Article

Effects of plasma aldosterone concentration and treatment with eplerenone on the survival of cats with chronic kidney disease

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Abstract — This study investigated the plasma aldosterone concentration (PAC) in cats with chronic kidney disease (CKD) and retrospectively evaluated the survival of cats with high PAC. Furthermore, this study prospectively examined eplerenone's effect on survival time in CKD cats with high PAC. The PAC was measured retrospectively in blood samples obtained from 156 client-owned cats that visited a veterinary hospital. The cats were designated into 2 groups: clinically healthy (n = 101) and those with CKD (n = 55). The PAC was measured by solid-phase radioimmunoassay; median (minimum–maximum) PAC in healthy cats was 97 pg/mL (range: 10 to 416 pg/mL) and the upper limit (95th percentile) was 243 pg/mL. In the CKD group, PAC [126 pg/mL (range: 10 to 981 pg/mL)] was higher ($P < 0.01$) than in the clinically healthy group. In cats with CKD, the survival time of those with high PAC (n = 16) (> 243 pg/mL) was shorter (P = 0.019) than that of those (n = 39) with normal PAC. Administering an aldosterone antagonist, eplerenone, at 2.5 to 5 mg/kg body weight prolonged survival (P = 0.005) in CKD cats with high PAC (n = 8). In conclusion, PAC could be a prognostic marker of CKD in cats and eplerenone may prolong survival in cats with CKD and a high PAC.

Résumé — Effets de la concentration plasmatique d'aldostérone et du traitement à l'éplérénone sur la survie des chats atteints d'insuffisance rénale chronique. Cette étude a examiné la concentration plasmatique d'aldostérone (PAC) chez les chats atteints d'insuffisance rénale chronique (IRC) et a évalué rétrospectivement la survie des chats ayant une PAC élevée. De plus, cette étude a examiné de manière prospective l'effet de l'éplérénone sur le temps de survie chez les chats IRC avec une PAC élevée. La PAC a été mesurée rétrospectivement dans des échantillons de sang prélevés sur 156 chats appartenant à des clients ayant visité un hôpital vétérinaire. Les chats ont été répartis en 2 groupes : cliniquement sains (n = 101) et ceux atteints d'IRC (n = 55). La PAC a été mesurée par radio-immunodosage en phase solide; la PAC médiane (minimale-maximale) chez les chats sains était de 97 pg/mL (plage : 10 à 416 pg/mL) et la limite supérieure (95^{e} centile) était de 243 pg/mL. Dans le groupe IRC, la PAC [126 pg/mL (plage : 10 à 981 pg/mL)] était plus élevée ($P < 0,01$) que dans le groupe cliniquement sain. Chez les chats atteints d'IRC, le temps de survie de ceux avec une PAC élevée (n = 16) (> 243 pg/mL) était plus court (P = 0,019) que celui de ceux (n = 39) avec une PAC normale. L'administration d'un antagoniste de l'aldostérone, l'éplérénone, à raison de 2,5 à 5 mg/kg de poids corporel a prolongé la survie (P = 0,005) chez les chats atteints d'IRC avec une PAC élevée (n = 8). En conclusion, la PAC pourrait être un marqueur pronostique de l'IRC chez le chat et l'éplérénone pourrait prolonger la survie des chats atteints d'IRC et d'une PAC élevée.

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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 has a major role in regulating body fluid volume and blood pressure (1). The MR is also present in other tissues besides the kidney, including cardiomyocytes and vascular endothelial cells. Aldosterone is locally produced in the vasculature, kidney, and heart in addition to the adrenal glands (2), and its actions may induce classical genomic, as well as rapid nongenomic effects (3). Nongenomic effects of aldosterone are proposed to potentiate angiotensin II-induced vasoconstriction and facilitate classical MR-mediated effects (2,3). In rodents, excessive secretion of aldosterone and activation of the MR cause cardiovascular inflammation, fibrosis and remodeling, and tubulointerstitial fibrosis and glomerular injury in the kidney (3).

There are several reports on plasma aldosterone concentration (PAC) in cats that are clinically healthy and in those with chronic kidney disease (CKD) and systemic hypertension (4–6). Measurement of urinary the aldosterone:creatinine ratio has also been reported in cats (7). However, it has been suggested that measuring aldosterone in feline urine using the available methodology has limited or no utility in investigating feline hypertension associated with kidney disease (7). The PAC in cats does not change significantly with age, sex, pregnancy, lactation, or circadian rhythm (4,8). Plasma renin activity (PRA) is also important as a biochemical marker of RAAS activation. Although 1 study reported PRA did not significantly differ between normotensive and hypertensive cats (5), another study reported that PRA was lower in hypertensive (both azotemic and nonazotemic) compared to control cats (6). Azotemic hypertensive cats have a significantly increased PAC and aldosterone-to-renin ratio independent of plasma renin activity (6). Furthermore, PRA in cats with reduced renal functions is reportedly less sensitive to salt intake than PAC (9). The PRA also differs with age and sex/neuter status (8). These findings propose the significance of measuring PAC in hypertensive CKD cats. However, PAC varies with dietary sodium and potassium intake (9–13) and a renal diet (sodium restriction) may be expected to elevate the PAC in cats. The RAAS is activated in cats with reduced renal function at the lowest salt intake and is associated with hypokalemia and a high excretion of potassium (9).

Angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) suppress the RAAS during hypertensive, renal, and cardiac diseases in cats (5,14–17). In the Randomized Aldactone Evaluation Study (RALES) conducted in humans, the MR antagonist, spironolactone, reduced mortality (by 30%) of patients with chronic heart failure that received ACEI and loop diuretics (18). Spironolactone also reportedly reduced the mortality rate in cats with congestive heart failure secondary to cardiomyopathy (19), but the authors were cautious in concluding this, as cats in the treatment group appeared to have less severe disease than the placebo group. Another selective MR antagonist, eplerenone, not only antagonizes MR (20) but also blocks the nongenomic effects of aldosterone in vascular tissues not susceptible to spironolactone (21,22). These effects of eplerenone might be more useful than spironolactone in treating hypertension due to vasoconstriction. However, the clinical relevance of this effect is still unclear in dogs and cats. Furthermore, although eplerenone reduces mortality and hospitalization in human patients with chronic heart failure (23,24), there are no available reports on eplerenone's use in feline practice. Hence, spironolactone is the only MR antagonist currently reported for its effect in cat-based clinical trials (19,25,26).

We hypothesized that if an elevated PAC is detectable in the early stages of CKD in cats, the use of MR antagonists may prolong lifespan. Although elevated PAC is a risk factor for kidney injury in humans, MR antagonists are beneficial in rodent models of CKD and human patients (26). However, the relationship between PAC and survival rate in CKD has not been investigated in cats. Therefore, this study investigated PAC in cats with CKD and evaluated survival of cats with high PAC. Furthermore, we examined the effect of eplerenone on survival time in CKD cats with high PAC.

Materials and methods

Animals

Records from client-owned cats that visited the Yasaka Animal Care Center, Japan, between October 2016 and December 2021 and had stored blood samples available, were reviewed retrospectively. This hospital provided general practice for local clients and referral services for veterinarians throughout the local area. Cats (N = 156) were identified with the diseases of interest including CKD, cardiac disease, and systemic hypertension, and categorized into the following groups: clinically healthy (n = 101) and those with CKD (n = 55). Cats diagnosed with other concurrent disease were excluded.

Grouping

Blood samples were collected from healthy cats and cats diagnosed with CKD by routine diagnostic procedures. Clinically healthy cats visited the hospital for routine health examinations, had no signs of illness, and received no medication. Subsequently, physical examination, hematological and biochemical examinations including blood urea nitrogen (BUN), creatinine, aspartate aminotransferase, alanine aminotransferase, glucose, and total protein and systemic blood pressure measurement were conducted for the health check. Informed consent was obtained for the use of the blood samples.

Hematological and biochemical examinations, urinalysis, blood pressure measurement, radiography, and abdominal ultrasonography were used to diagnose CKD. Urine protein/creatinine ratio was examined when urinary protein was detected on dipstick. When bacteria were detected in the urine sediment, urine culture was performed for antibiotic susceptibility test with disk diffusion method. Cats with CKD were subsequently classified using the International Renal Interest Society (IRIS) staging of CKD (modified 2019). Cats with a temporary and rapid increase in plasma creatinine exceeding the reference value, as occurs in acute kidney injury, were excluded from the study. Cats with hyperthyroidism were also excluded. Cats with kidney diseases including polycystic kidney disease, chronic urinary

tract obstruction, chronic urinary tract infection, urolithiasis, feline urological syndrome, and renal lymphoma, were included in the group. None of the cats was suspected of having toxic nephropathy. In cases such as chronic urinary tract obstruction and urinary tract infection, when the disease was treated and remitted, cats having chronic renal dysfunction on subsequent follow-up were included in the CKD group. The number of cats in IRIS Stages 1, 2, 3, and 4 were 0, 32, 18, and 5, respectively. Clinical signs in IRIS Stage 2 or higher included polyuria, oliguria, anuria, weight loss, dehydration, anemia, respiration abnormality, diarrhea, emesis, lethargy, or depression. Since this study mainly investigated the significance of PAC measurements associated with CKD, cats with primary hyperaldosteronism were excluded. Cats with primary hyperaldosteronism due to an adrenocortical tumor were excluded by abdominal ultrasonography.

Physical examination, hematological and biochemical examinations, electrocardiogram, blood pressure measurements, radiography, and echocardiography were used to diagnose cats with cardiac disease. A veterinary cardiologist certified by the Japanese Society of Veterinary Cardiology or a veterinarian with considerable experience and further training in cardiology conducted echocardiography.

Hypertension was diagnosed through non-invasive measurements using a Doppler or oscillometric device. Doppler was routinely used unless the cats were poorly tolerant, in which case oscillometric measurement was employed. After each cat was rested in a separate room, their blood pressure was measured multiple times until it became stable, and the average value was calculated from 5 to 10 stable measurements. A systolic blood pressure of $\geq$ 160 mmHg was defined as "hypertensive" based on the American College of Veterinary Internal Medicine (ACVIM) consensus statement on hypertension (27). Blood pressure was measured routinely in all cats with CKD and cardiac disease. It was also routinely measured in healthy cats for health examination, and if their systolic blood pressure was $<$ 160 mmHg, it was recorded as non-hypertension.

