CHAPTER 4

Prognostic factors for outcome after transsphenoidal hypophysectomy in dogs with pituitary-dependent hyperadrenocorticism

J M Hanson[a], E Teske[a], G Voorhout[b], S Galac[a], H S Kooistra[a], B P Meij[a]

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[a]*Department of Clinical Sciences of Companion Animals, and* [b]*Division of Diagnostic Imaging, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands*

Abstract

The aim of this study was to determine prognostic factors for outcome after transsphenoidal hypophysectomy in dogs with pituitary-dependent hyperadrenocorticism (PDH).

Transsphenoidal hypophysectomy was performed in 181 dogs with PDH by one veterinary neurosurgeon during a 12-year period. Survival analyses were performed with the Kaplan-Meijer estimate procedure. Prognostic factors (including patient, hormone and imaging data) were analyzed by univariate Cox's proportional-hazard analysis followed by stepwise multivariate analysis. The log-rank test was used to assess disease-free fractions in three groups categorized by the early postoperative urinary corticoid-to-creatinine ratio (UCCR).

Multivariate analysis revealed that old age, large pituitary size, and high plasma adrenocorticotropic hormone (ACTH) concentrations before surgery were associated with an increased relative risk for PDH-related mortality. Large pituitary size, relative thick sphenoid bone, high UCCR, and high plasma α-melanocyte-stimulating hormone (α-MSH) concentration before surgery were associated with an increased risk of recurrence in dogs that went into remission after hypophysectomy. Disease-free fractions were significantly higher in dogs with postoperative UCCR in the low normal range ($< 5 \times 10^{-6}$) than in dogs with postoperative UCCR in the upper normal range ($5\text{-}10 \times 10^{-6}$).

The results of this study indicate that pituitary size, thickness of the sphenoid bone, plasma α-MSH concentration, and urinary cortisol excretion before surgery are predictors for long-term remission after transsphenoidal hypophysectomy for the treatment of PDH in the dog. UCCR measured at 6 to 10 weeks after surgery can be used as guidance for predicting the risk of recurrence.

Pituitary-dependent hyperadrenocorticism (PDH) or Cushing's disease is a common endocrine disorder in dogs accounting for 85% of the cases of spontaneous hypercortisolism,[50] which is reported as an animal model for Cushing's disease in humans.[29]

In dogs, hypophysectomy is performed and this approach has proven to be effective.[24,34] Transsphenoidal selective adenomectomy is the treatment of choice for Cushing's disease in humans.[3,7,31] The short-term surgical outcome after pituitary surgery is better for surgeons and hospitals with a high case load.[3] Humans with Cushing's disease have a higher risk of complications after pituitary surgery compared to patients with other pituitary tumors.[3]

Although remission rates are initially high (70-90%, reviewed by others),[2,41,42,49] recurrences are common in both species especially in the long term.[2,7,12,13,24,25] Preoperative variables associated with decreased surgical outcome in humans are increased pituitary size,[6,8,11,12,18,22,32,41,49,60] and dural invasion of the pituitary adenoma.[6,11,14,18,35,41] Male gender, large pituitary tumor size, and extrasellar extension are risk factors for residual disease after surgery.[6,22,32] In both species, disease-free fractions are lower in cases with macroadenomas than in cases with microadenomas.[6,24,60] Furthermore, age, inability to identify pituitary tumor on preoperative computed tomography (CT) or magnetic resonance imaging (MRI), severity of clinical signs, depression and high pre-treatment urinary cortisol levels are reported to be risk factors for recurrence.[7,57]

Postoperative cortisol measurements are used for defining surgical failure and remission in human patients.[2,12,17,45,46,49,54,56,61,66] However, there is no consensus with regard to the interpretation of postoperative cortisol values.[15,44] Low postoperative serum cortisol concentration on the day after surgery before glucocorticoid therapy is initiated[54] or low cortisol concentrations at 3 months after pituitary surgery when glucocorticoids are routinely used[45] have been reported to be associated with a low risk for long-term recurrence. However, for a subgroup of patients, postoperative serum cortisol concentrations do not accurately predict surgical outcome.[2,45,49,66]

