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ORIGINAL RESEARCH

Survival of dogs with pituitary-dependent hyperadrenocorticism treated twice daily with low doses of trilostane

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Abstract

Background: Twice daily low trilostane doses have proven to be effective to manage canine Cushing's syndrome. However, survival and prognostic factors in dogs treated with this protocol have not been evaluated. The aim of the study was to evaluate survival and prognostic factors, including systolic blood pressure (SBP) at diagnosis, in dogs with pituitary-dependent hypercortisolism (PDH) treated with low trilostane doses.

Methods: Medical records of 91 dogs newly diagnosed with PDH initially treated with 0.2–1.1 mg/kg of trilostane twice daily were retrospectively included. Survival times were calculated using the Kaplan–Meier estimator. Univariable and multivariable analysis were performed using the Cox proportional hazard regression analysis.

Results: Overall, median survival was 998 days (range 26–1832 days, 95% confidence interval = 755–1241 days). In the multivariable analysis, age (hazard ratio [HR] = 1.337, $p < 0.001$), presence of calcinosis cutis (HR = 5.271, $p < 0.001$), body condition score (BCS) ≤3/9 (HR = 8.100, $p < 0.001$) and higher platelet count (HR = 1.002, $p = 0.022$) were negatively correlated with survival. SBP was not associated with survival.

Conclusions: Low-dose trilostane treatment twice daily provides slightly longer survival than previously reported for dogs with PDH treated once or twice daily at higher doses. Older age, presence of calcinosis cutis, low BCS and higher platelet count, but not systemic hypertension, are predictive of poorer prognosis in dogs with PDH.

INTRODUCTION

Trilostane, a competitive inhibitor of the 3β-hydroxysteroid dehydrogenase-isomerase enzyme system, is the most common drug used to treat canine Cushing's syndrome (CS).[1,2] This drug has proven to be effective in reducing cortisol concentrations and managing clinical signs both in dogs with pituitary-dependent hypercortisolism (PDH) and adrenal-dependent hypercortisolism (ADH).[3–5] Survival times of dogs with PDH treated with trilostane range from 549 to 930 days,[4–9] and factors associated with a poor prognosis include older age at diagnosis, high serum phosphate concentrations and higher weight at diagnosis.[6–9] The influence of systolic blood pressure (SBP) on survival of dogs with CS has not been frequently reported, but where it has, it does not seem to be associated with a poorer outcome.[3,7,8] However, in people with CS, hypertension is associated with higher mortality.[10,11]

Lower initial trilostane doses (0.2–1.1 mg/kg) administered twice daily have proven to be effective to control clinical signs in dogs with PDH, with fewer adverse effects than higher doses.[12,13] However, long-term survival of dogs with this treatment protocol has not been evaluated.

The aims of this study were to evaluate survival of dogs with PDH treated with low initial trilostane doses (0.2–1.1 mg/kg twice daily) and the influence of clinicopathological variables on survival time. A second aim was to evaluate the influence of SBP at diagnosis on survival in dogs with PDH.

MATERIALS AND METHODS

Clinical records of dogs with newly diagnosed naturally occurring CS that were presented to the Veterinary Teaching Hospital Complutense of Madrid between January 2013 and December 2018 were retrospectively reviewed. All owners signed a consent form authorising to use the data from their pets for research purposes. According to the governmental ethics committee consultation at the time of the study, no ethical approval was necessary to perform the study.

Suspicion of CS was based on the presence of compatible clinical signs, physical examination findings and laboratory abnormalities. Diagnosis of CS was confirmed when two of the following tests were positive: urinary cortisol:creatinine ratio (UCCR), adrenocorticotropic hormone-stimulation test (ACTH-st) and/or low-dose dexamethasone suppression test (LDDST). Ultrasonographic findings, LDDST results and/or endogenous ACTH concentrations were used to discriminate between PDH and ADH.[14]

Exclusion criteria were lack of a definitive diagnosis of CS, previous treatments for CS, refusal of treatment for CS, diagnosis of ADH, lack of compliance with treatment, less than 1 year of follow-up, as well as a long delay between diagnosis and treatment initiation.

Data from signalment (age, sex, reproductive status, breed and bodyweight), concurrent diseases, clinical signs (polydipsia, polyuria, polyphagia, panting, neurological signs), physical examination findings (coat and skin abnormalities, 'potbellied' appearance, macroscopically visible calcinosis cutis, body condition score [BCS]) and SBP were recorded.

In all dogs, SBP was measured at diagnosis using Doppler ultrasonography methodology (Vettex Uni 900, Huntleigh Diagnostics). The procedure was performed as described in the American College of Veterinary Internal Medicine (ACVIM) consensus recommendations.[15,16] Dogs were classified as hypertensive when SBP was 160 mmHg or higher and subclassified, according to the risk of target organ damage, as normotensive (SBP <140 mmHg), prehypertensive (SBP 140–159 mmHg), moderately hypertensive (SBP 160–179 mmHg) and severely hypertensive (SBP ≥180 mmHg).[15] Hypertensive dogs were treated with antihypertensive drugs following the guidelines for the management of systemic hypertension in dogs and cats.[15,16]

For dogs included in the study (i.e. with a definitive diagnosis of CS), results from complete blood count (CBC), complete biochemistry, serum electrolytes, urinalysis, urinary protein:creatinine ratio (UPC), urinary culture, ACTH-st, LDDST and UCCR at diagnosis were also reviewed when available. To avoid differences in measurement techniques, only those parameters evaluated at the laboratory of the Veterinary Teaching Hospital were included.

Serum biochemical parameters evaluated were glucose, urea, creatinine, total plasma proteins, alanine-aminotransferase, alkaline phosphatase, cholesterol, sodium, potassium, chloride and total calcium. Serum and urinary cortisol concentrations were measured by chemiluminescence immunoassay (Immulite 2000, Siemens Healthcare).

