Original Article

Plasma aldosterone concentration and the survival of dogs with chronic kidney disease

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Abstract

This study investigated plasma aldosterone concentration (PAC) in dogs with chronic kidney disease (CKD) and evaluated the survival of CKD dogs with high PAC, retrospectively. PAC was measured in blood samples obtained from 145 client-owned dogs. The dogs were divided into two groups: healthy (n = 106) and CKD (n = 39). In the healthy group, median (minimum-maximum) PAC was 56 (10–250) pg/mL and the upper limit (95 percentile) was 182 pg/mL. PAC (median 69 pg/mL; range 10–553 pg/mL) in the CKD group was significantly ($P < 0.05$) higher than in the healthy group. In dogs with CKD, the survival time (mean ± SD; 387 ± 270 days) of those with high PAC (n = 10) (> 182 pg/mL) was significantly ($P < 0.05$) shorter than that (742 ± 509 days) than those (n = 24) with normal PAC. In conclusion, high PAC might indicate a shorter survival time in dogs with CKD. However, further study on PAC level in CKD progression and treatment response in a larger population should be performed.

Keywords: Chronic kidney disease, dogs, plasma aldosterone concentration, survival

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Introduction

Aldosterone is a steroid hormone synthesized in the adrenal cortex and is part of the renin-angiotensin-aldosterone system (RAAS). It accelerates renal sodium retention and elimination of potassium through its action on the mineralocorticoid receptor (MR) and plays a major role in regulating body fluid volume and blood pressure (Ponda and Hostetter, 2006). The MR is also present in other tissues besides the kidneys, including cardiomyocytes and vascular endothelial cells. Aldosterone is locally produced in the vasculature, kidneys and heart in addition to the adrenal gland (Weber *et al*., 2003) and its actions may induce classical genomic, as well as rapid nongenomic, effects (Brown, 2013). Excessive secretion of aldosterone and activation of the MR cause cardiovascular inflammation, fibrosis and remodeling and tubulointerstitial fibrosis and glomerular injury in the kidneys (Brown, 2013). There have been several reports on plasma aldosterone concentration (PAC) in healthy animals, chronic kidney disease (CKD) and chronic heart failure in dogs (Knowlen *et al*., 1983; Grandt *et al*., 2022). Measurements of urinary aldosterone/creatinine ratio have also been reported in dogs (Lantis *et al*., 2015; Galizzi *et al*., 2021). Urinary aldosterone/creatinine ratio is not significantly different between healthy individuals and those with differing stages of myxomatous mitral valve disease in dogs and is influenced by individual factors such as breed, sex and age (Galizzi *et al*., 2021). It may be important to evaluate PAC in dogs with CKD associated with the activation of RAAS. Although angiotensin-converting enzyme inhibitors and angiotensin receptor blockers are used to suppress the RAAS during renal disease in dogs (Ames *et al*., 2019), the relationship between PAC and survival time in dogs with CKD has not been investigated. The purpose of this study was to investigate PAC in dogs with CKD and evaluate the survival time of CKD dogs with high PAC.

Materials and Methods

Animals: Records from client-owned dogs that visited the Yasaka Animal Care Center, Japan, between October 2016 and December 2021 that had stored blood samples available were reviewed retrospectively. One hundred and forty-five dogs were identified with diseases of interest including CKD and systemic hypertension, and categorized into the following groups: healthy (n = 106) and CKD (n = 39). Dogs diagnosed with other concurrent disease were excluded. Informed consent was obtained for the use of blood samples.

