The Prognostic Value of Perioperative Profiles of ACTH and Cortisol for Recurrence after Transsphenoidal Hypophysectomy in Dogs with Corticotroph Adenomas

S.J. van Rijn, J.M. Hanson, D. Zierikzee, H.S. Kooistra, L.C. Penning, M.A. Tryfonidou, and B.P. Meij

Background: Transsphenoidal hypophysectomy is an effective treatment for dogs with pituitary-dependent hypercortisolism (PDH). However, long-term recurrence of hypercortisolism is a well-recognized problem, indicating the need for reliable prognostic indicators.

Objectives: The aim of this study was to evaluate the prognostic value of perioperative plasma ACTH and cortisol concentrations for identifying recurrence of hypercortisolism after transsphenoidal hypophysectomy.

Animals: A total of 112 dogs with PDH that underwent transsphenoidal hypophysectomy met the inclusion criteria of the study.

Methods: Hormone concentrations were measured preoperatively and 1–5 hours after surgery. Both absolute hormone concentrations and postoperative concentrations normalized to preoperative concentrations were included in analyses. The prognostic value of hormone concentrations was studied with Cox's proportional hazard analysis.

Results: Median follow-up and disease-free period were 1096 days and 896 days, respectively. Twenty-eight percent of patients had recurrence, with a median disease-free period of 588 days. Both absolute and normalized postoperative cortisol concentrations were significantly higher in dogs with recurrence than in dogs without recurrence. High ACTH 5 hours after surgery, high cortisol 1 and 4 hours after surgery, high normalized ACTH 3 hours after surgery, high normalized cortisol 4 hours after surgery and the random slope of cortisol were associated with a shorter disease-free period.

Conclusions and clinical importance: Individual perioperative hormone curves provide valuable information about the risk of recurrence after hypophysectomy. However, because no single cutoff point could be identified, combination with other variables, such as the pituitary height/brain area (P/B) ratio, is still needed to obtain a good estimate of the risk for recurrence of hypercortisolism after hypophysectomy.

Key words: Canine; Cushing's disease; Hypercortisolism; Survival analysis.

Pituitary-dependent hypercortisolism (PDH) is a common endocrinopathy in dogs, with an estimated prevalence of 1 or 2 in 1000 dogs/y.[1] It is caused by an adrenocorticotropic hormone (ACTH)-secreting pituitary adenoma. Pituitary carcinomas are rare.[2] Clinical signs are caused by hypercortisolism and include polyuria, polydipsia, polyphagia, panting, heat intolerance, and enlargement of the abdomen caused by centripetal fat storage, atrophy of the skin, alopecia, muscle weakness, and lethargy.[3] Pituitary-dependent hypercortisolim in dogs has many similarities to Cushing's disease (CD)

From the Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands (van Rijn, Zierikzee, Kooistra, Penning, Tryfonidou, Meij); and the Department of Clinical Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden (Hanson).

This study was performed at the Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands.

Corresponding author: S.J. van Rijn, Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, PO Box 80154, 3508 TD Utrecht, The Netherlands; e-mail: s.j.vanrijn@uu.nl.

Abbreviations:

ACTH	adrenocorticotropic hormone
AUC	area under the curve
CD	Cushing's disease
CRH	corticotropin-releasing hormone
CT	computed tomography
MRI	magnetic resonance imaging
P/B ratio	pituitary height to brain area ratio
PDH	pituitary-dependent hypercortisolism
RI	random intercept
ROC	receiver operating curve
RS	random slope
UCCR	urinary corticoid-to-creatinine ratio

in humans, which has a much lower incidence, affecting only 1–2 persons per million.[4,5]

Pituitary surgery is the treatment of choice for CD in human patients, in whom preferably an adenomectomy is performed.[6] In dogs, transsphenoidal hypophysectomy has been shown to be an effective treatment for PDH.[7,8] Despite high initial remission rates, long-term recurrence is a well-recognized problem in both humans[9–13] and dogs,[7,14] with reported recurrence rates of 8.5% in humans and 25% in dogs.[7,12] Therefore, much effort has been expended to identify reliable predictors for completeness of tumor removal and recurrence, either preoperatively or postoperatively.