Complete urinalysis, UPC and urinary culture of a sample obtained by cystocentesis were also included. Results from the UPC were only considered if a negative urinary culture and inactive sediment were present. Proteinuria was considered if UPC was greater than 0.5.[17]

In all dogs, trilostane treatment was administered at an initial dose of 0.2–1.1 mg/kg twice daily. Monitoring and dose adjustments were performed based on clinical signs and the results of the ACTH-st and UCCR.[18] In those dogs with clinical signs consistent with CS and post-ACTH cortisol concentrations below 5.5 μg/dl but an increased UCCR, frequency of administration was increased to three times daily.[18,19] In dogs presenting with poor appetite, trilostane treatment was temporarily discontinued. Final trilostane dose and frequency were also recorded.

The 16 January 2020 was considered as the date of study closure, providing at least 1 year of follow-up for all dogs included. For this study, all causes of death were considered. Survival time was established as the time since trilostane treatment was started until death or losts for follow-up.

Variables assessed as potential prognostic factors were data from signalment, clinical signs at diagnosis, physical examination findings, laboratory results, SBP, hormonal tests results and trilostane dose through follow-up. Age and weight were also categorised as dichotomous variables, with the median used as the cut-off point. Concurrent diseases considered were neoplasia, chronic kidney disease (CKD), mitral valve disease and diabetes mellitus. For mitral valve disease and CKD, staging was also recorded.[17,20] Mitral valve disease diagnosis and staging was performed following the guidelines from the cardiology service of our clinic.[20] Single or multiple concurrent diseases were additionally analysed. Development of permanent hypoadrenocorticism or permanent trilostane discontinuation were also considered. The latter was established when trilostane dosage could be tapered down progressively until complete discontinuation of the drug and without recurrence of clinical signs of CS or hypercortisolemia by means of ACTH-st results performed routinely. Before the complete discontinuation of the drug, a once daily regimen was attempted in all

cases. Temporary trilostane discontinuation (i.e. due to anorexia, vomiting, etc.) of less than 1 week duration was also recorded, as well as the number of times it occurred in each dog.

Survival analysis was performed using the Kaplan–Meier estimator (results expressed as median and 95% confidence interval [95% CI]) and univariable Cox proportional hazards regression analysis, in order to screen potential predictor factors for a subsequent inclusion in a multivariable model (results expressed as hazard ratio [HR] and 95% CI). Differences between Kaplan–Meier curves were evaluated using the log-rank test. Multivariable analysis was also performed using the Cox proportional hazards regression analysis; variables with a p-value < 0.2 in the univariable analysis were included in the multivariable model using Wald forward stepwise selection. If a model could not be reached, variables were removed according to the number of missing data until a model was obtained. In all cases significance level was established as $p < 0.05$.

RESULTS

During the study period, 116 dogs were diagnosed with CS. Eighteen dogs were diagnosed with ADH. Seven dogs with PDH were excluded; two because other treatments (i.e. cabergoline and pituitary radiotherapy) were preferred, one because the owner refused to provide any treatment, two due to lack of owner's compliance with trilostane treatment, and two due to a lag in time between diagnosis and initiation of treatment. Thus, 91 dogs with PDH were finally included.

Population description

Signalment and concurrent diseases

Demographic characteristics are described in Table 1. At diagnosis 58/91 (63.7%) of the dogs had concurrent diseases (Table 2) and 17/91 (18.7%) had more than one concurrent disease.

Clinical signs and physical examination findings at diagnosis

Descriptive statistics of clinical signs and physical examination findings are described in Table 3. Of the four dogs with a BCS ≤3/9, one had CKD IRIS stage 3, one diabetes mellitus, one leishmaniosis and one did not have any concurrent disease.

Clinicopathological parameters

Complete descriptive statistics of CBC, biochemistry and hormonal tests are detailed in Table 4, and data from urinary parameters in Table 5.

TABLE 1 Signalment data in 91 dogs with pituitary-dependent hypercortisolism

Variable	Observed value	Dogs, *n* (%)
Age (years)	11 (range 6–18, IQR 10–13)	
Bodyweight (kg)	11 (range 1.8–42.5, IQR 6.6–16.55)	
Sex and reproductive status		
Neutered female		38 (41.8%)
Neutered male		23 (25.3%)
Entire female		14 (15.4%)
Entire male		16 (17.6%)
Breed		
Mixed breed		37 (40.7%)
Pure breed		54 (59.3%)
Yorkshire Terrier		10
Miniature Schnauzer		7
West Highland White Terrier		6
Cocker Spaniel		3
Dachshund		3
French Bulldog		3
Maltese		3
Miniature Poodle		3
Shih Tzu		2
Beagle		2
Boxer		2
Pit-bull		2
Scottish Terrier		2
Labrador Retriever		1
Pomeranian		1
Bichon Frise		1
English Bulldog		1
Dalmatian		1
Lhasa Apso		1

Note: Observed value is expressed as median (range, IQR as Q1–Q3). *N* denotes number of dogs.
Abbreviations: IQR, interquartile range; Q1, quartile 1; Q3, quartile 3.

Initial trilostane dose and adjustments of the treatment

Median daily initial trilostane dose was 1.02 mg/kg (range 0.6–2.24 mg/kg, interquartile range [IQR] = 0.9–1.42 mg/kg) divided in two equal doses administered twice daily.

In 4/91 (4.4%) dogs, trilostane was permanently discontinued; among them, one dog (1/91, 1.1%) developed permanent hypoadrenocorticism and required permanent treatment both with glucocorticoids and mineralocorticoids. In this particular dog, initial daily trilostane dose was 1.14 mg/kg which was increased up to 1.74 mg/kg 4 months before the development of hypoadrenocorticism. Median time since initiation of trilostane treatment until development of hypoadrenocorticism or permanent trilostane discontinuation was 415 days (range 96–1119 days,

TABLE 2 Concurrent diseases at diagnosis in 91 dogs with pituitary-dependent hypercortisolism