Most cats were receiving no medication at the time of hospital admission, but some cats had received treatment by the time blood sampling was performed. Eight cats in the CKD group received benazepril or amlodipine. Some cats had $>$ 1 blood sample available to measure PAC. Therefore, PAC values during health examination in the healthy group and at diagnosis in the diseased group were used to compare PACs between the groups.

Sample processing and analysis of PAC

Blood was mixed with EDTA and immediately centrifuged at room temperature, with plasma separated and frozen at −35°C until PAC analysis. Concentrations of PAC were measured by solid-phase radioimmunoassay, using a kit (SPAC-S Aldosterone Kit; Fujirebio, Tokyo, Japan). The radioimmunoassay method in the kit used was the same principle as previously validated for use with feline plasma in a commercially available human kit (6). 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 cats by adding 2 ranges of aldosterone control (53 to 88 pg/mL and 252 to 420 pg/mL) extracted from the human matrix to feline plasma. Lower and upper detection limits were 10 and 1600 pg/mL, respectively.

Determination of PAC reference range

The reference range for PAC was determined at a 95% confidence interval (CI) by a nonparametric statistic method using values from 101 healthy cats. With a median PAC of 89 pg/mL, the lower limit was 10 pg/mL at 5% percentile, and the upper limit was 243 pg/mL at 95% percentile. Therefore, the reference range for normal PAC was defined as 10 to 243 pg/mL, with PAC exceeding the upper limit defined as "high" PAC.

Survival rates of animals with high plasma aldosterone concentration (PAC) and effect of eplerenone

Survival days were calculated from the blood sampling date to compare long-term outcomes between high and normal PAC levels in the CKD group.

The eplerenone study was conducted prospectively on cats with a higher PAC than the reference value. Eight cats were used to examine the effect of eplerenone on the survival time of cats with CKD and a high PAC. Seven of these 8 cats had concurrent disease, including heart disease and/or arterial hypertension. Eighteen CKD cats with a high PAC were used as the non-eplerenone control group. Eight of these 18 had concurrent disease, including heart disease and/or arterial hypertension. Cats in the non-eplerenone group had similar clinical characteristics and biochemical variables to those in the eplerenone-treated group. Since no previous studies using eplerenone in cats were identified, informed consent was obtained from each cat owner regarding eplerenone administration. Owners and clinicians were not blinded to the eplerenone treatment (or not) group. The cats were alternately assigned to either the control group or eplerenone treatment group, in the order they visited the hospital. Informed consent was sought from owners regarding eplerenone administration, but some owners did not accept it. The reason for non-acceptance was that there was no report on eplerenone medication in cats. Therefore, the number of cats was greater in the non-eplerenone group than the eplerenone group. Eplerenone was orally administered at 2.5 to 5 mg/kg body weight (BW) once daily, based on previous dosages reported to have been effectively and safely used in dogs (28). Ethical approval was not required for the eplerenone study; however, informed consent was essential for inclusion. Veterinarians were responsible for any adverse reactions that may occur as a result of this unlicensed use for cats. They were also required to retain medical records including the name of the formulation, dosage, and manufacturer for at least 3 y.

Statistical analysis

Data were analyzed using statistical software (Prism 7.0; GraphPad, California, USA). Difference in sex frequencies between groups was compared using Fisher's Exact test. Data including PAC, age, systolic blood pressure, and biochemical variables, were tested for normality using the Shapiro-Wilk test. When the data were abnormally distributed, these nonparametric data were subjected to the Kruskal-Wallis test. When significant *P*-values

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

Variables	Healthy	CKD
Number of cats	101	55
Age (y)[a]	0.8 (range: 0.3 to 16.2)	15.3 (range: 1.7 to 23.1)*
Male/Female *(n)* (Castrated/Ovariohysterectomized)	50/51 (16/19)	23/32 (21/32)
Breed *(n)*	Domestic shorthair (92) Norwegian Forest (3) Scottish fold (3) Himalayan (2) Russian blue (1)	Domestic shorthair (49) Abyssinian (2) Norwegian Forest (1) Scottish fold (1) Russian blue (1) American shorthair (1)
Diagnosis *(n)*	None (101)	IRIS-2 (32) IRIS-3 (18) IRIS-4 (5)
Blood urea nitrogen (mg/dL)[a]	24 (range: 14 to 32)	36 (range: 18 to 140)*
Plasma creatinine (mg/dL)[a]	1.2 (range: 0.6 to 1.5)	2.6 (range: 1.7 to 9.6)*
Systolic blood pressure (mmHg)[b]	< 160	140 ± 10
Plasma aldosterone concentration (PAC) (pg/mL)[a]	89 (range: 10 to 416)	126 (range: 10 to 981)*

[a] Median (minimum–maximum).
[b] Mean ± standard deviation.
* $P < 0.01$, different from the healthy group.

were encountered, the *post-hoc* Dunn's multiple comparison test was used to determine significant differences between the groups. One-way analysis of variance (ANOVA) and *post-hoc* Tukey's multiple comparison test were used for intergroup comparisons when the data were normally distributed. The Mann-Whitney test or unpaired Student's *t*-test was used to compare data between the 2 groups to determine the difference. Kaplan–Meier curves were constructed to compare survival rates, and log-rank (Mantel-Cox) tests were used to compare survival curves because the curves were right-skewed and censored. The hazard ratio was expressed as 95% CIs. The significance level for each analysis was at $P < 0.05$.