In both humans and dogs, recurrence is more common in patients with an enlarged pituitary gland.[10,15] For postoperative evaluation of pituitary surgery in humans, different protocols have been used including

intraoperative and postoperative measurement of plasma cortisol[9,16–20] and ACTH[21–23] concentrations, as well as stimulation tests with corticotropin-releasing hormone (CRH)[6,24,25] and desmopressin.[25–27] Overall, postoperative basal hormone concentrations in humans are the most important predictor for recurrence,[12] but a test that is predictive for the individual patient remains to be developed.[28]

For postoperative evaluation of transsphenoidal hypophysectomy in dogs, the urinary corticoid-to-creatinine ratio (UCCR) has been used to define residual disease, remission, and recurrence. Urinary corticoid-to-creatinine ratio values in the upper half of the reference range 6–8 weeks after surgery are associated with a higher frequency of recurrence at long-term follow-up, whereas low UCCR values predict long-term remission.[15] The UCCR is an indirect reflection of ACTH production by the pituitary gland, and therefore the plasma ACTH concentration may give more direct information about the completeness of hypophysectomy and risk for recurrence. Because of the short plasma half-life of ACTH (approximately 20 minutes in both humans and dogs), the plasma ACTH concentration should decrease within a few hours after hypophysectomy.[29,30] This makes direct postoperative evaluation of ACTH concentrations potentially interesting and it is hypothesized that very low ACTH concentrations within a few hours after hypophysectomy will be associated with long-term remission. Indeed, in humans, it was shown that patients with increased plasma ACTH concentrations developed recurrence, although their postoperative serum cortisol concentrations were low.[22] In dogs with PDH, a combined stimulation test, including CRH and 3 other hypophysiotropic hormones, performed at 8 weeks after hypophysectomy, failed to identify dogs in which the disease would recur,[31] but plasma ACTH and cortisol concentrations collected immediately after hypophysectomy have not been evaluated yet.

The aim of this study was to evaluate the prognostic value of perioperative plasma ACTH and cortisol concentrations for recurrence of hypercortisolism after transsphenoidal hypophysectomy in dogs with PDH.

Materials and Methods

Animals

Between 2001–2013, 204 dogs with PDH, referred to the Department of Clinical Sciences of Companion Animals, Utrecht University, the Netherlands, underwent transsphenoidal hypophysectomy as primary treatment for PDH. All dogs were operated by the same veterinary neurosurgeon (BM). Medical records of these dogs were evaluated retrospectively, and dogs were included in this study when the postoperative follow-up period was ≥1 year and postoperative remission of PDH was confirmed within 8 weeks after surgery. Thirty-eight dogs were excluded because follow-up was <1 year, 25 dogs were excluded because remission was not confirmed within 8 weeks after surgery and 29 dogs were excluded because the immediate postoperative hormone measurements were not performed or were not available. In total, 112 dogs met the inclusion criteria, consisting of 10 Labrador Retrievers, 9

Maltese dogs, 8 Beagles, 8 Dachshunds, 5 Boxers, 4 Jack Russell Terriers, 3 Yorkshire Terriers, 3 Stabyhouns, 3 German Pointers, 2 Bearded Collies, 2 Cavalier King Charles Spaniels, 2 Hovawarts, 2 Scottish Terriers, 1 dog each of 33 other breeds, and 18 cross-bred dogs. There were 53 male dogs (21 castrated) and 59 female dogs (45 spayed). Age at time of surgery ranged from 3.7 to 14 years (median, 8.5 years). Body weight ranged from 3.8 to 61 kg (median, 19.5 kg).

Diagnosis

The diagnosis of hypercortisolism was based on clinical signs and increased UCCRs (reference range, $0.3–8.3 \times 10^{-6}$ [32]) in 2 consecutive morning urine samples collected at home.[33] After collection of the second urine sample, 3 doses of 0.1 mg dexamethasone per kg body weight were administered PO at 6–8 hours intervals, and the next morning a third urine sample was collected. When the UCCR in the third sample was <50% of the mean of that of the first 2 samples, the dog was categorized as being responsive to dexamethasone suppression, and PDH was diagnosed.[33] The median UCCR of the 112 dogs included in this study was 55×10^{-6} (range, $6–652 \times 10^{-6}$), and the median suppression after dexamethasone was 80% (range, −162 to 99%). In 36 cases, there was <50% suppression of the UCCR in the third sample, and in these dogs dexamethasone-resistant PDH was demonstrated by measurement of plasma ACTH concentration. The diagnosis of PDH was further supported by visualization of the adrenal glands by ultrasonography and pituitary gland imaging using computed tomography (CT) or magnetic resonance imaging (MRI).[34,35] In dogs with PDH, enlarged pituitary glands were distinguished from nonenlarged pituitary glands by their pituitary height/brain area (P/B) ratio.[36] A microadenoma in a nonenlarged pituitary gland can be visualized indirectly by the displacement of the 'pituitary flush' on dynamic CT imaging.[35] For the dogs included in this study, the median P/B ratio was 0.43 (range, 0.13–1.1). There were 24 dogs with nonenlarged pituitary glands (P/B ≤0.31) and 88 dogs with enlarged pituitary glands (P/B >0.31).