Concurrent diseases at diagnosis	Dogs, *n* (%)
None	33 (36.3%)
Neoplasia	15 (16.5%)
Mammary carcinoma/adenocarcinoma	5
Anal sac carcinoma	2
Mast cell tumour	2
Squamous cell carcinoma	1
Malignant melanoma	1
Chemodectoma	1
Hystiocitic sarcoma	1
Seminoma	1
Granular cell tumour of the tongue	1
Mitral valve disease	17 (18.7%)
Stage B1	10
Stage B2	7
Chronic kidney disease	9 (9.9%)
IRIS stage 2	7
IRIS stage 3	2
Diabetes mellitus	10 (11.0%)
Hypothyroidism	5 (5.5%)
Leishmaniosis (LeishVet stage I)[39]	3 (3.3%)
Neurological diseases	5 (5.5%)
Idiopathic epilepsy	2
Horner's syndrome	1
Idiopathic vestibular disease	1
Cognitive dysfunction syndrome	1
Pancreatitis	5 (5.5%)
Food allergy	3 (3.3%)
Congenital portosystemic shunt	2 (2.2%)
Abdominal masses involving other structures. Definitive diagnosis not reached	2 (2.2%)
Several concurrent diseases	17 (18.7%)
2	15
More than 2	2

Note: *N* denotes number of dogs.

TABLE 3 Clinical signs and physical examination findings at diagnosis in 91 dogs with pituitary-dependent hypercortisolism

Variable	Observed value	Dogs, *n* (%)
Duration of clinical signs before diagnosis (months)	7 (range 1–36, IQR 4–12)	
Polydipsia		83 (91.2%)
Polyuria		82 (90.1%)
Polyphagia		77 (84.6%)
Excessive panting at rest		50 (54.9%)
Coat abnormalities (i.e. dull coat, alopecia, hair regrowth retardation, changes in hair colour)		71 (78.0%)
Thin skin		62 (68.1%)
'Potbellied' appearance		56 (61.5%)
Neuromuscular signs (i.e. seizures, dullness, pseudomyotonia, Horner's syndrome, peripheral neuropathies, aggressiveness, vestibular disease)		14 (15.4%)
Calcinosis cutis		7 (7.7%)
BCS		
BCS ≤3/9		4 (4.4%)
BCS between 4 and 6		52 (57.1%)
BCS ≥7/9		35 (38.5%)
SBP (mmHg)	170 (range 120–280, IQR 150–200)	
Systemic hypertension (SBP ≥160 mmHg)		63 (69.2%)
Classification according to the risk of TOD		
Normotensive (SBP <140 mmHg)		14 (15.4%)
Prehypertensive (SBP between 140 and 159 mmHg)		14 (15.4%)
Moderately hypertensive (SBP between 160 and 179 mmHg)		23 (25.3%)
Severely hypertensive (SBP ≥180 mmHg)		40 (44.0%)

Note: Observed value is expressed as median (range, IQR as Q1–Q3). *N* denotes number of dogs.
Abbreviations: BCS, body condition score; IQR, interquartile range; Q1, quartile 1; Q3, quartile 3; SBP, systolic blood pressure; TOD, target organ damage.

IQR = 154–778 days). Trilostane was temporarily discontinued in 26/91 dogs (28.6%); once in 18/26 (69.2%), twice in 6/26 (23.1%) and three times in 2/26 (7.7%) dogs.

Among dogs in which trilostane was not permanently discontinued (n = 87), the final median daily trilostane dose was 1.3 mg/kg (range 0.27–8.85 mg/kg, IQR = 0.9–2.0 mg/kg) administered once daily (1/87, 1.2%), or divided in equal doses twice daily (81/87, 93.1%), or three times daily (5/87, 5.7%).

Survival analysis

At the time of study closure, 25/91 (27.5%) of dogs were still alive, 57/91 (62.6%) were dead and 9/91 (9.9%) were lost to follow-up. All dogs lost to follow-up were known to be alive 1 year after diagnosis, 6/9 (66.7%) 2 years after, 2/9 (22.2%) 3 years after and 1/9 (11.1%) 4 years afterwards.

Overall median survival was 998 days (range 26–1832 days, 95% CI = 755–1241 days) (Figure 1). More than 1 year survival was achieved in 68/91 dogs (74.7%), more than 2 years in 43/91 dogs (47.2%), more than 3 years in 24 dogs (26.4%), more than 4 years in 8/91 (8.8%) and one dog (1.1%) lived more than 5 years.

Most dogs were euthanised on welfare grounds (41/57). Postmortem examination was not available in most cases. Causes of death and reasons for euthanasia are described in Table 6.

TABLE 4 Descriptive statistics of hematological, biochemical and hormonal parameters of dogs with pituitary-dependent hypercortisolism (PDH) at the moment of diagnosis