Grouping: The healthy control dogs were animals that visited the hospital for routine health examinations or neutering, had no signs of illness and had received no medication. The dogs were fed a standard commercial diet chosen by each owner but the diet was not specified. Many blood samples in healthy dogs were collected before castration or ovariohysterectomy. Prior to neutering, the dogs were fasted for 8–12 hours but allowed to drink water freely. Physical examination, hematological and biochemical examinations including blood urea nitrogen (BUN) and creatinine and systemic blood pressure measurement were conducted for the health check. Hematological and biochemical examinations, urinalysis, blood pressure measurement, radiography and abdominal ultrasonography were used to diagnose CKD. Blood chemistries were analyzed using the IDEXX Catalyst One (IDEXX laboratories, Inc., ME). Electrolytes (sodium, potassium and chloride) were measured by ion-selective electrode potentiometry (i-STAT 1 analyzer; Abbott Point of Care, Inc., NJ). Urine protein/creatinine ratio (UPC) or symmetric dimethylarginine (SDMA) level was examined when any urinary protein was detected on the dipstick. Dogs with CKD were subsequently classified using the International Renal Interest Society (IRIS) staging of CKD (modified 2019). IRIS stage 1 in this study was defined as: (1) persistent normal plasma creatinine level (<1.4 mg/dL) or normal to mild increase (<18 µg/dL) in SDMA; (2) the presence of any renal abnormality including proteinuria of renal origin, UPC >0.5 persisting for a certain period of time and abnormal renal imaging findings. The study excluded dogs with a temporary and rapid increase in plasma creatinine exceeding the reference value as seen in acute kidney injury. Dogs with kidney diseases including polycystic kidney disease, chronic urinary tract obstruction, chronic urinary tract infection, urolithiasis and Fanconi syndrome were included in the group. In cases such as chronic urinary tract obstruction and urinary tract infection, when the disease was treated and remitted, dogs having chronic renal dysfunction on a subsequent follow-up were included in the CKD group. In such diseases, individuals whose CKD-related laboratory values were stable for a certain period of time, approximately one month, were included in the CKD group. Primary hyperaldosteronism, which was diagnosed by abdominal ultrasonography of the adrenal glands, higher PAC and lower renin activity, was not included in this study. Primary hyperaldosteronism due to an adrenocortical tumor was excluded by abdominal ultrasonography. Hypertension was diagnosed through non-invasive measurements using a Doppler (Vet-Dop, Vmed Technology, WA) or oscillometric (Pettrust, Aster Electric Co., Japan) device. Systolic blood pressure (SBP) of ≥160 mmHg was defined as "hypertensive" based on the ACVIM consensus statement (Acierno *et al*., 2018). Blood pressure was measured routinely in all dogs with CKD. It was also routinely measured in healthy dogs for health examination and if their SBP was non-hypertensive, it was recorded as <160 mmHg in almost of healthy dogs.

Most dogs were receiving no medication at the time of hospital admission but some dogs had received treatment by the time blood sampling was performed. Seven dogs in the CKD group had received alacepril (1.2–2.4 mg/kg, q12 h or q24 h) or telmisartan (1 mg/kg, q24 h) for 14 days to 3 years.

Sample processing and analysis of PAC: Blood was mixed with ethylenediaminetetraacetic acid and centrifuged. The plasma was then separated and frozen at –35°C until PAC analysis. Plasma samples for PAC measurement were measured collectively for

stored plasma within 6 months. PAC levels were measured by solid-phase radioimmunoassay, using a kit (SPAC-S Aldosterone Kit, Fujirebio, Tokyo, Japan). The RIA method in the kit used was the same principle as previously validated for use with canine plasma in a commercially available human kit (Hori *et al*., 2008). In this PAC kit, the cross-reactivity with cortisol, corticosterone and progesterone was 0.0002%, 0.03% and 0.008%, respectively, with excellent specificity. Intra- and inter-assay coefficients of variation were 1.8 to 8.3% and 2.4 to 3.2%, respectively. The kit was validated for use in dogs by adding two ranges of aldosterone control extracted from the human matrix to canine plasma. Lower and upper detection limits were determined to be 10 and 1600 pg/mL, respectively, by the standard curve of this kit with excellent sensitivity.

Determination of PAC reference range and survival rates of animals: The reference range for PAC was determined at a 95% confidence interval (CI) by a nonparametric statistic method using values from 106 healthy dogs. With a median PAC of 56 pg/mL, the lower limit was 10 pg/mL at 5% percentile and the upper limit was 182 pg/mL at 95% percentile. The reference range for normal PAC was defined as 10–182 pg/mL. PAC exceeding the upper limit was defined as "high" PAC. Survival days were calculated from the blood sampling date to compare long-term outcomes between high and normal PAC levels in the CKD group. Five of the 39 CKD dogs were excluded from the survival analysis because they could not be followed up due to transfer to a different hospital or to another region and their outcome was unknown.