Treatment and Follow-up

Transsphenoidal hypophysectomy was performed according to a microsurgical technique described previously.[37] After removal of the pituitary gland, treatment was started with 1 drop of 0.01% desmopressin[a] and continued every 8 hours in the conjunctival sac. Intravenous administration of 1 mg hydrocortisone[b] per kg body weight was started 5 hours after removal of the pituitary gland and continued every 6 hours until the dog resumed eating and drinking. Postoperative treatment followed the same protocol as described previously.[38] The dogs were kept on lifelong substitution therapy with cortisone acetate[c] at a dosage of 0.25 mg/kg PO q12h, and thyroxine[d] at a dosage of 15 μg/kg PO q12h. Desmopressin was administered routinely for 2 weeks and continued if polyuria because of central diabetes insipidus persisted.[38] The dogs were re-examined after 8 weeks. After surgery, UCCR was measured (in duplicate) at 2 weeks, 8 weeks, 6 months and thereafter once a year, or more frequently in dogs with suspected recurrence. All morning urine samples for UCCR measurements were collected at home by the owner 24 hours after cortisone acetate treatment. Follow-up reports were obtained from the routine follow-up examinations in the clinic, or during telephone conversations with the owner, referring veterinarian, or both. Remission was defined as UCCR $<10 \times 10^{-6}$ and resolution of clinical signs of hypercortisolism. Recurrence was defined as UCCR $\geq10 \times 10^{-6}$ and reappearance of clinical signs of hypercortisolism after initial remission.

Blood Sampling and Analysis

The protocol was approved by the Ethical Committee of the Faculty of Veterinary Medicine (Utrecht University, Utrecht, The Netherlands).

Two basal blood samples were collected on the day before surgery before 2008 and within 2 hours before the surgery after 2008. The average of the 2 values was included in this study as the preoperative value. After hypophysectomy, the first blood sample was collected when the dog was still on the operating table, approximately 1 hour after extraction of the pituitary gland. The remaining samples were collected at 2, 3, 4, and 5 hours after hypophysectomy when the dog was recovering in the intensive care unit. Desmopressin was started when the dog left the operating table. Hydrocortisone medication was started after the 5-hour sample was collected. Blood samples were collected in prechilled EDTA tubes and kept on ice, and centrifuged for 10–12 minutes at 4°C. Plasma was stored at −20°C until analyzed.

Plasma ACTH concentration was measured by a commercially available 2-site immunoradiometric assay.,[e,f] Plasma cortisol concentration was measured by a solid phase ^{125}I radioimmunoassay.[g] [39]

Data Analysis

Calculations were performed with SPSS[h] and R Studio.[i] For all postoperative time points, normalized plasma ACTH and cortisol concentrations were calculated by dividing the absolute plasma concentration by the preoperative concentration. These normalized plasma ACTH and cortisol concentrations also were used for analysis. Also, for each dog the area under the curve (AUC), random slope (RS), and random intercept (RI) of the ACTH and cortisol curves were calculated. Normality was assessed with the Kolmogorov–Smirnov Test, and nonparametric tests were used accordingly. Correlations were calculated using the Spearman's rho test. Differences between dogs with and without recurrence were evaluated with Mann–Whitney U-tests. Longitudinal comparisons were made using the Wilcoxon rank-sum test. Bonferoni corrections for multiple comparisons were applied.