	Dogs with PDH ($n = 91$)					
Parameter (units) [reference range]	***N***	**Median**	**Range (min–max)**	**IQR (Q1–Q3)**	**Number decreased**	**Number increased**
Haematocrit (%) [37.0–55.0]	90	48.45	28.60–67.50	45.85–52.50	4 (4.4%)	12 (13.3%)
Haemoglobin (g/dl) [12.0–18.0]	90	16.45	9.9–20.8	15.7–17.8	4 (4.4%)	20 (22.2%)
RBC ($\times 10^6/\mu$l) [5.5–8.5]	90	7.0	4.5–9.4	6.5–7.6	4 (4.4%)	5 (5.6%)
MCV (fl) [60.00–76.00]	90	68.55	51.20–79.00	66.15–68.55	3 (3.3%)	6 (6.7%)
MCH (pg) [19.50–24.50]	90	23.50	18.20–36.00	22.37–24.20	1 (1.1%)	20 (22.2%)
MCHC (g/dl) [32.00–36.00]	90	34.00	30.80–39.20	33.10–34.60	3 (3.3%)	7 (7.8%)
Platelets ($\times 10^3/\mu$l) [200–500]	89	436	133–1115	347—529	3 (3.4%)	29 (32.6%)
WBC ($\times 10^3/\mu$l) [6.00–17.00]	90	8.55	3.80–17.00	6.30–10.70	16 (17.8%)	1 (1.1%)
Mature neutrophils ($\times 10^3/\mu$l) [3.00–11.50]	87	6.38	2.86–14.26	4.72–8.00	4 (4.6%)	4 (4.6%)
Immature neutrophils ($\times 10^3/\mu$l) [0.00–0.30]	87	0.00	0.00–0.33	0.00–0.00	–	1 (1.1%)
Lymphocytes ($\times 10^3/\mu$l) [1.00–4.00]	87	1.33	0.30–4.90	0.84–1.98	33 (37.9%)	1 (1.1%)
Monocytes ($\times 10^3/\mu$l) [0.15–1.35]	87	0.37	0.00–2.28	0.24–0.60	12 (13.8%)	4 (4.6%)
Eosinophils ($\times 10^3/\mu$l) [0.10–1.25]	87	0.12	0.00–1.13	0.00–0.26	42 (48.3%)	0 (0.0%)
Basophils ($\times 10^3/\mu$l) [0.00–0.10]	85	0.00	0.00–0.09	0.00–0.00	0 (0.0%)	0 (0.0%)
Glucose (mg/dl) [70–125]	83	103	64–476	94–121	1 (1.2%)	19 (22.9%)
Urea (mg/dl) [10–58]	87	33	13–168	20–46	0 (0.0%)	16 (18.4%)
Creatinine (mg/dl) [0.3–1.4]	91	0.7	0.5–2.1	0.5–0.9	0 (0.0%)	8 (8.8%)
Cholesterol (mg/dl) [125–310]	54	326	157–1135	251–456	0 (0.0%)	31 (57.4%)
Total plasmatic proteins (g/dl) [5.5–7.8]	90	7.0	4.9–9.8	6.2–7.5	4 (4.4%)	17 (18.9%)
ALT (U/L) [10–60]	90	81	15–1764	46–138	0 (0.0%)	61 (67.8%)
ALKP (U/L) [25–110]	86	780	26–7452	178–1602	0 (0.0%)	74 (86.0%)
Sodium (mEq/L) [140–155]	42	149	141–160	146–150	0 (0.0%)	2 (4.8%)
Potassium (mEq/L) [3.8–5.8]	73	4.2	3.10–5.9	3.9–4.6	9 (12.3%)	1 (1.4%)
Sodium/potassium ratio	42	34.83	27.27–46.36	32.17–38.20	–	–
Chloride (mEq/L) [105–125]	37	110	102–122	108–114	3 (8.1%)	0 (0.0%)
Total calcium (mg/dl) [8–13]	54	9.7	7.3–11.9	9.0–10.2	1 (1.9%)	0 (0.0%)
Pre-ACTH cortisol (μg/dl) [0.5–5.5]	74	3.67	0.75–24.0	2.42–5.50	0 (0.0%)	18 (24.3%)
Post-ACTH cortisol (μg/dl) [8–18]	74	19.4	10.3–90.5	16.55–26.65	0 (0.0%)	52 (70.3%)
Basal (0 hour LDDST) cortisol (μg/dl) [0.5–5.5]	20	5.52	0.67–13.6	3.44–6.08	0 (0.0%)	10 (50%)
4 hours post-dexamethasone cortisol (μg/dl) [<1]	20	1.53	0.1–7.32	0.82–4.13	–	14 (70%)
8 hours post-dexamethasone cortisol (μg/dl) [<1]	20	3.22	1.01–5.54	1.72–4.52	–	20 (100%)
Urinary cortisol to creatinine ratio ($\times 10^{-6}$) [16–40]	63	164	8–1769	93–280	1 (1.6%)	62 (9.8.4%)

Note: *N* denotes number of dogs.
Abbreviations: ACTH, adrenocorticotropic hormone; ALKP, alkaline phosphatase; ALT, alanine aminotransferase; IQR, interquartile range; LDDST, low-dose dexamethasone suppression test; max, maximum; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentrations; min, minimum; Q1, quartile 1; Q3, quartile 3; RBC, red blood cells; WBC, white blood cells.

Univariable analysis

Clinical and clinicopathologic variables with a p-value <0.2 in the univariable analysis are detailed in Table 7. Figures 2 and 3 show differences in survival depending on the presence of calcinosis cutis and on the BCS, respectively.

Initial trilostane dose and adjustments of the treatment

Neither daily initial trilostane dosage (HR = 1.358, 95% CI = 0.687−2.681, p = 0.379) nor final daily trilostane dosage (HR = 0.852, 95% CI = 0.615−1.180, p = 0.335) influenced survival time. Frequency of trilostane administration at the end of treatment (twice daily vs. three times daily) did not influence survival.

Development of hypoadrenocorticism or lack of need of trilostane studied together did not influence survival time (HR = 0.980, 95% CI = 0.236−4.069, p = 0.978), as well as time since trilostane initiation until lack of need for trilostane (HR = 0.994, 95% CI = 0.982−1.007, p = 0.361). Neither temporary trilostane discontinuation nor the number of times the drug needed to be suspended affected survival.

TABLE 5 Descriptive statistics of urine parameters at diagnosis

Variable	*N*	Observed value	Dogs, *n* (%)
Urinary specific gravity	86	1.015 (range 1.000–1.058, IQR 1.010–1.022)	
Glycosuria	84		9 (10.7%)
Ketonuria	84		0 (0.0%)
Crystalluria	83		5 (6.0%)
Pyuria (≥5 WBC/hpf)	83		5 (6.0%)
Haematuria (≥10 RBC/hpf)	83		12 (14.5%)
Bacteriuria	83		11 (13.3%)
UPC	42	0.5 (range 0.05–14.50, IQR 0.20–1.82)	
UPC >0.5	42		13 (31.0%)
Positive urinary culture	74		13 (17.6%)

Note: The second column indicates the number of dogs in which the parameters were measured. Observed value is detailed as median (range, IQR as Q1–Q3). *N* denotes number of dogs.
Abbreviations: IQR, interquartile range; Q1, quartile 1; Q3, quartile 3; RBC, red blood cells; UPC, urinary protein:creatinine ratio; WBC, white blood cells.