Results

Age, sex, breed, blood biochemistry, systolic blood pressure, and diagnosis of cats in the clinically healthy and CKD groups are summarized in Table 1. Age [median (minimum to maximum)] in the healthy and CKD groups was 0.8 y (range: 0.3 to 16.2 y) and 15.3 y (range: 1.7 to 23.1 y), respectively. Cats in the clinically healthy group were younger ($P < 0.01$, by Mann–Whitney test) than those in the CKD group.

Comparison of PAC between healthy and CKD groups

The PAC in the clinically healthy and CKD groups was median (minimum–maximum); 89 pg/mL (range: 10 to 416 pg/mL) and 126 pg/mL (range: 10 to 981 pg/mL, respectively) (Table 1; Figure 1). Linear regression analysis observed no significant correlation (R squared = 0.00148; $P = 0.70$) between PAC and age in the healthy group (Appendix-Figure A1). PAC in the CKD group was higher ($P < 0.01$, by Mann–Whitney test) than the healthy group (Figure 1). Since cats in the clinically healthy group were significantly younger than those in the CKD group, age-matched control data for PAC analysis were pulled from the healthy group. The PAC, plasma creatinine, and BUN values in the age-matched control [≥ 10 y old; age 12.6 ± 1.9 y (mean ± SD), $n = 12$] and younger healthy cats (< 10 y old; age 2.1 ± 2.8 y, $n = 89$) were 85 ± 53 and 98 ± 68 pg/mL, 1.3 ± 0.2 and 1.2 ± 0.2 mg/dL, and 24.5 ± 5.0 and 23.9 ± 3.3 mg/dL, respectively. There were no significant differences ($P = 0.72$, 0.15, and 0.56, respectively; by Mann–Whitney test or unpaired Student's *t*-test) in those values between young-healthy cats and old-healthy cats age-matched to CKD cats. The PAC was greater ($P = 0.03$, by Mann–Whitney test) in CKD cats than in age-matched healthy cats.

Blood biochemistry and SBP in cats in the CKD group as classified by IRIS stage are summarized in Table 2. The PACs were higher ($P = 0.03$, Kruskal–Wallis test followed by Dunn's multiple comparison test) in IRIS stage 2 than in the healthy group (Figure 2). Similarly, PACs in IRIS stage 3 and 4 cats were higher ($P = 0.01$) than in the healthy group. At IRIS Stages 3 and 4, individual differences in PACs were great. In particular, IRIS Stage 4 cats ($n = 5$) had a large variation in PAC (median = 98 pg/mL; minimum–maximum = 10–981 pg/mL).

Survival analysis in cats with high *versus* normal PAC

Blood biochemistry, systolic blood pressure and treatments of cats with normal and high PAC used for evaluating the survival time in CKD group are shown in Table 3. In the CKD group, cats with high PAC had shorter ($P = 0.019$, by log-rank test) survival periods than those with normal PAC (Figure 3). Median (minimum–maximum) survival of cats with high PAC and normal PAC was 446 d (range: 29 to 1586 d) and 1233 d (range: 11 to 1850 d), respectively. Hazard ratio (high/normal PAC) for risk of death was 2.21. On Day 0, normal and high

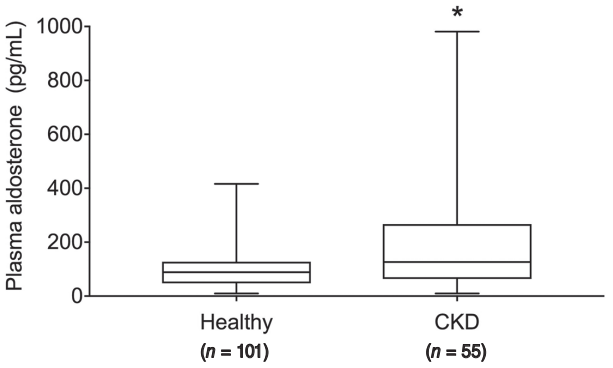


Figure 1. Plasma aldosterone concentration in healthy ($n = 101$) and chronic kidney disease (CKD) ($n = 55$) groups. The boxes represent the 25th and 75th quartiles, with the horizontal line representing the median. The whiskers represent the data range. * $P < 0.01$, showed significant difference from the healthy group by the Mann–Whitney test.

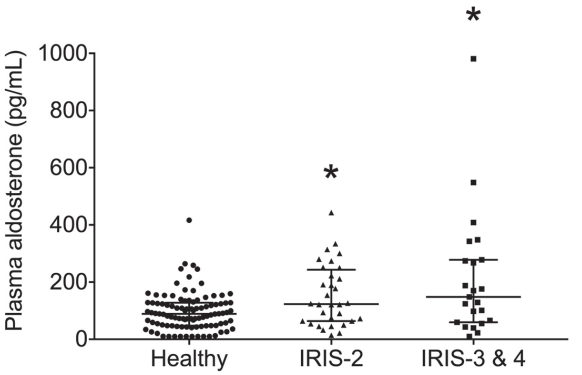


Figure 2. Plasma aldosterone concentrations in healthy ($n = 101$), IRIS stage 2 ($n = 32$), and IRIS stage 3 ($n = 18$) and 4 ($n = 5$) cat groups. The bars and whiskers indicate the median, 25th, and 75th quartiles. * $P < 0.05$, showed significant difference from the healthy group by the Kruskal–Wallis test, followed by Dunn's multiple comparison test.