The disease-free period was calculated for all dogs and was defined as the interval between the date of surgery and the date on which, the dog was last known to be remission, or the date of recurrence. Dogs that had died from nonrelated causes and dogs that were still alive and in remission at the time of follow-up were counted as censored cases. To assess the prognostic value of the different factors, variables were first analyzed with univariate Cox's proportional hazard analysis. Variables with a P-value <.05 were then entered in a multivariate Cox's proportional hazard analysis (Reverse Stepwise [Conditional LR]). For variables significant in the multivariate analysis, cutoff values to create the binary variables 'low' versus 'high' were calculated based on a receiver operating curve (ROC).[40] Disease-free analysis was performed using Kaplan–Meier curves and the Log-rank test was used to assess significance between 'low' and 'high' values. Significance was set at P < .05.

Results

Follow-up Data

Median follow-up for the 112 patients included in this study was 1096 days (range, 373–3799 days). At last follow-up, 35 dogs were alive. Median disease-free period was 896 days (range, 80–3340 days). Of the 112 patients, 31 (28%) had a recurrence of hypercortisolism, whereas 81 dogs were still in remission at last follow-up (alive or dead because of nonrelated cause). Median disease-free period for the dogs with recurrence was 588 days (range, 80–1688 days).

Basal Hormone Concentrations and Clinical Variables

Correlation was calculated between preoperative plasma ACTH and cortisol concentrations and clinical variables (P/B ratio, preoperative UCCR, survival time and disease-free period). A significant correlation was only found between preoperative plasma ACTH concentration and P/B ratio ($\rho = 0.28$, $P = .003$) and between preoperative plasma cortisol concentration and preoperative UCCR ($\rho = 0.31$, $P = .003$).

Hormone Profiles after Hypophysectomy

For each dog, plasma ACTH and cortisol concentrations were plotted as an individual curve (Fig 1) and compared with follow-up information. A significant correlation was found between absolute plasma ACTH and cortisol concentrations at 1–5 hours after surgery, normalized plasma ACTH and cortisol concentrations at 1–5 hours after surgery, AUC of ACTH and cortisol, and RI of ACTH and cortisol. For each time point of ACTH and cortisol measurement, plasma concentrations, normalized plasma concentrations, and curve characteristics were compared between the group of dogs with recurrence and the group of dogs without recurrence. Plasma cortisol concentrations at 1–5 hours after surgery were significantly higher in dogs with recurrence than those in dogs without recurrence (Fig 2). Also, significant differences were found in normalized plasma cortisol concentrations at 1–5 hours after surgery and for RS and RI of cortisol. When comparing the lowest postoperative result, dogs with recurrence had a significantly higher plasma cortisol concentration ($P = .008$), and plasma ACTH concentration tended to be higher in these dogs ($P = .07$). Data also were analyzed longitudinally, both for dogs with recurrence and dogs without recurrence. Plasma ACTH and cortisol concentrations decreased significantly over time after surgery in both groups (Fig 2).