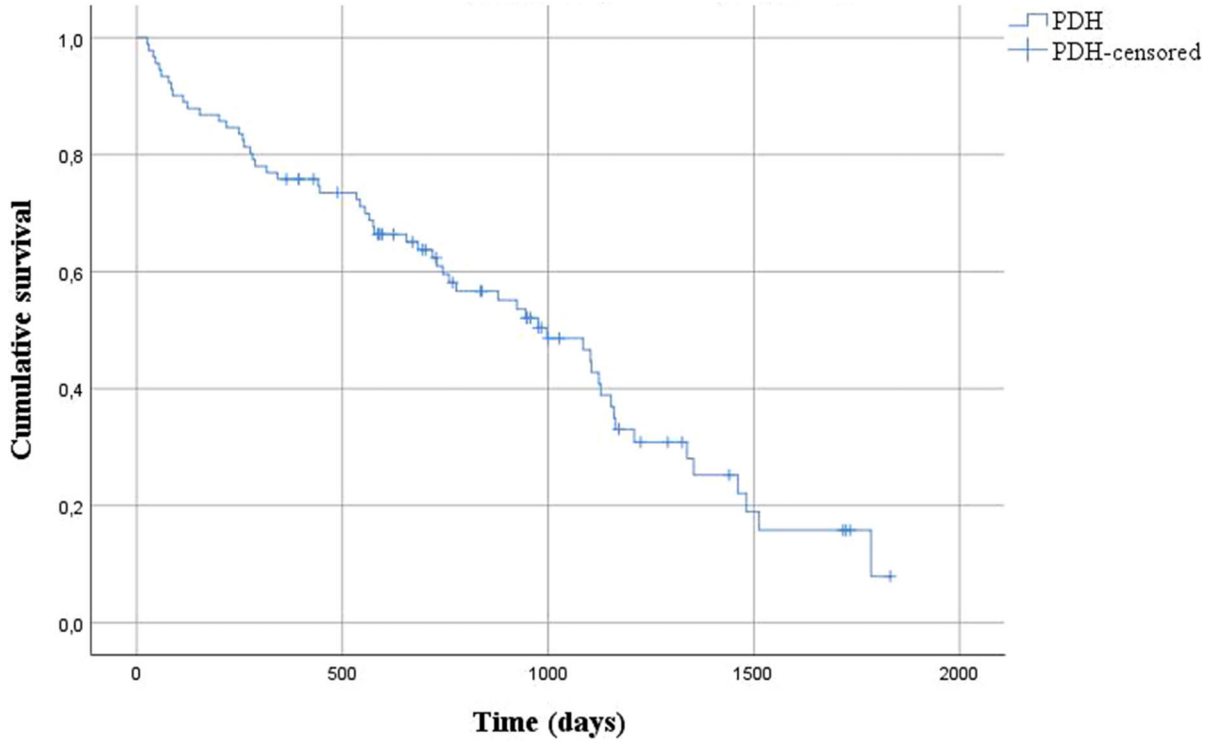

FIGURE 1 Overall survival of dogs with pituitary-dependent hypercortisolism (PDH) initially treated with low doses of trilostane

Multivariable risk analysis

Variables with a *p*-value <0.2 in the univariable analysis were included in the model. The final multivariable model included: age at diagnosis (HR = 1.337, 95% CI = 1.186−1.506, $p < 0.001$), presence of calcinosis cutis (HR = 5.271, 95% CI = 2.237−12.418, $p < 0.001$), BCS ≤3/9 (HR = 8.100, 95% CI = 2.498−26.267, $p < 0.001$) and higher platelet count (HR = 1.002, 95% CI = 1.000−1.003, $p = 0.022$).

DISCUSSION

The overall median survival time for dogs with PDH treated with low initial trilostane doses administered twice daily was 998 days (95% CI = 755−1241 days), which is slightly longer than that reported for higher doses of trilostane administered twice (833−930 days) or once daily (532−766 days).[4–9] Dogs with PDH left untreated have a reported median survival time of 506 days,[21] which is nearly half of the survival time reported in the present study, reinforcing the importance of treating this disease. As lower trilostane doses are effective in controlling clinical signs,[12] are associated with fewer adverse effects[13] and survival time is somewhat longer than previously reported, initial low doses of trilostane administered twice daily are a good option for the management of dogs with PDH.

As experience with trilostane has increased, the recommended starting dose has decreased. The initial trilostane recommended dose was 3–6 mg/kg administered twice daily, but some studies have

