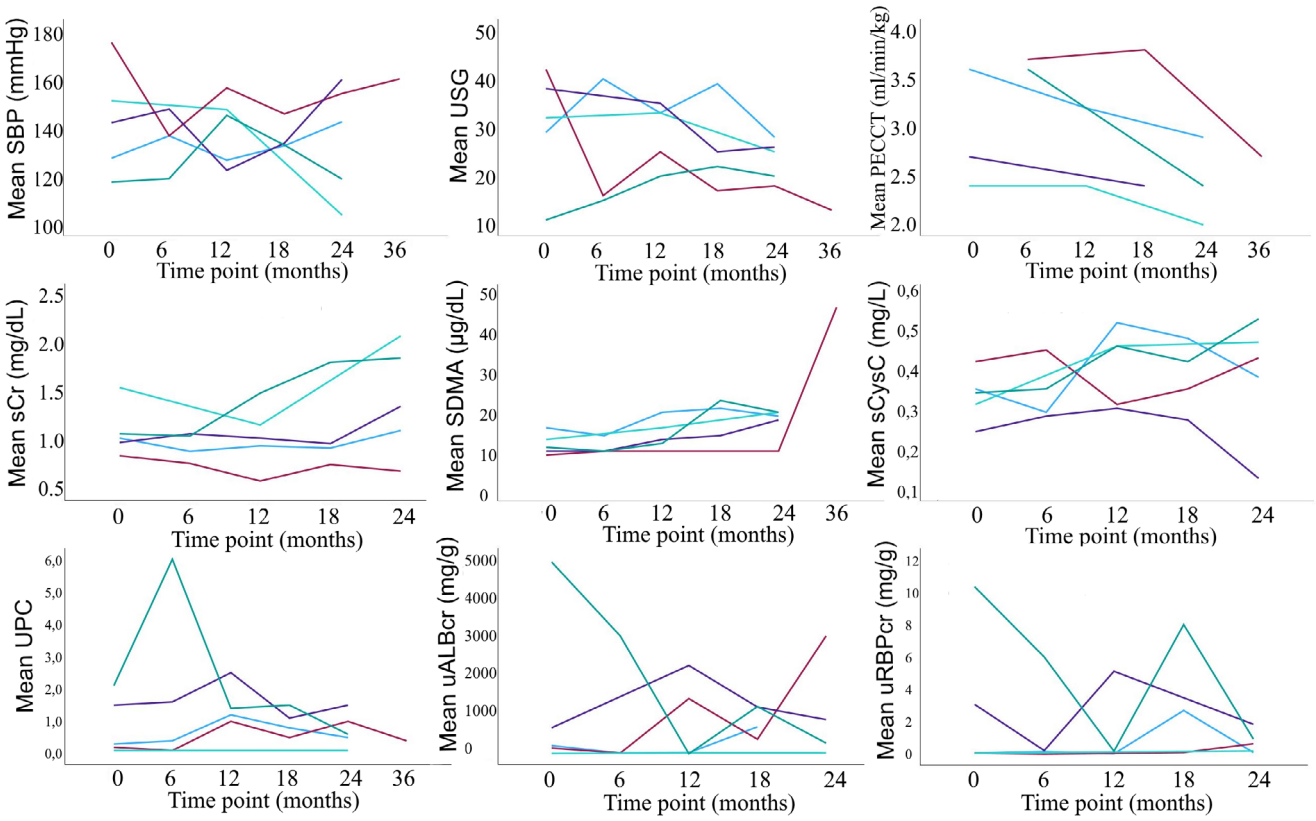

FIGURE 4 | Graphic representation, using line graphs, of evolution of assessed renal biomarkers (SBP, USG, PECCT measurements, sCr, SDMA, sCysC, UPC, uALBcr, and uRBPcr) over time for five dogs that developed azotemic CKD during or after 2 year follow-up in which PECCT measurements were available. Each dog was assigned a color. PECCT, glomerular filtration rate value; SBP, systolic blood pressure; sCr, serum creatinine; sCysC, serum cystatin C; SDMA, symmetric dimethyl arginine; UPC, urine protein:creatinine ratio; USG, urine specific gravity; uALBcr, urinary albumin:creatinine ratio; uRBPcr, urinary retinol-binding protein:creatinine ratio.