Disease-free Period Analysis

Clinical variables (P/B ratio, age, body weight, sex), absolute plasma ACTH and cortisol concentrations, normalized plasma ACTH and cortisol concentrations and curve characteristics (AUC, RI and RS) were entered in the univariate Cox's proportional hazard analysis for disease-free period of the 112 dogs. The variables that were significant ($P < .05$, Table 1) were entered in a multivariate Cox's proportional hazard model, grouped in absolute plasma hormone concentrations, normalized plasma hormone concentrations, and curve characteristics. The P/B ratio was excluded from further analysis, for a better appreciation of the influence of the hormone concentrations in the different models. High plasma ACTH concentration 5 hours after surgery, high plasma cortisol concentrations 1 and 4 hours after surgery, high
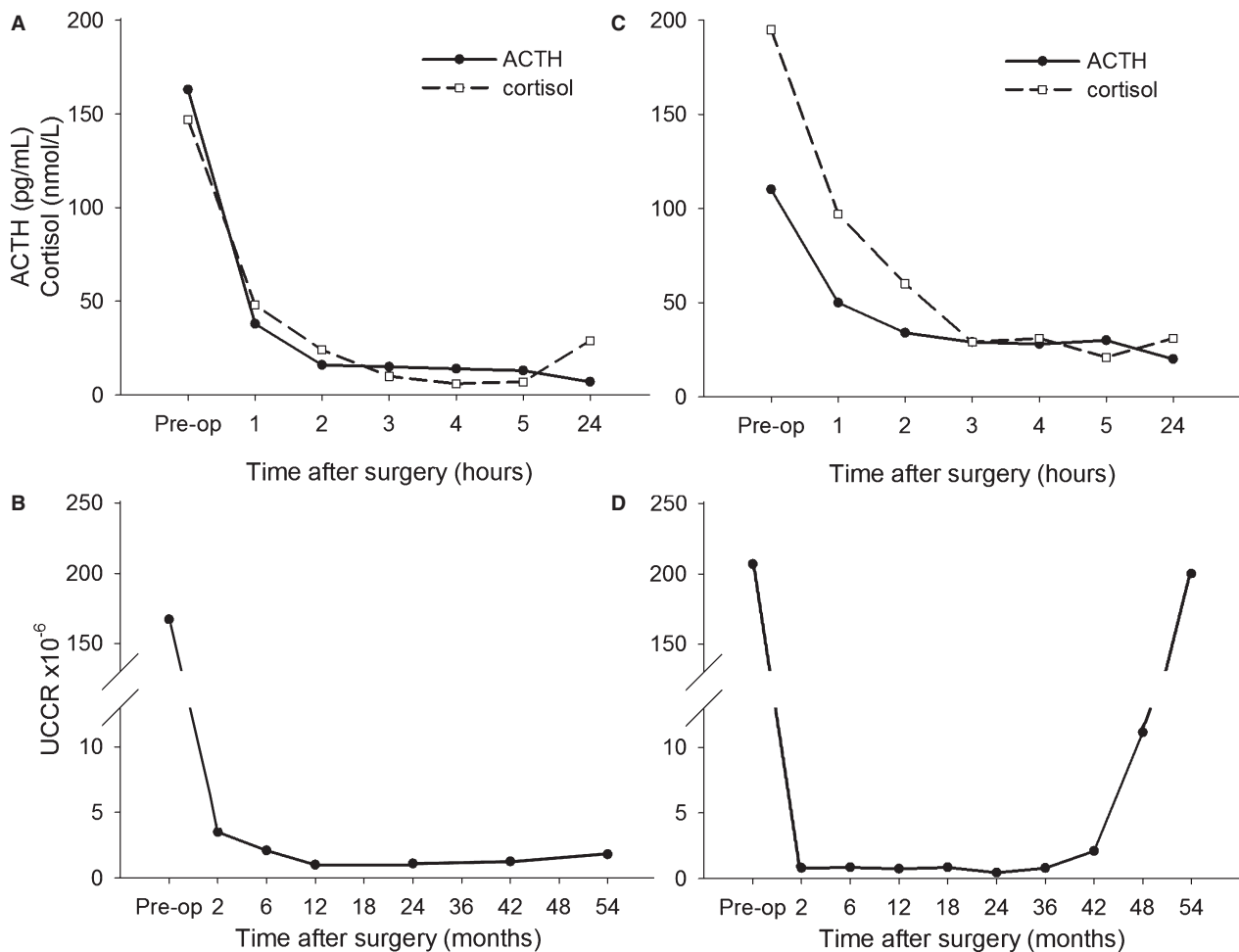
Fig 1. Typical example of perioperative individual profiles of plasma ACTH and cortisol concentrations in dogs that underwent hypophysectomy for treatment of PDH. Panels **A** and **C** show the plasma concentrations of ACTH and cortisol from 24 hours (Preoperative) before to 5 hours after surgery. Panels **B** and **D** show the UCCRs of the same dogs over time. The dog in panel **A** and **B** remained in remission for 54 months, and the plasma ACTH and cortisol concentrations decreased within 5 hours after hypophysectomy to values close to zero. The dog in panel **C** and **D** had a recurrence of hypercortisolism at 48 months after surgery. In this dog plasma, ACTH and cortisol concentrations did not decrease to values close to zero within 5 hours after hypophysectomy.

normalized plasma ACTH concentration 3 hours after surgery, high normalized plasma cortisol concentration 4 hours after surgery and the RS of cortisol were associated with shorter disease-free period (Hazard Ratio >1.0, $P < .05$); (Table 2). No significant differences were found between Kaplan–Meier survival curves for disease-free periods of these variables (ACTH 5 hours after surgery [$P = .36$], cortisol 1 hours after surgery [$P = .12$], cortisol 4 hours after surgery [$P = .07$, Fig 3], normalized cortisol 4 hours after surgery [$P = .15$], and RS of cortisol [$P = .13$]). The ROC curve of normalized ACTH 3 hours after surgery was not of sufficient quality to calculate a cutoff value.