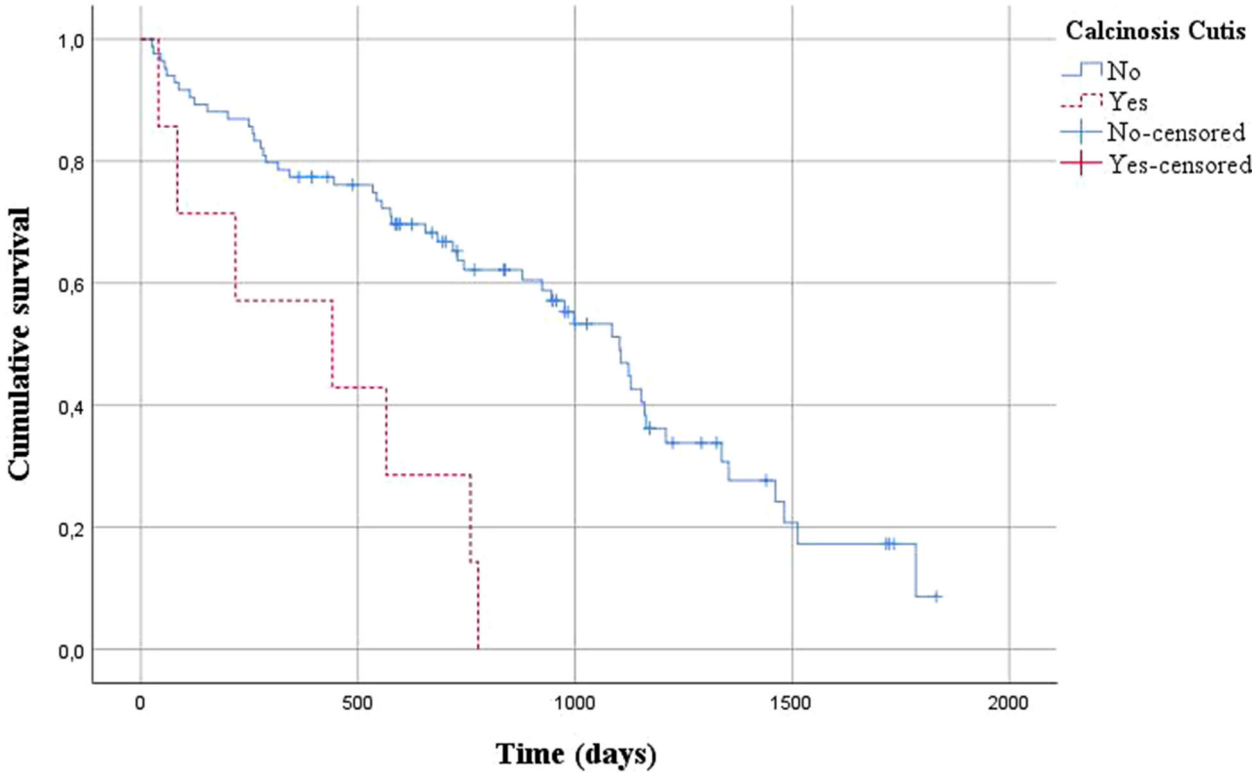

FIGURE 2 Influence of calcinosis cutis on survival time of dogs with pituitary-dependent hypercortisolism

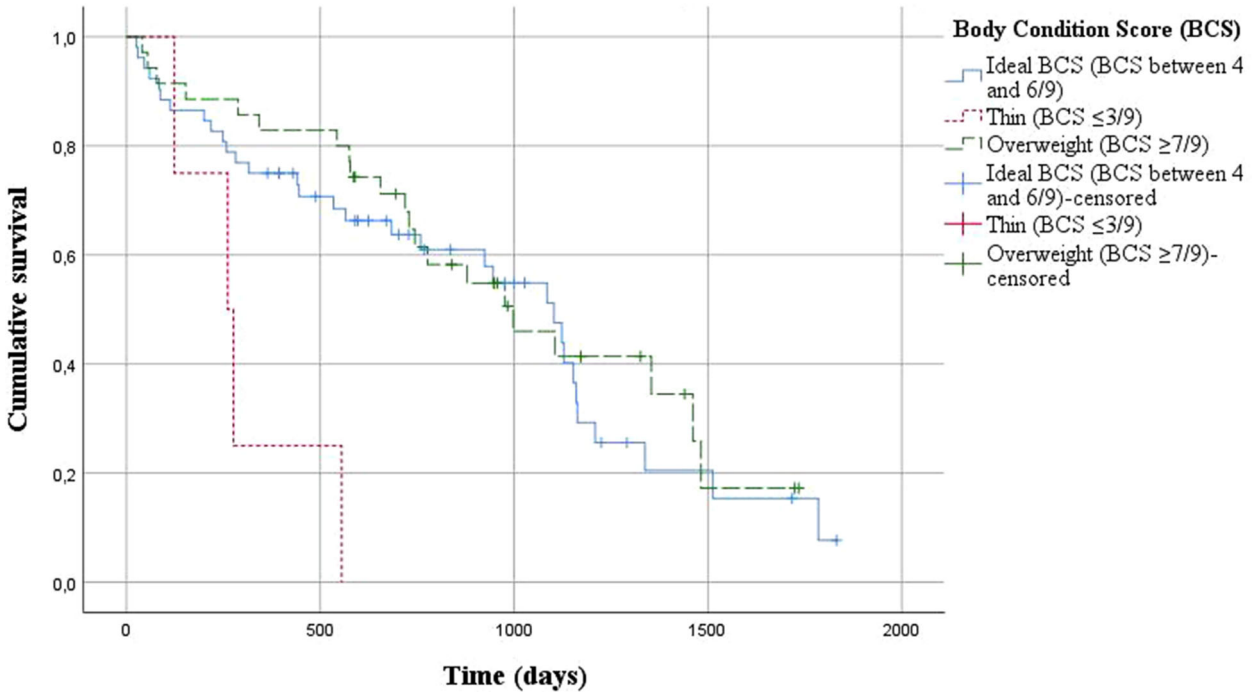

FIGURE 3 Influence of body condition score on survival time of dogs with pituitary-dependent hypercortisolism

demonstrated that using lower doses (0.21−1.1 mg/kg every 12 hours) was effective.[12] Nowadays, the use of low trilostane doses is more common, with a dose of 2 mg/kg daily (either administered once or divided twice daily)[22] or even lower (0.5 mg/kg every 12 hours)[18] usually recommended. This 2 mg/kg daily dose is in the upper range of the doses used in the present study and can be considered nowadays more as a standard dose rather than a low dose. However, to the authors' knowledge, this is the first survival study consistently including only dogs initially treated with this low dose twice daily regimen and is more likely reflecting the actual recommendations for dogs with CS.

Final median daily trilostane dose in the present study was 1.3 mg/kg, which is lower than previously

TABLE 6 Causes of death recorded in 57 dogs with pituitary-dependent hypercortisolism

Causes of death	Dogs, *n* (%)
Unknown	4/57 (7.0%)
Sudden death	4/57 (7.0%)
Miscellaneous (rodenticide intoxication, road traffic accident)	2/57 (3.5%)
Rupture of gallbladder mucocele	1/57 (1.7%)
Status epilepticus	1/57 (1.7%)
Euthanasia	45/57 (78.9%)
Neoplastic diseases (mast cell tumour, gastric carcinoma, prostatic carcinoma, thyroid carcinoma, parathyroid carcinoma, oral melanoma, nasal carcinoma, ovarian neoplasia, lymphoma, intracranial neoplasia other than pituitary tumour)	13
Poor quality of life	11
Chronic kidney disease	8
Neurological signs. Definitive diagnosis not reached	5
Pituitary macrotumour	2
Osteoarthrosis	2
Diabetic ketoacidosis	1
Pulmonary fibrosis	1
Pancreatitis	1
Pseudomyotonia	1

Note: Death was considered as 'unknown' when the owners or the veterinary practitioners consulted were unable (or unwilling) to explain why did the dog died but was not a sudden event; on the other hand, it was considered as 'sudden death' when the owners found their dog dead without previous clinical signs of deterioration. *N* denotes number of dogs.

reported (2.68–6.4 mg/kg/day).[4,12,13] This indicates that hypercortisolemia and its clinical signs can be controlled long term using low doses of trilostane, with the advantage of achieving slightly longer survival times and fewer side effects.