TABLE 4 | Age-specific RIs including 90% CIs for the LL and UL for sCr, sCysC, uALBcr, and uRBPcr in a population of healthy appearing older dogs ($N = 121$).

Biomarker	Mean (±SD)	RI LL (90% CI)	RI UL (90% CI)
sCr (mg/dL) $N = 122$	1.02 (±0.26)	0.52 (0.46–0.60)	1.60 (1.47–1.93)
< 20 kg, $N = 70$	0.96 (±0.28)[a]	0.38 (0.26–0.50)	1.49 (1.37–1.60)
≥ 20 kg, $N = 52$	1.10 (±0.21)[b]	0.66 (0.58–0.74)	1.53 (1.46–1.61)
sCysc (mg/L) $N = 121$	0.27 (±0.06)	0.17 (0.16–0.18)	0.43 (0.36–0.48)
< 20 kg, $N = 69$	0.27 (±0.06)[a]	N/A	N/A
≥ 20 kg, $N = 52$	0.28 (±0.06)[a]	N/A	N/A
uALBcr (mg/g) $N = 76$	26.54 (±49.20)	N/A	89.03 (45.46–126.42)
uRBPcr (mg/g) $N = 72$	0.27 (±0.54)	N/A	1.07 (0.54–1.49)

Note: Statistical differences between weight groups are shown with letters in superscript: Weight groups having a common letter do not differ significantly from each other. For sCr, weight-based (< 20 or ≥ 20 kg) age-specific RIs were obtained as a significant difference ($p < 0.05$) was found between both groups. This was not the case for sCysC measurements; therefore, only a global RI was calculated.
Abbreviations: CIs, confidence intervals; LL, lower limit; N, number; N/A, not available; RI, reference interval; sCr, serum creatinine; sCysC, serum cystatin C; SD, standard deviation; UALBcr, urinary albumin:creatinine ratio; UL, upper limit; uRBPcr, urinary retinol-binding protein:creatinine ratio.

reflects largely the current inability to diagnose CKD in dogs in an early phase of the disease. The fact that no correlation was found between age and MCS and that age group was not associated with the development of azotemic CKD leads us to conclude that this is not just an age-related change. Our finding highlights the importance of a complete nutritional assessment, including body weight, BCS, and MCS, as part of a health screening protocol in aging dogs, especially when screening for renal health

[49, 51]. It additionally shows that detection of muscle atrophy in elderly dogs is a clear indication to perform laboratory screening for CKD.

Several correlations were found among assessed renal biomarkers. Age was moderately correlated with sCysC and SDMA, but not with sCr. Age might therefore affect SDMA and sCysC, emphasizing the importance of age-specific RIs for both biomarkers. This is available for SDMA [24] and the current study provides RI for sCysC measured through PENIA. Large breed dogs (≥20 kg) have a significantly higher sCysC (mean 0.34 ± 0.05) compared with small breed dogs (<20 kg) (mean 0.27 ± 0.07), suggesting body weight might affect sCysC, in contrast to humans and cats [37]. This is supported by the finding that sCysC is superior to sCr and a better prognostic renal marker in small breed dogs (<15–20 kg) [52], but inferior to sCr in a group of larger-sized dogs (median 20 kg) [10]. In the present study, no correlation was found between body weight and sCysC. When comparing mean values between dogs <20 kg versus ≥20 kg, no significant difference in sCysC values could be observed. Therefore, only a global RI of the complete study cohort was calculated. Differences in study design could have contributed to this apparently contradictory result. In our study, BCS and MCS were not taken into account, although they might influence sCysC measurements [53]. However, the vast majority had an optimal BCS and MCS (almost 90% and 95%, respectively) and no significant correlation was found between sCysC and both variables. As sCr between dogs <20 kg versus ≥20 kg significantly differed, this suggests that a weight-based age-specific RI is most suitable for this biomarker.