Table 2. Blood biochemistry and systemic blood pressure in cats in the chronic kidney disease (CKD) group as classified by IRIS stage.

Variables	Healthy	IRIS-2	IRIS-3 and 4
Number of cats	101	32	23
Age (y)[a]	0.8 (range: 0.3 to 16.2)	13.9 (range: 4.0 to 23.1)**	17.4 (range: 1.7 to 20.8)**
Male/Female *(n)* (Castrated/Ovariohysterectomized)	50/51 (16/19)	16/16 (15/16)	7/16 (6/16)
Blood urea nitrogen (mg/dL)[a]	24 (range: 14 to 32)	29 (range: 18 to 48)**	57 (range: 25 to 140)**††
Plasma creatinine (mg/dL)[a]	1.2 (range: 0.6 to 1.5)	2.2 (range: 1.7 to 2.8)**	3.7 (range: 2.9 to 9.6)**†
Systolic blood pressure (mmHg)[b]	< 160	139 ± 10	142 ± 10
Plasma aldosterone concentration (PAC) (pg/mL)[a]	89 (range: 10 to 416)	123 (range: 14 to 443)*	148 (range: 10 to 961)*

IRIS — International Renal Interest Society.
[a] Median (minimum–maximum).
[b] Mean ± standard deviation.
* $P < 0.05$, ** $P < 0.01$, different from healthy group.
† $P < 0.05$, †† $P < 0.01$, different from IRIS-2 group.

PACs [median (minimum–maximum)] were 94 pg/mL (range: 10 to 222 pg/mL) and 307 pg/mL (range: 250 to 981 pg/mL), respectively. There were no significant differences in age, plasma creatinine, BUN, and systolic blood pressure values between normal and high PAC groups (Table 3). Treatments and IRIS stages between normal and high PAC groups were similar (Appendix-Table A1).

Effects of eplerenone on survival in cats with high PAC

Age, sex, diagnosis, blood biochemistry, systolic blood pressure, and medications of the eplerenone and non-eplerenone treatment groups in high PAC cats are shown in Table 4. In cats with high PAC and CKD, eplerenone administration prolonged ($P = 0.005$, by log-rank test) survival compared to cats not receiving eplerenone (Figure 4). Median (minimum–maximum) survival of cats with eplerenone and non-eplerenone was 1109 d (range: 333 to 1391 d) and 243 d (range: 17 to 1312 d), respectively. Hazard ratio (eplerenone/non-eplerenone) was 0.35.

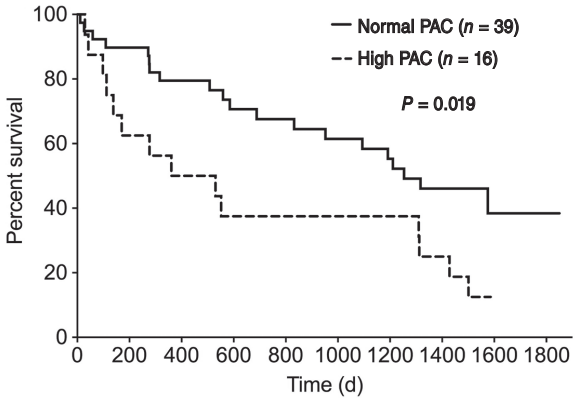


Figure 3. Kaplan–Meier survival curves comparing survival time of cats with normal (solid line) and high (dashed line) plasma aldosterone concentration (PAC) in the chronic kidney disease (CKD) group. Although high PAC was set at > 243 pg/mL, normal PAC was 10 to 243 pg/mL. *P*-value, showing statistically significant differences, is presented above the graph.

Table 3. Blood biochemistry, systemic blood pressure, and medications of cats with normal and high PAC used for survival analysis in chronic kidney disease (CKD) group.

Variables	Normal PAC	High PAC
Number of cats	39	16
Age (y)[a]	14.2 (range: 1.7 to 23.1)	16.1 (range: 7.3 to 20.8)
Male/Female *(n)* (Castrated/Ovariohysterectomized)	20/19 (18/19)	3/13* (3/13)
IRIS stage (% incidence)	IRIS-2 (61%) IRIS-3 (31%) IRIS-4 (8%)	IRIS-2 (50%) IRIS-3 (38%) IRIS-4 (12%)
Plasma aldosterone concentration (PAC) (pg/mL)[a]	94 (range: 10 to 222)	307 (range: 250 to 981)**
Blood urea nitrogen (mg/dL)[a]	34 (range: 18 to 119)	42 (range: 18 to 140)
Plasma creatinine (mg/dL)[a]	2.6 (range: 1.7 to 9.6)	3.0 (range: 2.1 to 8.0)
Systolic blood pressure (mmHg)[b]	140 ± 8	141 ± 15
Treatment *(n)*	Kidney support diet (n = 14)[c], Fluid infusion (n = 8), Benazepril 0.5 to 0.6 mg/kg, q24h (n = 4), Amlodipine 0.2 to 0.4 mg/kg, q24h or q12h (n = 1), Potassium gluconate (n = 2), Phosphate binder (n = 2), or antibiotics (n = 1)	Kidney support diet (n = 9)[c], Fluid infusion (n = 4), Benazepril 0.5 to 0.6 mg/kg, q24h (n = 4), Amlodipine 0.2 to 0.4 mg/kg, q24h or q12h (n = 3), Potassium gluconate (n = 2), or Phosphate binder (n = 1)
Survival time (day)[a]	1233 (range: 11 to 1850)	446 (29 to 1586)*