Negative prognostic factors identified in the multivariable analysis were increased age at diagnosis, presence of calcinosis cutis, a BCS ≤3/9 and an elevated platelet count; but not systemic hypertension (SH). Age at diagnosis has been widely described as a factor associated with poor prognosis in dogs with PDH, regardless of the treatment administered.[6–9] However, presence of calcinosis cutis, decreased BCS or elevated platelet count have not been previously described.

It is not unexpected that age is correlated with a shorter survival, as older dogs, independently of PDH, are at higher risk of all-cause mortality. Also, concurrent diseases are more likely as age increases. Even though concurrent diseases were not selected as a negative prognostic factor, these conditions can impact on quality of life, which can promote euthanasia. Causes of death recorded in this study also support this, as many dogs died of causes not attributable to PDH.

Dogs with PDH and calcinosis cutis had shorter survival times (441 days) than dogs without (1103 days). Serum phosphate concentrations and parathyroid hormone concentrations in dogs with CS are significantly higher than in healthy dogs.[23,24] This has been hypothesised to be one of the possible mechanisms for the development of soft tissue calcification, and therefore calcinosis cutis.[24] Dogs with PDH treated with trilostane with serum phosphate concentrations >1.45 mmol/L have lower survival times than those with values ≤1.45 mmol/L.[7] Unfortunately, serum ionised calcium and phosphate concentrations were not routinely measured and were not included in the study, therefore it is unclear if the relation between calcinosis cutis and survival might be related to increased phosphate concentrations.

Thin dogs (i.e. BCS ≤3/9) had shorter survival times (261 days) than those with an ideal BCS (1103 days) and overweight dogs (998 days). Influence of BCS on survival of dogs with CS has not been well studied, and a low BCS has not been described to be a negative prognostic factor previously.[25] However, lower BCS has been associated with lower survival times in dogs with other diseases such as CKD,[26,27] lymphoma[28] or heart failure.[29] A lower BCS might indicated increased protein catabolism, secondary to chronic hypercortisolism or other diseases.[18,30] In the univariable analysis, concurrent diseases were not correlated with survival time and did not fit in the multivariable model; however, as three of the four dogs with a low BCS did have concurrent diseases, their influence cannot be excluded. In dogs with ADH, it has been reported that muscle weakness is associated with shorted survival times[3]; unfortunately, in the present study weakness or degree of sarcopenia were not recorded, and thus correlations among them and BCS were not possible.

As platelet count increased so did the hazards for all-cause mortality in the present study. This has not been previously described in dogs with CS. In dogs, a direct link between thrombocytosis and increased risk of thrombosis has not been proven, but an incidence of thromboembolic disease of 8% in dogs with thrombocytosis secondary to other diseases, including CS, has been reported.[31] It might be possible that dogs with a higher platelet count were more likely to develop complications related to a hypercoagulative status; however, the lack of necropsy in most cases and the difficulty of achieving a definitive diagnosis of a thromboembolic disease, makes it difficult to confirm this theory.

Development of permanent hypoadrenocorticism can occur in 15% of dogs during the first 2 years of trilostane treatment and in 26% of dogs after 4 years of treatment. This does not seem to be related with trilostane dose, and it is reported to be permanent in 6% of the dogs.[32] In the present study, trilostane was permanently discontinued in 4.4% of the dogs but only one (1.1%) developed permanent hypoadrenocorticism. Thus, the incidence of permanent hypoadrenocorticism was at the lower end of the reported range.[4,12,32,33] Permanent hypoadrenocorticism and permanent or temporarily trilostane discontinuation were not related with survival time.

In the present study, prevalence of SH (69.2%) and prevalence of severe SH (44%) were similar to previously reported.[34,35] Hypertension is known to cause

TABLE 7 Univariable survival analysis of clinicopathological variables of dogs with pituitary-dependent hypercortisolism