Discussion

We studied the prognostic value of perioperative ACTH and cortisol concentrations in predicting recurrence of hypercortisolism in dogs after hypophysectomy. With multivariate analysis, we identified several variables with significant prognostic value (ACTH 5 hours after surgery, cortisol 1 and 4 hours after surgery, normalized ACTH 3 hours after surgery, normalized cortisol 4 hours after surgery, and the RS of cortisol). However, Kaplan–Meier survival analysis showed that single time point measurements were not sufficient to predict a significant difference in disease-free period, as was also shown in a meta-analysis of studies of humans.[12] Therefore, perioperative hormone concentrations must be combined with other clinical information, such as pituitary size (P/B ratio), to provide reliable information about the prognosis of dogs with PDH.

The long-term recurrence rate in this study was 28% (31 in 112 dogs). The dogs included in this study had a median P/B ratio of 0.43, indicating a high proportion of dogs with enlarged pituitary glands. We previously showed that high P/B ratio is predictive of recurrence,[15] and also in this study, the P/B ratio had a high hazard ratio in the Cox's proportional hazard's analysis.
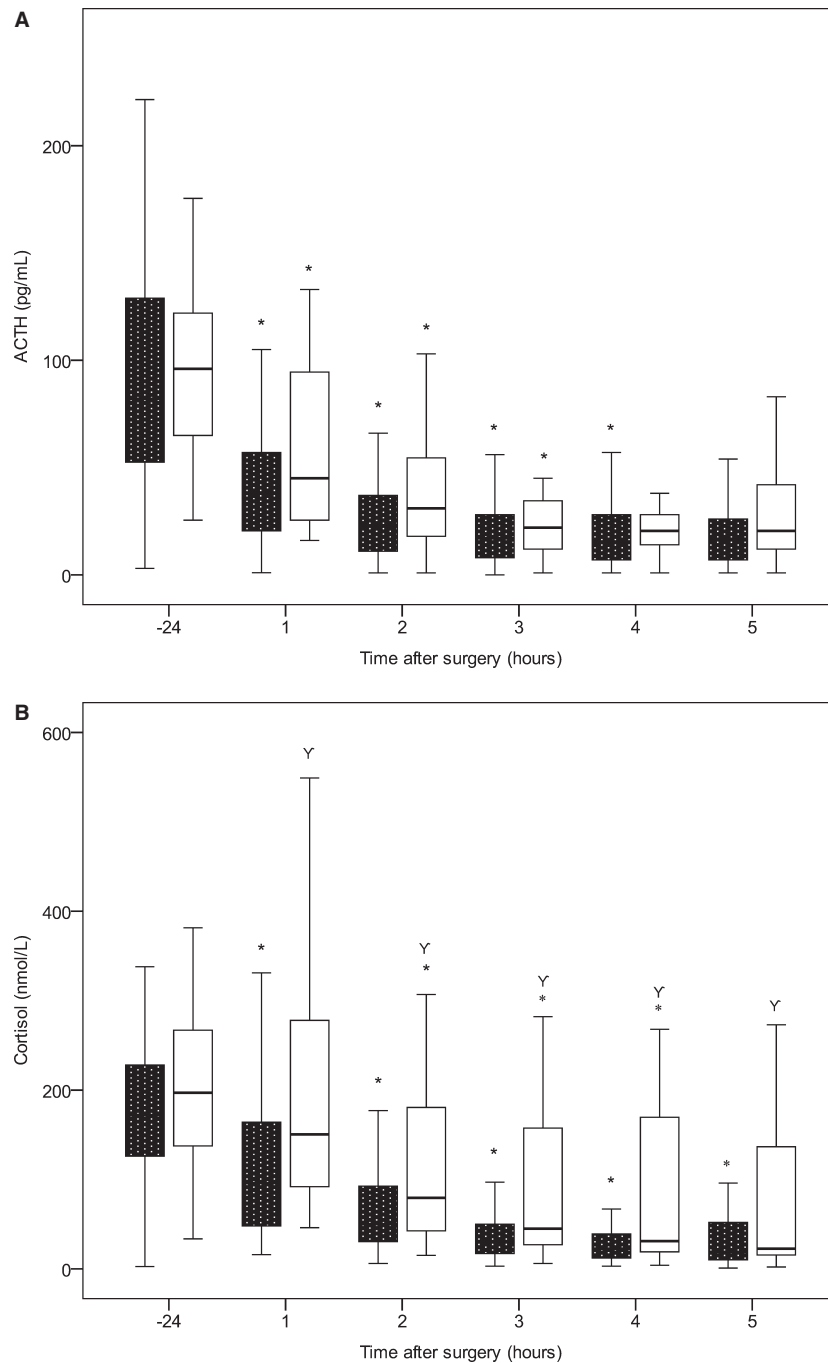
Fig 2. Boxplots demonstrating the change in plasma ACTH (**A**) and cortisol (**B**) concentrations over time in dogs with recurrence (open boxes, n = 31) and dogs without recurrence (black boxes, n = 81) of hypercortisolism after hypophysectomy. * indicates a significant difference to the previous time point. ϒ indicates a significant difference between plasma cortisol concentrations in dogs with and without recurrence.