Statistical analysis: Data was analyzed using statistical software (Prism 7.0, GraphPad, CA). Numerical data was tested for normality using the Shapiro–Wilk test. The Mann–Whitney test or unpaired t-test was used to determine the difference between two groups. For multiple group comparison, the nonparametric data was analysed by the Kruskal–Wallis test followed by the post-hoc Dunn's multiple comparison test and parametric data by one-way analysis of variance and post hoc Tukey's multiple comparison test. Kaplan–Meier curves were constructed to compare survival rates and log-rank (Mantel-Cox) tests were used to compare survival curves. The significance level for each analysis was at $P < 0.05$. When the numerical data in all groups was abnormally distributed, the data was presented as median and range, whereas normally distributed data was presented as mean ± standard deviation (SD).

Results

Table 1 summarizes the age, sex, breed, blood biochemistry, SBP and diagnosis of dogs in the healthy and CKD groups as classified by the IRIS stage. Age in the healthy group (median 5.9 years) was significantly ($P < 0.01$) younger than that in the CKD group (median 13.8 years). A linear regression analysis showed no significant correlation ($r = 0.008$; $P = 0.94$) between PAC and age in the healthy group. When data in healthy dogs age-matched to CKD group was pulled from the healthy group, there were no significant differences ($P > 0.05$) in PAC, plasma creatinine and BUN values between young-healthy dogs (< 12 years old; age 5.3 ± 4.1 (mean ± SD), n = 92) and old-healthy dogs age-matched to CKD dogs (≥ 12 years old; age 13.7 ± 1.4, n = 14). The reason that old-healthy dogs age-matched to CKD dogs was chosen as ≥ 12 years old was because their mean age was most similar to that of the CKD dogs (mean 13.5 years old), with the highest P (= 0.89) value. Five dogs of 106 healthy dogs had a high PAC over the upper limit (>182 pg/mL). The breeds of 5 dogs were 1 Labrador Retriever, 1 Miniature Dachshund, 2 Toy Poodles and 1 Pug, ranging in age from 6 to 12 years.

Comparison of PAC between healthy and CKD groups: The number of CKD dogs in IRIS stages 1, 2, 3 and 4 was 18, 18, 3, and 0, respectively. Clinical signs in IRIS stage 2 or 3 included emesis, anorexia, anemia, lethargy, polydipsia and polyuria, weight loss, dehydration, or depression. Seven dogs with CKD had hypertension. PAC (median 69 pg/mL; range 10–553 pg/mL) in the CKD group was significantly ($P = 0.03$) higher than that (median 56 pg/mL; range 10–250 pg/mL) in the healthy group (Table 1; Fig. 1). The mean ± SD values of PAC in healthy and CKD groups were 73 ± 59 pg/mL and 131 ± 139 pg/mL, respectively. In the CKD group as classified by IRIS stage, PAC (median 97 pg/mL) in IRIS stage 2 and 3 was significantly ($P = 0.03$) higher than in the healthy group (Fig. 1). Furthermore, PAC in CKD dogs was also significantly ($P = 0.03$) greater than that (median 43 pg/mL; range 10–177 pg/mL) in age-matched healthy dogs. In the CKD group ($n = 39$), a linear regression analysis showed a significant positive correlation ($r = 0.360$; $P = 0.02$) between PAC and IRIS stage but no significant correlation ($r = 0.235$; $P = 0.15$) between age and IRIS stage. In the CKD dogs ($n = 34$) used for the survival analysis, a linear regression analysis also showed a significant correlation ($r = 0.351$; $P = 0.03$) between PAC and IRIS stage but no significant correlation ($r = 0.179$; $P = 0.31$) between age and IRIS stage.

Table 1 Age, sex, breed, blood biochemistry, systemic blood pressure and diagnosis of dogs in the healthy and chronic kidney disease (CKD) groups