Dogs with PDH and enlarged pituitary glands have significantly lower survival and disease-free fractions after transsphenoidal hypophysectomy than dogs with normal sized pituitary glands.[24] Also, the presence of adrenocorticotropic hormone (ACTH) pulses after hypophysectomy is a risk factor for the recurrence of hyperadrenocorticism.[23] The aim of the present study was to report the outcome of hypophysectomy at one center by one veterinary neurosurgeon for treatment of 181 dogs with PDH with a follow-up time up to 12 years. Prognostic factors were analyzed for early post-operative mortality and recurrences of canine PDH after transsphenoidal hypophysectomy.

Materials and Methods

Animals

One-hundred-and-eighty-one dogs with PDH, referred to the Department of Clinical Sciences of Companion Animals, Utrecht University, over a 12-year (1993–2005) period, underwent transsphenoidal hypophysectomy as primary treatment for PDH. Purebred dogs of 57 different breeds and crossbred dogs were represented. The most common breeds were Dachshund (n=16), Minature Poodle (n=13), Maltese (n=8) and Yorkshire Terrier (n=8), which together comprised 25% of the dogs. Eighty dogs were male (25 castrated) and 101 were female (62 spayed). The age at the time of surgery ranged from 3 to 14 years

(median, 9 years). The body weights ranged from 4 to 61 kg (median, 15 kg) with 88 dogs in the group 0 to ≤ 15 kg, 60 dogs in the category > 15 to ≤ 30 kg and 33 dogs weighed > 30 kg. In addition, the dogs were divided into three groups according to the shape of the skull: brachycephalic (*n*=8), mesaticephalic (*n*=125) and dolicocephalic (*n*=10). The 38 crossbred dogs were not categorized by skull shape.

Most dogs had the classical features of canine hyperadrenocorticism such as polyuria, polyphagia, truncal obesity, pot-belly appearance, muscle atrophy, and skin changes such as skin atrophy, alopecia, and calcinocis cutis (Figure 1A).[50] A few dogs also showed neurological signs due to tumor mass effect.[50]

Diagnosis

The diagnosis of hyperadrenocorticism was based upon urinary corticoid-to-creatinine ratio (UCCR) in two consecutive morning urine samples combined with a high-dose dexamethasone test, as described earlier.[53,59] After collection of the second urine sample, three oral doses of 0.1 mg dexamethasone per kg body weight were administered at 8-h intervals and the next morning a third urine sample was collected. When the UCCR in the third sample was less than 50% of the mean in the first 2 samples, the dog was categorized as being responsive to dexamethasone suppression and PDH was diagnosed.[20] In cases with less than 50% suppression dexamethasone-resistent PDH was confirmed by measurements of plasma ACTH concentrations and further supported by visualization of the adrenals by ultrasonography and pituitary imaging.[10,52,63,64]

Imaging

Pituitary imaging was performed in anesthetized dogs with a third generation CT scanner (Tomoscan CX/S, Philips NV, Eindhoven, The Netherlands) (155 dogs) (Figure 2) or with a 0.2 T open field MRI scanner (Magnetom Open Viva, Siemens AG, München, Germany) (26 dogs) using protocols described previously.[24,62] The height and width of the pituitary gland were measured on transverse images. The length of the pituitary gland was estimated from the number of images containing a section of the gland (CT) or on sagittal reconstructions of the transverse images (MRI). The ratio between height of the pituitary gland and the area of the brain (P/B ratio) was calculated to correct for the large differences in dog size.[30] Pituitaries with a P/B ratio > 0.31×10^{-2} mm^{-1} were enlarged and those with a P/B ratio ≤ 0.31×10^{-2} mm^{-1} nonenlarged.[30] The pituitary height-to-width, height-to-length, and width-to-length ratios were determined to estimate pituitary shape. Pituitary volume was calculated with an ellipsoid approximation model ($\pi/6$ x the product of the length, width and height).[4] The thickness of the sphenoid bone was measured on the transverse image on which pituitary height was measured.