The moderate correlation found between body weight and uALBcr could be suggestive of a size effect. Obesity can affect urinary biomarkers; however, the size effect was not mentioned before [53]. In our study, neither BCS nor MCS were correlated with any of the urinary renal biomarkers. The high percentages of dogs with optimal BCS and MCS could have influenced our findings. In contrast to a recently published study, uALBcr was not correlated with any of the functional biomarkers, neither with SBP nor age [17]. This difference might be explained by a different definition of early renal disease (US findings vs. urinalysis), the low % of persistent hypertension in our study, different methodology, as well as the fact that the age effect found in the previous study might be rather a group effect, given that their control dogs and dogs with nonsystemic disease were considerably younger than their CKD and chronic systemically ill dogs [17]. However, the obtained upper RI limit for uALBcr in our study (median 62, [range 39–85] mg/g) is considerably higher compared with a reported (median 0–19, [range 13–28] mg/g) RI in a younger group of dogs (i.e., median age 3.6 years) [54]. An older study using the same methodology as the current study also found a, albeit not significantly, higher uALBcr in 10 healthy older dogs (median 17.8 [range 3.3–296.5] mg/g) compared with 10 healthy young dogs (median 4.7 [range, 1.5–46.3] mg/g) [14]. Despite different study designs and methodology, overall these findings indicate an age effect on uALBcr, emphasizing the need for age-specific RI and potentially also for a weight-specific RI [54].

This study has some limitations. Our cohort included healthy appearing older dogs which were allowed medicinal/surgical treatment during follow-up. Despite their healthy appearances, underlying diseases were diagnosed and treated, including severe dental disease, neoplasia, or urolithiasis, possibly contributing to proteinuria or CKD. However, as only persistent increased UPC values were included in the BP or OP group at baseline, we could reevaluate the dogs after appropriate treatment. Moreover, this limitation is inherent to the study design and the study group. As we depended on owners' ability to assess health in their dogs, it cannot be excluded that early signs of CKD, for example, polyuria/-dipsia, remained undetected. Assessment of GFR and medical imaging was not routinely done for each dog, as this was practically unfeasible. As a result, some cases with CKD IRIS Stage 1 might have remained unnoticed. However, strict diagnostic laboratory criteria for CKD were applied, and dogs considered at risk for CKD were monitored more closely.

In conclusion, this prospective study shows that older, apparently healthy dogs are prone to the development of non-azotemic or azotemic CKD, emphasizing the importance of health screening protocols, including urinalysis. Muscle atrophy might be an early indicator of CKD in older dogs. We propose uRBPcr combined with functional markers to be preferred over uALBcr as a screening tool to identify which apparently healthy older dogs will develop azotemic CKD.

Disclosure

Authors declare no off-label use of antimicrobials.

Ethics Statement

The owners signed an informed consent, and the study was approved by the local and national ethical committee (EC 2019/39; DWZ/EV/19/115/75). Authors declare human ethics approval was not needed.

Conflicts of Interest

The authors declare no conflicts of interest.

References

1. F. L. Metzger, "Senior and Geriatric Care Programs for Veterinarians," *Veterinary Clinics of North America: Small Animal Practice* 35 (2005): 743–753.

2. Banfield Pet Hospital, "Global Pet Health Report 2022," 2022, https://www.healthforanimals.org/wp-content/uploads/2022/04/Global-State-of-Pet-Health-Draft.

3. A. Willems, D. Paepe, S. Marynissen, et al., "Results of Screening of Apparently Healthy Senior and Geriatric Dogs," *Journal of Veterinary Internal Medicine* 31 (2017): 81–92.

4. D. Paepe, G. Verjans, L. Duchateau, K. Piron, L. Ghys, and S. Daminet, "Routine Health Screening Findings in Apparently Healthy Middle-Aged and Old Cats," *Journal of Feline Medicine and Surgery* 15 (2013): 8–19.

5. D. Paepe and S. Daminet, "Feline CKD: Diagnosis, Staging and Screening—What Is Recommended?," *Journal of Feline Medicine and Surgery* 15 (2013): 15–27.

6. G. Lees, "Early Diagnosis of Renal Diseases and Renal Failure," *Veterinary Clinics of North America: Small Animal Practice* 34 (2004): 867–885.

7. J. Hall, M. Yerramilli, E. Obare, et al., "Nutritional Interventions That Slow the Age-Associated Decline in Renal Function in a Canine

Geriatric Model for Elderly Humans," *Journal of Nutrition, Health & Aging* 20 (2016): 1010–1023.