Variables	Healthy	CKD	IRIS-1	IRIS-2 and 3
Number of dogs	106	39	18	21
Age (y)[a]	5.9 (0.3–17.0)	13.8 (5.2–19.6)**	13.2 (5.2–15.9)**	14.2 (9.4–19.6)**
Male/Female (n)	58/48	15/24	5/13	10/11
(Castrated/Ovariohysterectomized)	(36/35)	(5/21)	(1/12)	(4/9)
Breed (n)	Toy Poodle (21), Mixed (14), Miniature Dachshund (12), Shiba (8), Chihuahua (5), Pomeranian (4), Labrador Retriever (3), Pembroke Welsh Corgi (3), Bernese Mountain Dog (3), Maltese (3), Shetland Sheepdog (2), Golden Retriever (2), Jack Russell Terrier (2), Pug (2), Shih Tzu (2), Papillon (2), Yorkshire Terrier (2), French Bulldog (2), Poodle (1), Miniature Schnauzer (1), Bulldog (1), American Pit Bull Terrier (1), Border Collie (1), Bichon Frise (1), Samoyed (1), German Shepherd Dog (1), Beagle (1), Chesapeake Bay Retriever (1), Doberman Pinscher (1), Cavalier King Charles Spaniel (1), Miniature Pinscher (1), Italian Greyhound (1)	Miniature Dachshund (7), Labrador Retriever (4), Toy Poodle (4), Pembroke Welsh Corgi (4), Mixed (3), Shiba (3), Miniature Schnauzer (3), Border Collie (2), Yorkshire Terrier (2), Shetland Sheepdog (1), Poodle (1), Golden Retriever (1), Maltese (1), Chihuahua (1), Hokkaido dog (1), Weimaraner (1)	Labrador Retriever (3), Toy Poodle (2), Pembroke Welsh Corgi (2), Yorkshire Terrier (2), Mixed (1), Shiba (1), Miniature Schnauzer (1), Border Collie (1), Shetland Sheepdog (1), Maltese (1), Chihuahua (1), Hokkaido dog (1), Weimaraner (1)	Miniature Dachshund (7), Toy Poodle (2), Pembroke Welsh Corgi (2), Mixed (2), Shiba (2), Miniature Schnauzer (2), Labrador Retriever (1), Border Collie (1), Poodle (1), Golden Retriever (1)
Diagnosis (n)	None (106)	IRIS-1 (18) IRIS-2 (18) IRIS-3 (3)	IRIS-1 (18)	IRIS-2 (18) IRIS-3 (3)
Cause of CKD (n)		Glomerulonephritis (8), Urolithiasis (5), Polycystic kidney disease (5), Fanconi syndrome (3), Chronic urinary tract infection (2), Pyelonephritis (1), Atrophy of left kidney (1), Unknown including aging (14)	Glomerulonephritis (6), Fanconi syndrome (2), Chronic urinary tract infection (2), Unknown including aging (8)	Urolithiasis (5), Polycystic kidney disease (5), Glomerulonephritis (2), Fanconi syndrome (1), Pyelonephritis (1), Atrophic kidney (1), Unknown including aging (6)

Medication (n)		Alacepril (1.2–2.4 mg/kg, q12 h or q24 h) for 14 days to 3 years (6), Telmisartan (1 mg/kg, q24 h) for 90 days (1), Antibiotics (1)	Alacepril (1.4–2.4 mg/kg, q12 h or q24 h) for 30 days to 7 months (3), Telmisartan (1 mg/kg, q24 h) for 90 days (1), Antibiotics (1)	Alacepril (1.2–2 mg/kg, q12 h or q24 h) for 14 days to 3 years (3)
Blood urea nitrogen (mg/dL)[a]	18 (6–40)	23 (10–188)**	22 (11–52)*	30 (10–188)**
Plasma creatinine (mg/dL)[a]	0.9 (0.4–1.4)	1.4 (0.7–4.3)**	1.2 (0.7–1.4)	1.8 (1.4–4.3)**†
Systolic blood pressure (mmHg)[b]	<160	147 ± 15	150 ± 16	143 ± 13
Plasma aldosterone concentration (PAC) (pg/mL)				
Median (range)[a]	56 (10–250)	69 (10–553)*	58 (10–355)	97 (10–553)*
Mean ± SD[b]	73 ± 59	131 ± 139	99 ± 97	159 ± 165

IRIS – International Renal Interest Society; [a] Median (minimum–maximum); [b] Mean ± standard deviation.
* $P < 0.05$, ** $P < 0.01$, significantly different from healthy group.
† $P < 0.01$, significantly different from IRIS-1 group.