We showed a significant decrease in the plasma ACTH concentrations after surgery in both dogs with and without recurrence. The short half-life of ACTH explains this rapid decrease. Plasma ACTH concentrations have been measured intra-operatively in humans,[21–23] but are not commonly used as prognostic indicators. A recent study of humans did however show the usefulness of the combination of ACTH and

cortisol measurements.[22] Plasma cortisol has a longer half-life (in humans approximately 50 minutes[41]), and the decrease in plasma cortisol concentration after hypophysectomy is expected to be less steep than that of ACTH. In humans, plasma and urinary cortisol concentrations most commonly are used as prognostic variables.[9,12,16–20] We showed also that cortisol concentrations decreased significantly in the postoperative

Table 1. Significant variables ($P < .05$) in the univariate Cox's proportional hazard analysis for disease-free period after transsphenoidal hypophysectomy. Variables with $P > .05$ are not included in this table.

Variable	HR	95% CI		P-Value
P/B value	22.664	4.478	114.712	<.001
ACTH2	1.008	1.002	1.014	.007
ACTH3	1.007	1.003	1.010	<.001
ACTH4	1.016	1.008	1.025	<.001
ACTH5	1.019	1.004	1.035	.014
Cort1	1.004	1.002	1.005	<.001
Cort2	1.004	1.002	1.005	<.001
Cort3	1.002	1.001	1.004	<.001
Cort4	1.004	1.003	1.006	<.001
normACTH2	4.477	1.416	14.152	.011
normACTH3	2.241	1.469	3.420	<.001
normACTH4	4.788	1.770	12.954	.002
normCort1	1.826	1.318	2.530	<.001
normCort2	1.888	1.286	2.772	.001
normCort3	1.498	1.176	1.908	.001
normCort4	2.067	1.407	3.035	<.001
RI ACTH	1.011	1.003	1.018	.005
AUC Cort	1.000	1.000	1.000	<.001
RI Cort	1.005	1.003	1.008	<.001
RS Cort	1.171	1.093	1.256	<.001

AUC Cort, area under the curve for cortisol; CI, confidence interval; HR, hazard ratio; normACTH, normalized plasma ACTH concentration to preoperative value; normCort, normalized plasma cortisol concentration to preoperative value; P/B value, pituitary height/brain area value; RI, random intercept; RS, random slope.

period, with the median plasma cortisol concentration being significantly higher in dogs that developed recurrence than in dogs without recurrence at all postoperative time points.

Evaluating individual hormone curves for patients with and without recurrence, we observed a faster decrease in plasma ACTH concentrations in dogs without recurrence, but mean plasma ACTH concentrations were not different between groups, probably because of large variation, especially in the recurrence group. Very low plasma ACTH concentrations suggest complete removal of the corticotroph adenoma, but do not exclude presence of remaining normal pituitary cells, because these cells have been suppressed by the negative feedback of high cortisol concentrations for a long period. Very low plasma ACTH concentrations within 5 hours as an indication of complete hypophysectomy is even more noteworthy because at 1 hour after hypophysectomy desmopressin was administered which, besides replacing the vasopressin deficiency, is a well-known stimulant of ACTH secretion.[25–27] Because of this, measurement of high postoperative plasma ACTH concentrations indicates remnant functional corticotroph adenoma tissue, increasing the risk for recurrence of hypercortisolism over time. Indeed, evaluating the lowest postoperative plasma concentrations of ACTH and cortisol for each patient, we showed significantly higher plasma cortisol concentrations in dogs with recurrence.