8. F. L. Metzger and A. H. Rebar, "Clinical Pathology Interpretation in Geriatric Veterinary Patients," *Veterinary Clinics of North America: Small Animal Practice* 42 (2012): 615–629.

9. R. Dhaliwal, E. Boynton, S. Carrera-Justiz, et al., "2023 AAHA Senior Care Guidelines for Dogs and Cats," *Journal of the American Animal Hospital Association* 59 (2023): 1–21.

10. L. Pelander, J. Häggström, A. Larsson, et al., "Comparison of the Diagnostic Value of Symmetric Dimethylarginine, Cystatin C, and Creatinine for Detection of Decreased Glomerular Filtration Rate in Dogs," *Journal of Veterinary Internal Medicine* 33 (2019): 630–639.

11. S. J. J. Marynissen, P. M. Y. Smets, L. F. E. Ghys, et al., "Longterm Follow-Up of Renal Function Assessing Serum Cystatin C in Dogs With Diabetes Mellitus or Hyperadrenocorticism," *Veterinary Clinical Pathology* 45 (2016): 1–10.

12. T. Kongtasai, D. Paepe, E. Meyer, et al., "Renal Biomarkers in Cats: A Review of the Current Status in Chronic Kidney Disease," *Journal of Veterinary Internal Medicine* 3 (2022): 379–396.

13. R. G. Price, "Early Markers of Nephrotoxicity," *Comparative Clinical Pathology* 11 (2002): 2–7.

14. P. M. Smets, E. Meyer, B. E. Maddens, et al., "Urinary Markers in Healthy Young and Aged Dogs and Dogs With Chronic Kidney Disease," *Journal of Veterinary Internal Medicine* 24, no. 1 (2010): 65–72.

15. G. D'Amico and C. Bazzi, "Pathophysiology of Proteinuria," *Kidney International* 63 (2003): 809–825.

16. A. Bacic, M. M. Kogika, K. C. Barbaro, C. S. Iuamoto, D. M. N. Simões, and M. L. Santoro, "Evaluation of Albuminuria and Its Relationship With Blood Pressure in Dogs With Chronic Kidney Disease," *Veterinary Clinical Pathology* 39 (2010): 203–209.

17. K. Paukner, Z. Filipejova, J. Mares, et al., "A Comprehensive Analysis of Albuminuria in Canine Chronic Kidney Disease," *Veterinary Medicine and Science* 10 (2024): e1403.

18. F. Chacar, M. Kogika, T. R. Sanches, et al., "Urinary Tamm-Horsfall Protein, Albumin, Vitamin D-Binding Protein, and Retinol-Binding Protein as Early Biomarkers of Chronic Kidney Disease in Dog," *Physiological Reports* 5 (2017): e13262.

19. M. Nabity and J. Hokamp, "Urinary Biomarkers of Kidney Disease in Dogs and Cats," *Veterinary Clinics: Small Animal Practice* 53 (2023): 53–71.

20. J. Raila, L. Brunnberg, F. J. Schweigert, and B. Kohn, "Influence of Kidney Function on Urinary Excretion of Albumin and Retinol-Binding Protein in Dogs With Naturally Occurring Renal Disease," *American Journal of Veterinary Research* 71 (2010): 1387–1394.

21. M. Gizzarelli, X. Roura, P. Scarpa, et al., "Prevalence of Proteinuria in Owned Dogs From Italy: A Multicentric Study," *Veterinary Medicine International* 2019, no. 1 (2019): 6073624.

22. M. C. Lopez, V. Aybar, A. Zatelli, et al., "Is Proteinuria a Rare Condition in Apparently Healthy and Sick Cats? A Feline Practice Experience (2007–2018)," *Open Veterinary Journal* 11 (2021): 508–516.

23. S. Marynissen, A. Willems, D. Paepe, et al., "Proteinuria in Apparently Healthy Elderly Dogs: Persistency and Comparison Between Free Catch and Cystocentesis Urine," *Journal of Veterinary Internal Medicine* 31 (2017): 93–101.

24. S. Marynissen, G. Junius, E. Van den Steen, et al., "Serum Symmetric Dimethylarginine in Older Dogs: Reference Interval and Comparison of a Gold Standard Method With the Enzyme-Linked Immunosorbent Assay," *Journal of Veterinary Internal Medicine* 38 (2024): 960–970.