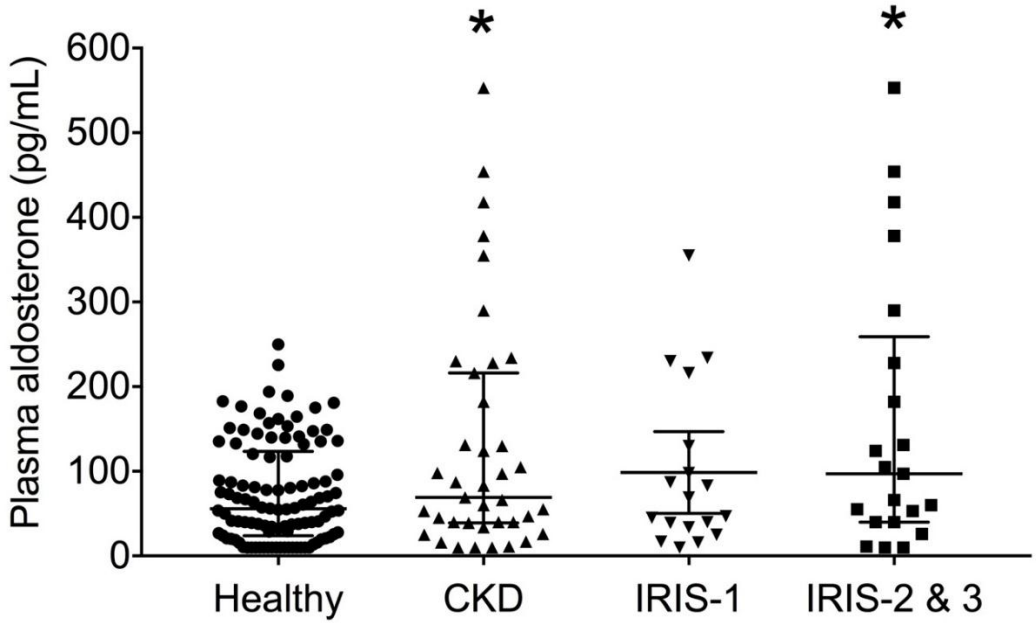

Figure 1 Plasma aldosterone concentration in healthy (n = 106) and chronic kidney disease (CKD) (n = 39) groups, and groups as classified by IRIS stage 1 (n = 18), and IRIS stage 2 and 3 (n = 21). The bars and whiskers indicate the median, 25th, and 75th quartiles. * $P < 0.05$, showed significant difference from the healthy group.

Survival analysis in CKD dogs with high versus normal PAC: Table 2 shows the blood biochemistry, SBP and treatments of dogs with normal and high PAC used for evaluating the survival time in the CKD group. Two dogs in the high PAC group had apparent dehydration. Three dogs in the normal PAC group and two dogs in the high PAC group had mild anemia (PCV < 35%). Six dogs in the normal PAC group and five dogs in the high PAC group had a mild increase in ALT (> 125 IU/L). Treatments and IRIS stages between normal and high PAC groups were similar (Table 2). On day 0, normal and high PACs (mean ± SD) were 57 ± 36 and 330 ± 121 pg/mL, respectively. In the CKD group, dogs with high PAC had significantly (P = 0.013) shorter survival periods than those with normal PAC (Fig. 2). Survival time (mean ± SD) of dogs with high PAC and normal PAC was 387 ± 270 and 742 ± 509 days, respectively. Hazard ratio (high/normal PAC) expressed as 95% CIs for risk of death was 2.35. There were no significant differences in age, body condition score (BCS), plasma creatinine, BUN, plasma potassium, sodium, chloride, inorganic phosphorus, alanine aminotransferase (ALT), packed cell volume (PCV), total protein, albumin and SBP values between normal and high PAC groups (Table 2). When the cause of death was judged clinically, deaths in the normal PAC group were due to natural or sudden causes in 14 dogs and renal failure in 10 dogs. Deaths in the high PAC group were due to natural or sudden causes in 4 dogs and renal failure in 6 dogs.