Surgery

Transsphenoidal hypophysectomy was performed according to a microsurgical technique described previously.[39] Postoperative hormone substitution therapy consisted of cortisone acetate and thyroxine.[38] Desmopressin was administered for 2 weeks and continued if polyuria due to central diabetes insipidus persisted.[24,38,39] Re-examination after 8 weeks included physical examination, routine blood chemistry, measurements of basal plasma thyroxine concentration at 10 to 12 h after L-thyroxine medication, and basal UCCR urine samples

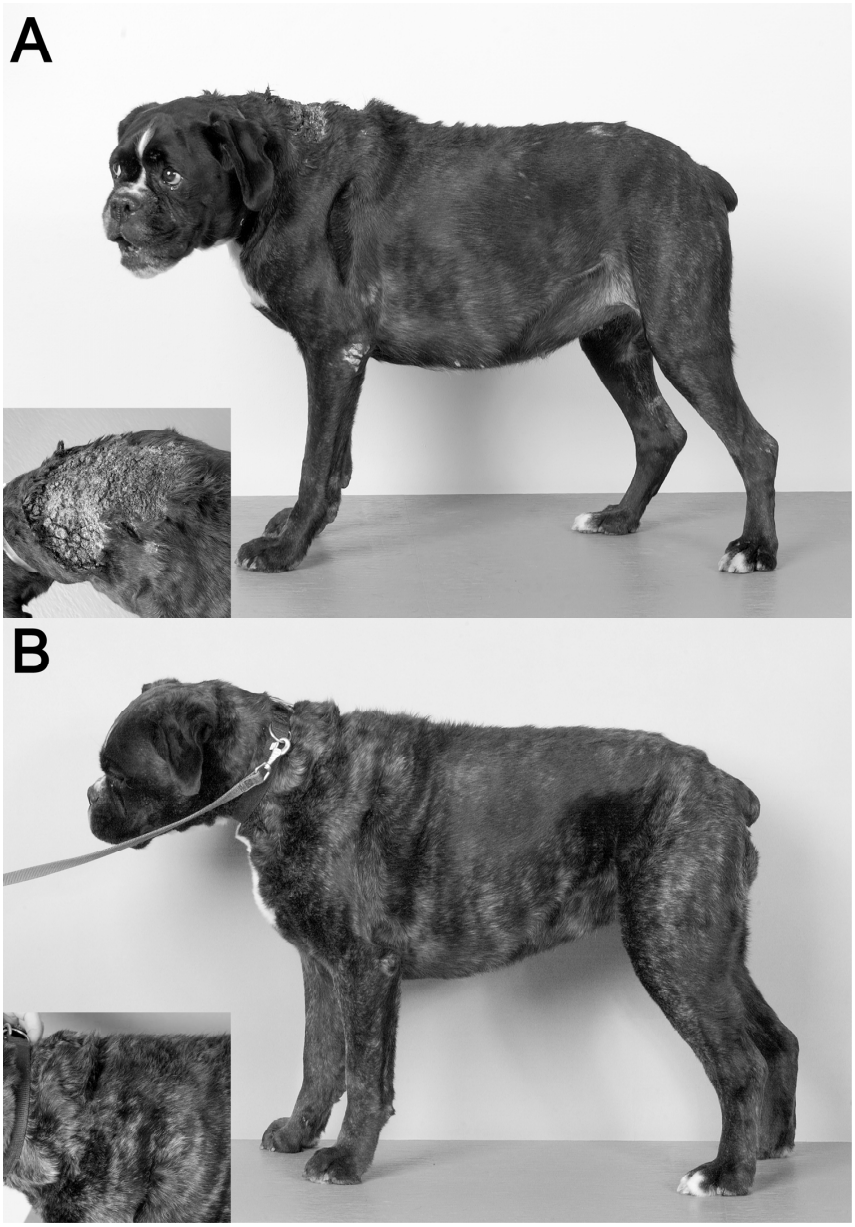

Figure 1. (A) A 5-year-old female Boxer dog with features of (pituitary-dependent) hyperadrenocortisim: abdominal enlargement, atrophy of the thigh muscles, some hair loss in the groins, and severe