25. K. R. Friedrichs, K. E. Harr, K. P. Freeman, et al., "Reference Interval Guidelines: Determination of De Novo Reference Intervals in Veterinary Species and Other Related Topics," *Veterinary Clinical Pathology* 41 (2012): 441–453.

26. W. D. Fortney, "Implementing a Successful Senior/Geriatric Health Care Program for Veterinarians, Veterinary Technicians, and Office Managers," *Veterinary Clinics of North America: Small Animal Practice* 42 (2012): 823–834.

27. M. J. Acierno, S. Brown, A. E. Coleman, et al., "ACVIM Consensus Statement: Guidelines for the Identification, Evaluation, and Management of Systemic Hypertension in Dogs and Cats," *Journal of Veterinary Internal Medicine* 32 (2018): 1803–1822.

28. G. Rossi, L. Giori, S. Campagnola, A. Zatelli, E. Zini, and S. Paltrinieri, "Evaluation of Factors That Affect Analytic Variability of Urine Protein-to-Creatinine Ratio Determination in Dogs," *American Journal of Veterinary Research* 73 (2012): 779–788.

29. IRIS, "IRIS Staging Guidelines of CKD," 2023, http://iris-kidney.com.

30. S. Marynissen, G. Schils, L. Stammeleer, S. Daminet, P. Smets, and D. Paepe, "Systolic Blood Pressure Measurements With Doppler Ultrasonic Flow Detector and High-Definition Oscillometry Are Comparable on Population Level but Show Large Intra-Individual Differences in Apparently Healthy Elderly Dogs," *Journal of the American Veterinary Medical Association* 261 (2023): 1–8.

31. S. A. Brown, "Management of Chronic Kidney Disease," in *BSAVA Manual of Canine and Feline Nephrology and Urology*, 2nd ed., ed. J. Elliott and G. F. Grauer (British Small Animal Veterinary Association, 2007), 223–230.

32. D. G. O'Neill, J. Elliott, D. B. Church, P. D. McGreevy, P. C. Thomson, and D. C. Brodbelt, "Chronic Kidney Disease in Dogs in UK Veterinary Practices: Prevalence, Risk Factors, and Survival," *Journal of Veterinary Internal Medicine* 27 (2013): 814–821.

33. M. Sosnar, "Retrospective Study of Renal Failure in Dogs and Cats Admitted to University of Veterinary and Pharmaceutical Sciences Brno During 1999–2001," *Acta Veterinaria Brno* 72 (2003): 593–598.

34. J. Bartges and D. J. Polzin, "Chronic Kidney Disease," in *Nephrology and Urology of Small Animals* (Wiley-Blackwell, 2011), 433–471.

35. J. A. Hall, M. Yerramilli, E. Obare, M. Yerramilli, K. Almes, and D. E. Jewel, "Serum Concentrations of Symmetric Dimethylarginine and Creatinine in Dogs With Naturally Occurring Chronic Kidney Disease," *Journal of Veterinary Internal Medicine* 30 (2016): 794–802.

36. F. Baumgartner, F. Boretti, and B. Gerber, "Prognostic Factors in Dogs With Common Causes of Proteinuria," *Schweizer Archiv für Tierheilkunde* 164, no. 7 (2022): 525–533.

37. A. Larsson, J. Helmersson, L. O. Hansson, and S. Basu, "Increased Serum Cystatin C Is Associated With Increased Mortality in Elderly Men," *Scandinavian Journal of Clinical and Laboratory Investigation* 65 (2005): 301–305.

38. N. Iwasa, R. Kumazawa, S. Nomura, et al., "Prognostic Value of Serum Cystatin C Concentration in Dogs With Myxomatous Mitral Valve Disease," *Journal of Veterinary Internal Medicine* 37 (2023): 412–419.

39. N. Iwasa, S. Takashima, T. Iwasa, et al., "Serum Cystatin C Concentration Measured Routinely Is a Prognostic Marker for Renal Disease in Dogs," *Research in Veterinary Science* 119 (2018): 122–126.