Discussion

The PAC from 106 healthy, unmedicated dogs was investigated in this study to establish normal PAC values. Results showed that the median PAC was 56 pg/mL and the upper limit as defined by the 95% percentile was 182 pg/mL. To the best of our knowledge, there are no published reports on the reference value of PAC using more than 100 healthy dogs. However, median PAC in this study was similar to those (mean 43–76 pg/mL, n=12–24) of healthy dogs reported previously (Knowlen *et al*., 1983; Javadi *et al*., 2003). Furthermore, the upper limit of PAC in the present study was similar to the maximum value (143–190 pg/mL) in small populations of healthy dogs (n=10–24) in previous studies (Knowlen *et al*., 1983; Baumstark *et al*., 2014; Gójska-Zygner and Zygner, 2015), suggesting that the upper limit is appropriate as a reference value in healthy dogs. The upper limit of PAC in healthy dogs of this study was also similar to that (195 pg/mL) in a large population of healthy cats (n=130) reported previously (Javadi *et al*., 2004). On the other hand, five dogs of 106 healthy dogs had a high PAC over the upper limit in this study. The PAC in dogs is influenced by individual factors such as breed, sex and age (Pedersen *et al*., 1995; Galizzi *et al*., 2021). Although breed differences in PAC levels of healthy dogs have been reported (Pedersen *et al*., 1995), the five dogs are not always included in the breed having a high PAC in that report. Furthermore, from history taking and physical examination, these dogs showed no evidence of dietary management or hydration status problems. However, four out of these five dogs were over 10 years of age, thus subclinical adrenal or renal abnormalities that could not be diagnosed by physical examination, renal panel, and imaging should not be completely ignored. The PACs in the present study were measured collectively from stored plasma within 6 months. Although it cannot be ruled out that long-term storage could have affected PAC measurements, previous studies in humans have demonstrated the stability of steroid hormones in plasma samples frozen at -25°C for 1 to 10 years (Kley *et al*., 1985). Therefore, the effect of different storage time might be minimal in the present study.

In the present study, age in the healthy group was significantly younger than that in the CKD group. This difference is mainly because many blood samples of the healthy dogs were from young healthy dogs referred for health screening before neutering. However, linear regression analysis showed no significant correlation between PAC and age in the healthy group. This result in healthy dogs was consistent with a previous report in cats (Yu and Morris, 1998). Additionally, when data in healthy dogs age-matched to CKD group was pulled from the healthy group, there was no significant difference in PAC between young, healthy dogs and age-matched old, healthy dogs in this study.

The present study revealed that dogs with CKD, especially classified by IRIS stage 2 and 3, had significantly higher PACs than healthy dogs. PACs in dogs with IRIS stage 2 and 3 in this study were similar to those (median 83 pg/mL, n=10) in canine CKD reported recently (Grandt *et al*., 2022). Likewise, it has been reported that cats with hypertension and concurrent CKD had increases in PACs and plasma aldosterone/renin ratio (Jensen et al., 1997; Jepson *et al*., 2014), suggesting RAAS activation.

In dogs with CKD, this study revealed that the survival time of dogs with high PAC was significantly shorter than that of those with normal PAC. Previously, multiple pathophysiological mechanisms have been proposed to have caused shortened survival of cats with high PAC in CKD via MR activation, including aldosterone-induced vasculopathy, tubulointerstitial fibrosis and glomerular injury (Brown, 2013; Spencer *et al*., 2020). On the other hand, hypovolemia directly can activate the RAAS and elevates PAC (Papagiannopoulos-Vatopaidinos *et al*., 2020). It is possible that hypovolemia associated with the higher stages of CKD may be responsible for the short survival of dogs with a high PAC. Therefore, it is suggested that measuring PAC may be useful for a prognostic marker of dogs with CKD. The MR antagonists may prolong the survival of CKD dogs with high PAC, although future investigations are required. Additionally, in the present study, a significant positive correlation between PAC and IRIS stage was observed in CKD dogs used for survival analysis, suggesting that the lower survival rate in high PAC group may be related to severity of CKD as well as effect of aldosterone.

Table 2 Blood biochemistry, systemic blood pressure and medications of dogs with normal and high PAC used for survival analysis in chronic kidney disease (CKD) group