secondary crusty dermatitis along the dorsum with calcinosis cutis (see insert from shoulder). The dog had a pronounced polydipsia, polyuria and a ravenous appetite. (B) 3 months after hypophysectomy with less sagging abdomen, full recovery of the hair coat, and regain of muscle mass (see insert from shoulder) (See Color section).

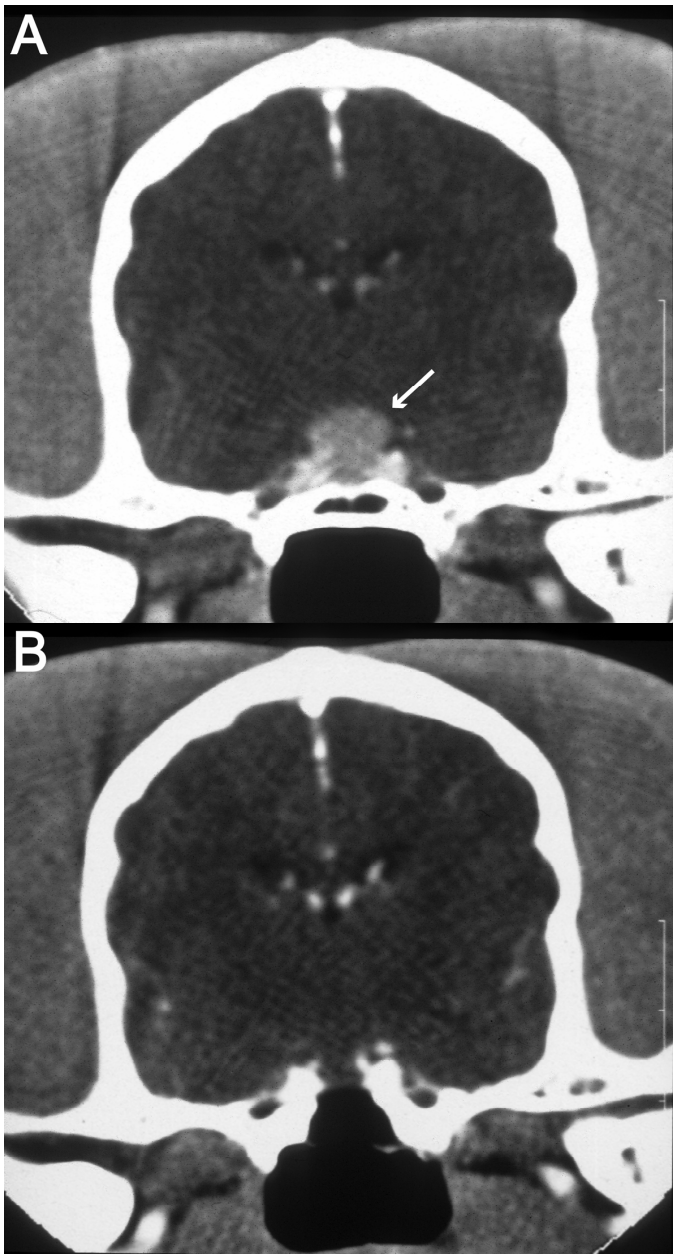

Figure 2. (A) A typical computed tomography image of the skull of 13-year-old female crossbred dog with hyperadrenocorticism due to a large pituitary adenoma (arrow). (B) The same dog 8 weeks after hypophysectomy.

collected at home at 24 h after cortisone medication. UCCRs were measured again 6 months after surgery and thereafter once a year. In case of suspicion of recurrence, UCCR was determined earlier.