40. F. Cai, L. Zhang, P. Zhao, et al., "Urinary RBP as an Independent Predictor of Renal Outcome in Diabetic Nephropathy," *Disease Markers* 2022 (2022): 1–13, https://doi.org/10.1155/2022/9687868.

41. T. Rezk, R. Salota, J. J. Gan, et al., "Urinary Retinol Binding Protein Predicts Renal Outcome in Systemic Immunoglobulin Light-Chain (AL) Amyloidosis," *British Journal of Haematology* 194 (2021): 1016–1023.

42. Y. Yuan, C. Wang, X. Shao, et al., "Urinary Retionol-Binding Protein as a Risk Factor of Poor Prognosis in Acute-on-Chronic Renal Injury," *Journal of Nephrology* 29 (2016): 827–833.

43. A. Hillaert, D. J. X. Liu, S. Daminet, et al., "Serum Symmetric Dimethylarginine Shows a Relatively Consistent Long-Term

Concentration in Healthy Dogs With a Significant Effect of Increased Body Fat Percentage," *PLoS One* 16 (2021): e0247049.

44. D. J. X. Liu, E. Meyer, B. J. G. Broeckx, et al., "Variability of Serum Concentrations of Cystatin C and Urinary Retinol-Binding Protein, Neutrophil Gelatinase-Associated Lipocalin, Immunoglobulin G, and C-Reactive Protein in Dogs," *Journal of Veterinary Internal Medicine* 32 (2018): 1659–1664.

45. F. Jacob, D. J. Polzin, C. A. Osborne, et al., "Evaluation of the Association Between Initial Proteinuria and Morbidity Rate or Death in Dogs With Naturally Occurring Chronic Renal Failure," *Journal of the American Veterinary Medical Association* 226 (2005): 393–400.

46. H. Miyakawa, M. Ogawa, A. Sakatani, R. Akabane, Y. Miyagawa, and N. Takemura, "Evaluation of the Progression of Non-Azotemic Proteinuric Chronic Kidney Disease in Dogs," *Research in Veterinary Science* 138 (2021): 11–18.

47. E. A. Fulton, A. R. McBrearty, D. J. Shaw, and A. E. Ridyard, "Response and Survival of Dogs With Proteinuria (UPC > 2.0) Treated With Angiotensin Converting Enzyme Inhibitors," *Journal of Veterinary Internal Medicine* 37 (2023): 2188–2199.

48. F. Formiga, R. Moreno-Gonzalez, A. Corsonello, et al., "Diabetes, Sarcopenia and Chronic Kidney Disease; the Screening for CKD Among Older People Across Europe (SCOPE) Study," *BMC Geriatrics* 22 (2022): 254–264.

49. WSAVA Nutritional Assessment Guidelines Task Force Members, L. Freeman, I. Becvarova, N. Cave, et al., "WSAVA Nutritional Assessment Guidelines," *Journal of Feline Medicine and Surgery* 52 (2011): 385–396.

50. A. Sabatino, L. Cuppari, P. Stenvinkel, B. Lindholm, and C. M. Avesani, "Sarcopenia in Chronic Kidney Disease: What Have We Learned So Far?," *Journal of Nephrology* 34 (2021): 1347–1372.

51. A. Santarossa, J. M. Parr, and A. Verbrugghe, "The Importance of Assessing Body Composition of Dogs and Cats and Methods Available for Use in Clinical Practice," *Journal of the American Veterinary Medical Association* 251 (2017): 521–529.

52. Y. Miyagawa, R. Akabane, M. Ogawa, et al., "Serum Cystatin C Concentration Can Be Used to Evaluate Glomerular Filtration Rate in Small Dogs," *Journal of Veterinary Medical Science* 82 (2020): 1828–1834.

53. A. Tvarijonaviciute, J. J. Ceron, S. L. Holden, V. Biourge, P. J. Morris, and A. J. German, "Effect of Weight Loss in Obese Dogs on Indicators of Renal Function or Disease," *Journal of Veterinary Internal Medicine* 27 (2013): 31–38.

54. F. A. Falus, Z. Vizi, K. E. Szabo, et al., "Establishment of a Reference Interval for Urinary Albumin-to-Creatinine Ratio in Dogs," *Veterinary Clinical Pathology* 51 (2022): 585–590.

Supporting Information

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