Variables	Normal PAC	High PAC
Number of dogs	24	10
Age (y)[a]	13.4 ± 2.2	14.6 ± 1.7
Male/Female (n) (Castrated/Ovariohysterectomized)	10/14 (2/12)	3/7 (3/6)
Body condition score (five-point system)[b]	3 (2.5–4)	3 (2.5–4)
IRIS stage (% incidence)	IRIS-1 (42%) IRIS-2 (54%) IRIS-3 (4%)	IRIS-1 (30%) IRIS-2 (50%) IRIS-3 (20%)
Cause of CKD (n)	Glomerulonephritis (7), Urolithiasis (4), Fanconi syndrome (3), Polycystic kidney disease (2), Chronic urinary tract infection (1), Pyelonephritis (1), Atrophy of left kidney (1), Unknown including aging (5)	Polycystic kidney disease (3), Glomerulonephritis (1), Urolithiasis (1), Chronic urinary tract infection (1), Unknown including aging (4)
Duration of disease (months)[a]	12.1 ± 8.7	10.5 ± 6.8
Plasma aldosterone concentration (PAC) (pg/mL)[a]	57 ± 36	330 ± 121**
Blood urea nitrogen (mg/dL)[b]	28 (10–88)	26 (11–188)
Plasma creatinine (mg/dL)[b]	1.6 (0.7–4.1)	1.5 (1.0–4.3)
Plasma potassium (mmol/L)[a]	4.6 ± 0.4	4.7 ± 0.8
Plasma sodium (mmol/L)[a]	146 ± 3	144 ± 5
Plasma chloride (mmol/L)[a]	114 ± 4	115 ± 3
Plasma inorganic phosphorus (mg/dL)[a]	4.4 ± 1.1	5.7 ± 4.1
Plasma alanine aminotransferase (IU/L)[a]	111 ± 106	162 ± 130
Packed cell volume (%)	43.4 ± 6.0	43.2 ± 7.3
Total protein (g/dL)	6.9 ± 0.6	6.7 ± 0.5
Systolic blood pressure (mmHg)[a]	147 ± 18	148 ± 11
Treatment (n)	Fluid infusion (7), Alacepril 1.2-2.4 mg/kg, SID or BID (6), Telmisartan 1 mg/kg, SID (3), Phosphate binder (3), Kidney support diet[c] (2), or Antibiotics (1)	Fluid infusion (4), Alacepril 1.4-2.0 mg/kg, SID or BID (5), Phosphate binder (1), or Antibiotics (1)
Survival time (day)[a]	742 ± 509	387 ± 270*

PAC, Plasma aldosterone concentration; IRIS, International Renal Interest Society; [a] Mean ± standard deviation; [b] Median (minimum–maximum); [c] Specific diet formulated with low phosphorous and restricted or highly digestible protein of high quality (Royal Canin; Canine Renal Support A Dry Dog Food or Canine Multifunction Renal Support + Hydrolyzed Protein Dry Dog Food).
* $P < 0.05$, ** $P < 0.01$, significantly different from normal PAC.