With multivariate analysis, not only absolute hormone concentrations were included but also concentrations normalized to the preoperative result and characteristics of the complete hormone curve. The definitive models included several variables with significant prognostic value. The advantage of hourly repeated measurements is that curve characteristics could be included in the analysis. Kaplan–Meier survival analysis showed that single time point measurements were not sufficient to predict recurrences, as was also shown in a meta-analysis of studies of humans,[12] making a combination of different data essential.

Based on a cohort of 112 patients with a follow-up of >1 year, a combination of absolute hormone concentrations, normalized hormone concentrations and curve characteristics has predictive value. Therefore, use of the hormone curve of a patient, in combination with other clinical variables (such as P/B ratio) is needed in the postoperative evaluation of dogs with PDH to

Table 2. Grouped variables entered in multivariate Cox's proportional hazard analysis (Reverse LR Conditional) for disease-free period after transsphenoidal hypophysectomy resulted in a final model.

Grouped Variables Entered	Variables in Final Model	HR	95% CI		P-Value
ACTH2, ACTH3, ACTH4,	ACTH3	1.032	0.999	1.066	.060
ACTH5, Cort1, Cort2, Cort3, Cort4	ACTH4	0.921	0.845	1.005	.064
	ACTH5	1.055	1.006	1.106	.026*
	Cort1	1.005	1.000	1.010	.041*
	Cort2	0.993	0.986	1.001	.072
	Cort4	1.007	1.002	1.013	.008*
normACTH2, normACTH3,	normACTH3	2.082	1.237	3.504	.006*
normACTH4, normCort1,	normCort4	1.805	1.182	2.756	.006*
normCort2, normCort3, normCort4					
RI ACTH, RI Cort, RS Cort, AUC Cort	RI ACTH	1.011	0.999	1.022	.067
	RS Cort	1.150	1.064	1.244	<.001*

AUC Cort, area under the curve for cortisol; CI, confidence interval; HR, hazard ratio; normACTH, normalized plasma ACTH concentration to preoperative value; normCort, normalized plasma cortisol concentration to preoperative value; RI, random intercept; RS, random slope.

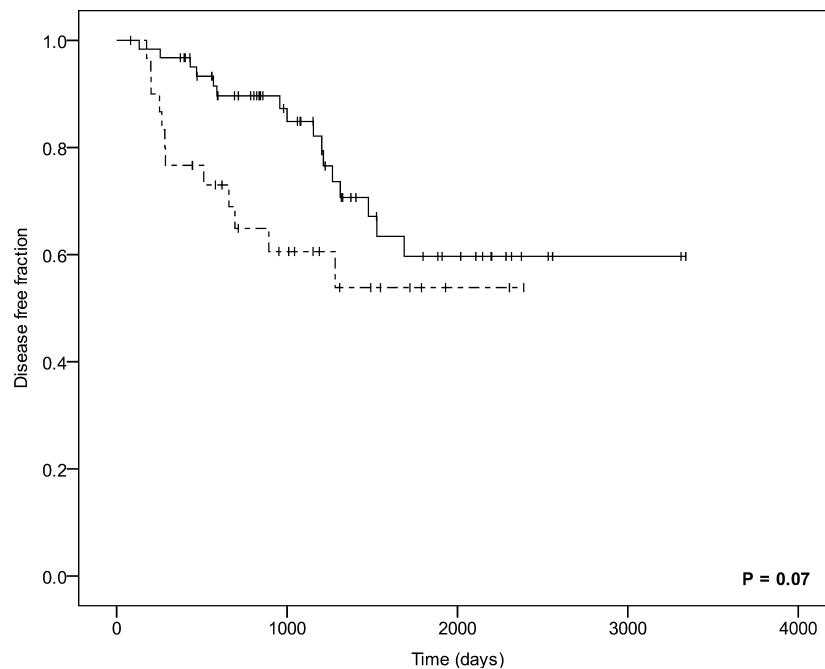Significant variables ($P < .05$) are marked with *.

Fig 3. Kaplan–Meier curves comparing the disease-free period in dogs with high (>33 nmol/L), dotted line, n = 31) and low (<33 nmol/L), continuous line, n = 62) plasma cortisol concentrations at 4 hours after hypophysectomy (P = .07). Censored cases are represented by vertical bars.

provide a reliable estimation of the prognosis. Close follow-up of the patients, over the long-term, also remains crucial.

Footnotes

Acknowledgments

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