PAC — Plasma aldosterone concentration; IRIS — International Renal Interest Society.
[a] Median (minimum–maximum).
[b] Mean ± standard deviation.
[c] Specific diet formulated with low phosphorous and a moderate level of highly digestible protein (Royal Canin, Renal with chicken).
Analytical constituents are as follows: Protein — 8%; Fat content — 8%; Crude ash — 1.3%; Crude fiber — 0.8%; Moisture — 77%; Calcium — 0.15%; Phosphorus — 0.08%; Potassium — 0.2%; Sodium — 0.11%; Magnesium — 0.015%; Iron — 0.003%; Copper — 0.0005%; Zinc — 0.003%; EPA and DHA — 0.15%; Taurine — 0.14%; Arginine — 0.4%; Vitamin E — 0.015%; Vitamin C — 0.007%; Vitamin B — 0.007%; and others.
* $P < 0.05$, ** $P < 0.01$, different from normal PAC.

The PACs [median (minimum–maximum)] on Day 0 in cats receiving and not receiving eplerenone were 356 pg/mL (range: 269 to 540 pg/mL) and 338 pg/mL (range: 252 to 548 pg/mL), respectively. There was no significant difference between the groups in age, PAC, plasma creatinine, BUN, or systolic blood pressure. Treatments, IRIS stages, and complications were similar between groups. There was no significant difference in median BUN values between the groups, but the non-eplerenone group included 3 cats with extremely high BUN values (range: 88 to 127 mg/dL), whereas the eplerenone group included no cats with such high BUN values (maximum 46 mg/dL). When the cause of death was judged clinically, deaths in the non-eplerenone group were due to natural or sudden cause in 13 cats, heart failure in 5 cats, and renal failure in 2 cats. Deaths in the eplerenone group were due to natural cause in 1 cat, heart failure in 3 cats, and renal failure in 4 cats.

Discussion

The PAC from 101 healthy, unmedicated cats was investigated in this study to establish normal PAC values. Median PAC was 89 pg/mL, and the upper limit as defined by the 95% percentile was 243 pg/mL. These PACs were very similar to those reported previously (4,8), indicating that the upper limit is appropriate as a reference value in clinically healthy cats. Plasma samples for PAC used in this study were measured collectively from stored plasma within 6 mo. Although it cannot be ruled out that long-term storage would 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 y (29).

In the present study, the age in the healthy group was significantly lower than that in the CKD group. This difference is mainly because many blood samples in healthy cats were collected before neutering. There was no significant correlation between age and PAC in this study in healthy cats, consistent with a previous report (4). In addition, there was no significant difference in PAC between young, healthy cats and age-matched old healthy cats in this study.

The present study revealed that cats with CKD had significantly higher PACs than healthy cats. Likewise, it has been reported that cats with hypertension and concurrent CKD had increases in PACs and plasma aldosterone:renin ratio (5,6), suggesting RAAS activation. In addition, a previous study detected no difference in PAC between normotensive cats with CKD and age-matched controls (6). However, in our investigation, PAC was significantly higher in cats with IRIS Stage 2 of CKD than healthy cats. Cats with IRIS Stages 3 and 4 had also significantly higher PAC than healthy cats. In IRIS Stage 4, however, PACs varied greatly among individuals, and some individuals had a low PAC. It is unclear why some cats at the terminal stage of CKD had low a PAC. It is reported that cats excrete very small concentrations of free aldosterone and its metabolite, 18-glucuronidated aldosterone, in urine (7). Polar metabolites

Table 4. Age, sex, diagnosis, blood biochemistry, systemic blood pressure, and medications of eplerenone and non-eplerenone treatment groups in high PAC cats.