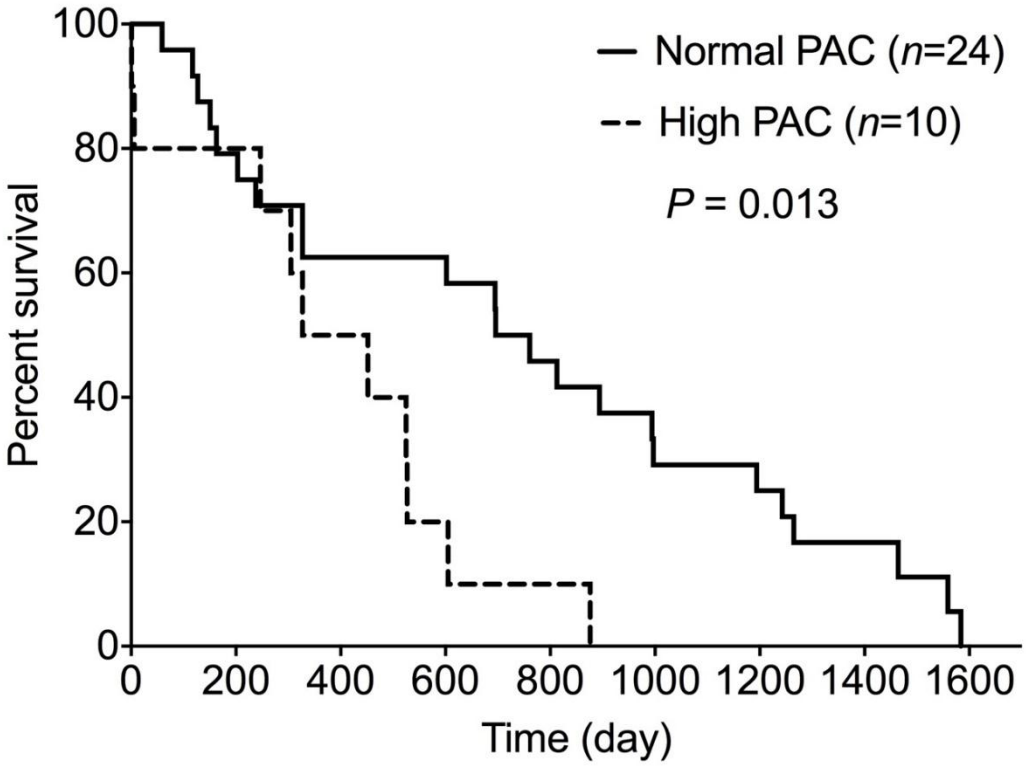

Figure 2 Kaplan–Meier survival curves comparing survival time of dogs with normal (solid line) and high (dashed line) plasma aldosterone concentration (PAC) in the chronic kidney disease (CKD) group. While high PAC was set at > 182 pg/mL, normal PAC was 10–182 pg/mL. *P*-value, showing statistically significant differences, is presented above the graph.

Diet effect on the PAC level has also been reported in previous dog studies. For example, potassium-depleted diet and high sodium intake decreased PAC (Hulter *et al*., 1980; Kjolby *et al*., 2005), while sodium-depletion diet elevated PAC in dogs (Lovern *et al*., 2001; Bie *et al*., 2009). In the present study, feed for the dogs was not a sodium or potassium restricted diet. In addition, there were no significant differences in breed, sex, age and plasma sodium, potassium and chloride levels between the high PAC and normal PAC groups, suggesting that these factors may not have largely influenced the PAC level in the CKD dogs of this study. On the other hand, PAC is reported to be increased by anemia (Anand *et al*., 1993), obesity (Dinh Cat *et al*., 2016), azotemic hypertention (Jepson *et al*., 2014) and chronic liver disease (El-Raziky *et al*., 2005). Chronic liver dysfunction may reflect the inability of the diseased liver to metabolize and clear plasma aldosterone from the circulation (El-Raziky *et al*., 2005). In the present study, some of CKD dogs in both high PAC and normal PAC groups had azotemia, mild anemia, mild increase in hepatic enzyme (ALT) and increase in BCS, which may have influenced the PAC value. However, there were no significant differences in blood biochemistry and BCS between the high and normal PAC groups in CKD dogs of this study. Additionally, age of dog and duration of CKD as well as other complications may affect the survival time in CKD dogs. In the present study, the age of dog and duration of CKD at the time of blood sampling for PAC measurement did not significantly differ between the high and normal PAC groups, suggesting that these factors may not have had a significant effect on the difference in survival time between the two groups.

There are some limitations in this study. In the present study, using abdominal ultrasonography, primary hyperaldosteronism caused by adrenal tumors is deemed unlikely in the CKD group. However, as plasma renin activity was not measured in most cases of CKD dogs, idiopathic hyperplasia of zona glomerulosa might not have been completely excluded. It has been mentioned that PAC value should be measured after the discontinuation of medications affecting this value; the duration of discontinuation is recommended >2 weeks for angiotensin converting enzyme inhibitors and angiotensin II receptor antagonists (Tamura *et al*., 2021). In the present study, some dogs in the CKD group had received these medications at the time of blood sampling for PAC evaluation. In addition, since many dogs used for survival analysis in this study were receiving medications such as alacepril and telmisartan that could potentially affect PAC or stimulate aspects of the RAAS, further analyses of PAC and survival time in a large population of CKD dogs under certain conditions with and without medications should be performed. Small sample size and uneven numbers of dogs between groups in this study might also be included a problem for statistical power.

In conclusion, dogs with CKD had significantly higher PAC than healthy dogs. In CKD, the survival of dogs with high PAC was significantly shorter than those with normal PAC. A significant positive correlation between PAC and IRIS stage was observed in CKD dogs, suggesting that the lower survival rate in high PAC group may be related to the severity of CKD. Therefore, high PAC might indicate a shorter survival time in dogs with CKD. However, further study on

PAC level in CKD progression and treatment response in a larger population should be performed.

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