Variable	Categories	Median survival (days) (95% CI)	Hazard ratio (95% CI)	*p*-Value		*p*-Value (log-rank)
Age (years)		–	1.386 (1.225–1.586)	<0.001		–
Age	≤11 years	1338 (1053–1623)	Baseline	<0.001		<0.001
	>11 years	577 (389–765)	3.614 (2.061–6.340)			
Sex	Male	1153 (1031–1275)	Baseline	0.010		0.009
	Female	719 (493–945)	2.064 (1.186–3.592)			
Sex and reproductive status	Entire male	1153 (909–1398)	Baseline	–	0.028	.020
	Entire female	445 (258–632)	3.178 (1.308–7.721)	0.011		
	Neutered male	1128 (750–1506)	1.135 (0.454–2.834)	0.787		
	Neutered female	777 (308–1247)	1.919 (0.861–4.279)	0.111		
Neoplasia	No	1085 (814–1356)	Baseline	0.130		0.126
	Yes	577 (0–1310)	1.625 (0.867–3.044)			
Diabetes mellitus	No	976 (658–1294)	Baseline	0.164		0.158
	Yes	1482 (500–2464)	0.536 (0.223–1.289)			
Coat abnormalities	No	1163 (495–1831)	Baseline	0.072		0.068
	Yes	946 (590–1302)	1.897 (0.944–3.813)			
Thin skin	No	1123 (742–1504)	Baseline	0.175		0.172
	Yes	976 (643–1309)	1.519 (0.831–2.777)			
Calcinosis cutis	No	1103 (931–1275)	Baseline	0.001		<0.001
	Yes	441 (0–1013)	3.982 (1.743–9.098)			
BCS	BCS ≤3/9	261 (112–409)	4.750 (1.588–14.207)	0.005	0.010	0.004
	BCS 4–6/9	1103 (875–1331)	Baseline	–		
	BCS ≥7/9	998 (643–1353)	0.843 (0.484–1.470)	0.547		
Haematocrit (%)			0.958 (0.916–1.003)	0.066		–
Hb (g/dl)			0.837 (0.727–0.963)	0.013		–
Decreased Hb (Hb < 12 g/dl)	Normal	1085 (896–1274)	Baseline	0.001		<0.001
	Decreased	59 (0–135)	6.564 (2.273–18.957)			
MCH (pg)			0.843 (0.716–0.993)	0.040		–
PLT ($\times 10^3/\mu$l)			1.003 (1.001–1.004)	<0.001		–
Thrombocytosis (PLT ≥$500 \times 10^3/\mu$l)	No	1105 (920–1290)	Baseline	0.007		0.006
	Yes	655 (470–840)	2.121 (1.226–3.669)			
Lymphocytes ($\times 10^3/\mu$l)			1.000 (0.999–1.000)	0.073		–
Glucose (mg/dl)			0.997 (0.993–1.001)	0.154		–
Urea (mg/dl)			1.011 (1.004–1.019)	0.003		–
Increased urea (>58 mg/dl)	No	1085 (890–1281)	Baseline	0.031		0.027
	Yes	575 (535–615)	2.005 (1.067–3.766)			
Cholesterol (mg/dl)			1.001 (1.000–1.003)	0.053		–
ALT (U/L)			1.001 (1.000–1.002)	0.009		–
Potassium (mEq/L)			1.908 (0.950–3.833)	0.069		–
Chloride (mEq/L)			0.810 (0.780–0.928)	0.002		–
Total calcium (mg/dl)			0.682 (0.446–1.044)	0.078		
USG			0.982 (0.960–1.004)	0.104		–
Glycosuria	No	976 (650–1301)	Baseline	0.133		0.126
	Yes	1482 (534–2430)	0.480 (0.184–1.251)			
Pyuria (≥5 WBC/hpf)	No	998 (733–1263)	Baseline	<0.001		<0.001
	Yes	84 (30–138)	9.291 (2.954–29.225)			
Bacteriuria	No	998 (629–1366)	Baseline	0.070		0.064
	Yes	543 (0–1249)	2.030 (0.944–4.366)			
Urinary culture	Negative	1123 (923–1323)	Baseline	0.003		0.002
	Positive	235 (0–737)	3.246 (1.482–7.111)			
1 hour post-ACTH cortisol (μg/dl)	–	–	1.023 (0.994–1.053)	0.129		–

Note: Only variables with a *p*-value <0.2 are shown. *N* denotes number of dogs.
Abbreviations: ACTH, adrenocorticotropic hormone; ALT, alanine aminotransferase; BCS, body condition score; CI, confidence interval; Hb, haemoglobin; MCH, mean corpuscular haemoglobin; PLT, platelet count; USG, urinary specific gravity; WBC, white blood cells.

damage in target organs[15]; which worsens concurrent diseases and quality of life and could potentially impact on lifespan. In human medicine, hypertension is known to increase mortality in patients with CS.[10,11] However, in the present study neither SBP nor presence of severe SH at diagnosis were associated with a poorer outcome. It has to be considered that, in human medicine, the cause of death is usually available, which is not the case in veterinary medicine, as is the case in the present study. Furthermore, mortality in people with CS is usually related to cardiovascular diseases such as strokes and myocardial infarction,[10] for which hypertension is known to be a risk factor.[36] These cardiovascular diseases are not usually reported in veterinary medicine, and causes of death in this study do not support this theory. Despite the lack of a direct correlation between SH and survival time of dogs with CS, it is strongly recommended to routinely measure SBP as well as to treat hypertension, due to its detrimental effects. Hypertension was treated and monitored since diagnoses in all of the hypertensive dogs included, which might have influenced the lack of relationship with mortality.

This study has some limitations that should be acknowledged. Due to its retrospective nature, complete laboratory data were not available in all dogs. This might have affected the final multivariable model, as variables with lower number of observations were excluded first. Second, computed tomography or magnetic resonance imaging were available only in a few dogs with PDH; thus, data on pituitary size or other variables have not been included. The importance of this limitation is derived mainly from the fact that the survival time described for dogs with large pituitary tumors treated with radiotherapy or hypophysectomy is low,[37,38] and it might be reduced as well in dogs treated with trilostane. Also, the study was conducted in a partially referral institution; however, the study population was similar to that generally reported for dogs with CS, and thus results should be applicable to the general population. Finally, despite not being one of the aims of the study, it would have been interesting to evaluate the clinical control of the disease in these dogs under this treatment protocol. However, the low median final trilostane dose, considering that monitoring was based on clinical signs, and the long median survival time achieved can be interpreted as a good clinical control as previously described by other authors using this treatment protocol.[12] Moreover, in a previous study of the same research group involving 51 of the dogs included in the present one, all dogs included shown a good clinical control of the disease at some point during the first year of treatment.[19]

In conclusion, low dose twice daily trilostane treatment provides a slightly longer survival time than previously reported in dogs with CS treated with once or twice daily higher trilostane doses. Older age, calcinosis cutis, a thin BCS (i.e. ≤3/9) and higher platelet count are predictive of a poorer prognosis in dogs with PDH. Hypertension, when treated with antihypertensive medication, does not seem to affect the prognosis in dogs with PDH.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

Hypothesis generation and experimental design: Paula García San José, Carolina Arenas Bermejo and María Dolores Pérez Alenza. *Data collection*: Paula García San José, Daniel Alonso Miguel, Sandra González Sanz, Irene Clares Moral, Miriam Portero Fuentes and María Dolores Pérez Alenza. *Data analysis and interpretation*: Paula García San José, Carolina Arenas Bermejo, Daniel Alonso Miguel and María Dolores Pérez Alenza. *Writing and revising the manuscript*: Paula García San José, Carolina Arenas Bermejo and María Dolores Pérez Alenza.

ETHICS STATEMENT

Data were collected in the course of routine clinical treatment. According to the governmental ethics committee consultation at the time of the study, no ethical approval was necessary to perform the study. All owners signed a consent form authorising use of their pets' data for research purposes.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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