Variables	Non-eplerenone	Eplerenone
Number of cats	18	8
Age (y)[a]	16.6 ± 3.4	15.0 ± 1.8
Male/Female *(n)* (Castrated/Ovariohysterectomized)	8/10 (8/9)	3/5 (3/4)
IRIS stage *(n)*	IRIS-2 (*n* = 9), IRIS-3 (*n* = 9)	IRIS-2 (*n* = 5), IRIS-3 (*n* = 3)
Diagnosis *(n)*	CKD (*n* = 10) CKD and hyperthyroidism (*n* = 1) CKD and HCM (CHF) (*n* = 2) CKD and hypertension (*n* = 3) CKD, hyperthyroidism, and hypertension (*n* = 1) CKD, HCM (CHF), and hypertension (*n* = 1)	CKD (*n* = 1) CKD and MI (*n* = 1) CKD and hypertension (*n* = 2) CKD and HCM (CHF) (*n* = 1) CKD and RCM (CHF) (*n* = 1) CKD, MI, and hypertension (*n* = 1) CKD, HCM (CHF) and hypertension (*n* = 1)
Plasma aldosterone concentration (PAC) (pg/mL)[a]	360 ± 95	381 ± 100
Blood urea nitrogen (mg/dL)[b]	42 (range: 19 to 127)	34 (range: 26 to 46)
Plasma creatinine (mg/dL)[a]	2.9 ± 0.9	2.8 ± 0.6
Systolic blood pressure (mmHg)[a]	164 ± 32	167 ± 22
Treatment	Kidney support diet[c], Fluid infusion, Benazepril (0.5 to 0.6 mg/kg BW, PO, q24h), Amlodipine (0.2 to 0.4 mg/kg BW, PO, q12h or q24h), Pimobendan (0.2 to 0.3 mg/kg BW, PO, q12h), Methimazole (0.34 to 0.54 mg/kg BW, PO, q12h or q24h), Furosemide (0.5 to 1 mg/kg BW, PO, q12h), Potassium gluconate, or Phosphate binder	Kidney support diet[c], Fluid infusion, Benazepril (0.5 to 0.6 mg/kg BW, PO, q12h or q24h), Amlodipine (0.22 mg/kg BW, q24h), Pimobendan (0.26 to 0.3 mg/kg BW, PO, q12h), or Furosemide (1 to 2 mg/kg BW, PO, q12h or q24h)
Survival time (day)[b]	243 (range: 17 to 1312)	1109 (range: 333 to 1391)*

PAC — Plasma aldosterone concentration; IRIS — International Renal Interest Society; CKD — Chronic kidney disease; HCM — Hypertrophic cardiomyopathy; CHF — Congestive heart failure; MI — Mitral insufficiency; RCM — Restrictive cardiomyopathy; BW — Body weight.
[a] Mean ± standard deviation.
[b] Median (minimum–maximum).
[c] Specific diet formulated with low phosphorous and a moderate level of highly digestible protein (Royal Canin, Renal with chicken).
* $P < 0.01$, different from non-eplerenone group.

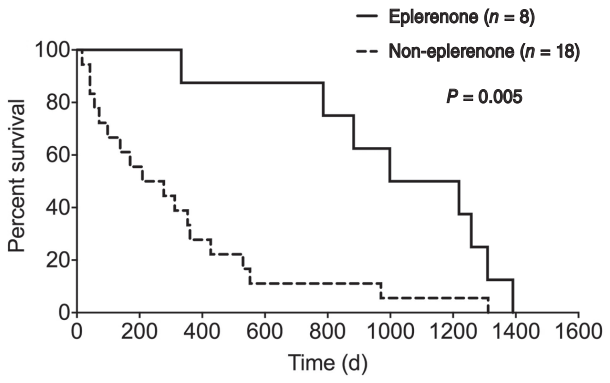


Figure 4. Kaplan–Meier survival curves comparing the survival time of cats treated with eplerenone (solid line) and those that did not receive eplerenone (dashed line) within cats having high plasma aldosterone concentration (PAC) and chronic kidney disease (CKD). *P*-value, showing the statistically significant difference, is presented above the graph.

of aldosterone interfere with PAC quantification, associated with deteriorating renal function in humans (30,31). Although the influence of polar metabolites on PAC in cats is unknown, it could be important to examine further on aldosterone metabolism and accumulation of polar aldosterone metabolites in feline blood associated with deteriorating renal function. Therefore, when treating with anti-aldosterone drugs based on PAC levels, attention should be given for evaluation of PAC at the terminal stage of CKD in cats.

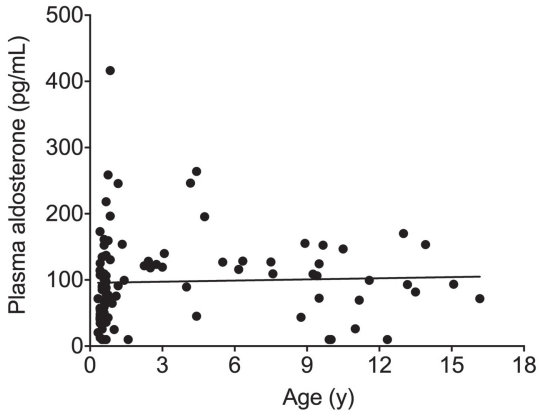
In cats with CKD, this study revealed that the survival time of cats with high PAC was significantly shorter than that of those with normal PAC. Previously, multiple pathophysiological mechanisms have been proposed to cause shortened survival of cats with high PAC in CKD *via* MR activation, including aldosterone-induced vasculopathy, tubulointerstitial fibrosis, and glomerular injury (3,26). Conversely, hypovolemia can directly activate the RAAS and elevate PAC (32). It is possible that hypovolemia associated with the terminal stages of CKD may be responsible for the short survival in cats with a high PAC.

The MR antagonist, spironolactone, reduces the mortality rate in cats with cardiomyopathy (19), but the authors were cautious in concluding this as the cats in the treatment group appeared to have less severe disease than the placebo group. However, the effect of MR antagonist on the mortality rate in cats with CKD has not been fully investigated. Also, the selective MR antagonist, eplerenone, has not yet been applied to cats. The present study suggests that eplerenone administration significantly prolonged survival of cats with high PAC in

CKD. Several of the cats in the prospective study had concurrent cardiac disease and/or arterial hypertension. Positive effects of eplerenone may, at least partially, be due to effects of eplerenone on blood pressure and the heart. Eplerenone reduces hospitalization and mortality in human patients with chronic heart failure, including myocardial infarction (23,24). Using MR antagonists to treat end-stage renal disease reduces the 3-year mortality rate and is associated with lower blood pressure, reduced left ventricular mass, and improved left ventricular ejection fraction in human patients (33). In addition, eplerenone effectively reduced blood pressure compared with other agents, such as spironolactone, enalapril, losartan, and amlodipine in humans (34). Our results in cats also indicated that although the pathophysiological mechanisms require future studies, eplerenone may reduce mortality rate in cats with high PAC in CKD with cardiovascular disease. Therefore, it is suggested that measuring PAC may be useful, particularly given that eplerenone could be an effective treatment for hyperaldosteronism in cats.

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 PRA was not measured in most cases of CKD cats, idiopathic hyperplasia of zona glomerulosa tissue might not have been completely excluded. In addition, since the echocardiography was not routinely performed in cats with CKD in this study, asymptomatic heart abnormalities such as HCM may have been overlooked in cats with CKD. Since many cats in this study were receiving medications such as benazepril, furosemide, and amlodipine that could potentially affect PAC or stimulate aspects of the RAAS, further analyses of PAC and survival time in CKD cats under certain conditions with and without medications may be needed. In the eplerenone study, there was no safety data on eplerenone in healthy cats or those with the conditions studied. Although cats were alternately assigned to either the control or eplerenone treatment, they were not randomly assigned eplerenone treatment, and the control group did not receive a placebo treatment. As cats whose owners refused eplerenone treatment were included in the control group, arguably these owners were more cautious or less willing to try treatments, which could have influenced survival. Owners and clinicians were not blinded to the eplerenone treatment. In addition, more than half of the cats in the non-eplerenone treated group had CKD only, compared to only 1/8 in the eplerenone-treated group. The higher BUN in some cats of non-eplerenone group may be an indicator that cats were more severely affected in this group, *e.g.,* more dehydrated and/or worse CHF. These findings might have contributed to a difference in survival times between the eplerenone and non-eplerenone groups. Further studies with double-blind, placebo-controlled trials would be required.

In conclusion, cats with CKD had significantly higher PAC than clinically healthy cats. In CKD, the survival of cats with high PAC was significantly shorter than those with normal PAC. The use of eplerenone also significantly prolonged the survival of cats with high PAC in CKD complicated with cardiac disease or hypertension. Although this study proposes PAC as a prognostic marker of CKD cats, eplerenone may be useful in prolonging cats' survival with high PAC in CKD complicated with cardiac disease or hypertension. Our results warrant further studies with double-blind, placebo-controlled trials.



Appendix-Figure A1. Correlation between plasma aldosterone concentration and age in 101 healthy cats. Data were analyzed using linear regression analysis.

Appendix-Table A1. Medications of cats with normal and high plasma aldosterone concentration (PAC) used for survival analysis in chronic kidney disease (CKD) group.

Group	IRIS stage *(n)*	Treatment *(n)*
Normal PAC	IRIS-2 (*n* = 24)	None (*n* = 20) Kidney support diet (*n* = 2) Benazepril 0.5 to 0.6 mg/kg, q24h (*n* = 2) Fluid infusion (*n* = 1)
	IRIS-3 (*n* = 12)	None (*n* = 2) Kidney support diet (*n* = 10) Fluid infusion (*n* = 5) Benazepril 0.5 to 0.6 mg/kg, q24h (*n* = 2) Potassium gluconate (*n* = 2) Phosphate binder (*n* = 2) Amlodipine 0.2 to 0.4 mg/kg, q24h or q12h (*n* = 1)
	IRIS-4 (*n* = 3)	Kidney support diet (*n* = 2) Fluid infusion (*n* = 2) Antibiotics (*n* = 1)
High PAC	IRIS-2 (*n* = 8)	None (*n* = 5) Kidney support diet (*n* = 3) Benazepril 0.6 mg/kg, q24h (*n* = 2) Amlodipine 0.2 mg/kg, q24h (*n* = 1) Potassium gluconate (*n* = 1) Fluid infusion (*n* = 1)
	IRIS-3 (*n* = 6)	Kidney support diet (*n* = 5) Benazepril 0.6 mg/kg, q24h (*n* = 2) Amlodipine 0.2–0.4 mg/kg, q24h (*n* = 2) Potassium gluconate (*n* = 1) Phosphate binder (*n* = 1) Fluid infusion (*n* = 2)
	IRIS-4 (*n* = 2)	Kidney support diet (*n* = 1) Fluid infusion (*n* = 1)

IRIS — International Renal Interest Society.

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