Postoperative mortality was defined as death within 4 weeks after surgery irrespective of the cause of death. Residual disease was defined as early postoperative (< 2 months after surgery) UCCR $\geq 10 \times 10^{-6}$ and no resolution of clinical signs and/or remnant pituitary tumor tissue on early postoperative CT or MRI scans. Remission was defined as UCCR $< 10 \times 10^{-6}$ and resolution of clinical signs of hyperadrenocorticism. Recurrence was defined as UCCR $\geq 10 \times 10^{-6}$ and return of clinical signs of hyperadrenocorticism after initial complete remission (Figure 1B).

Hormone Determinations

Plasma ACTH concentration was measured by two different methods during the course of the study. In 59 dogs a radioimmunoassay (RIA) without extraction was used, according to the procedure validated for the dog and described previously.[1,10,40] This antiserum also cross reacted with ACTH precursors. The tracer was purchased from International CIS (St Quentin-Yvelines, France), and the standard was obtained from the NIH (Bethesda, MD, USA). The intra-assay coefficient of variation (CV) was 8%, the inter-assay CV was 12%, and the sensitivity was 2.2 pmol/l. The cross-reactivity with α-melanocyte-stimulating hormone (α-MSH) was < 0.1%.[10]

In 112 dogs plasma ACTH concentrations were measured using a commercially available two-site immunoradiometric assay (IRMA) (Nichols Institute, Wijchen, The Netherlands). The antiserum is highly specific for ACTH (1-39). The intra-assay CV was 3.2%, the inter-assay CV was 7.8%, and the sensitivity was 0.22 pmol/l. There was no cross-reactivity between the antiserum and α-MSH or ACTH precursors.[10,47]

Plasma cortisol concentrations were measured with two comparable methods. In 72 dogs a RIA was used, using cortisol antiserum as described previously.[51] The intra-assay CV was 5%, the inter-assay CV was 10% and the sensistivity was 1 nmol/l. In 93 dogs plasma cortisol concentrations were measured by a solid phase ^{125}I RIA (Coat-A-Count® Cortisol, Diagnostic Products Corporation, Los Angeles, USA). The antiserum is highly specific for cortisol, with very low cross-reactivity to other compounds that are present in patient samples. The intra-assay CV was 4% the inter-assay CV was 4.5-6.3%, and the sensitivity was 5.5 nmol/l.

The urinary corticoid concentration was measured by RIA as described previously.[53] The intra-assay CV was 6%, the inter-assay CV 8%, and the sensitivity was 1 nmol/l. The urinary corticoid concentration was related to the urinary creatinine concentration (Jaffé kinetic method, initial rate reaction) and the UCCR was calculated.[53,59]

Plasma concentration of α-MSH was measured by RIA without extraction according to methods described previously.[51] The intra-assay CV was 10%, the inter-assay CV was 23%, and the sensitivity was 3 pmol/l. The antiserum had less than 0.1% cross-reactivity with ACTH (1-39) and 4% cross-reactivity with ACTH (1-24). Plasma α-MSH concentrations equal to or more than 36 pmol/l were considered increased.[10]

According to protocol previously described, plasma growth hormone (GH) concentration was measured by a homologous RIA[16] plasma prolactin (PRL) and luteinizing hormone (LH) concentration were measured by heterologous RIAs,[43,58] plasma thyroid stimulating hormone (TSH) concentration was measured by a homologous IRMA using a commercially available kit (Diagnostic Products Corp., Los Angeles, USA),[65] and plasma total thyroxine (T_4) con-

centration was measured by RIA, with essentially the same method as was described by Belshaw and Rijnberk.[5]

Statistical Analysis

Survival analyses were performed with EGRET (Cytel Inc., Cambridge, MA, USA) statistical packages. Survival and disease-free fractions were calculated according to the Kaplan-Meier estimate procedure.[19] The survival period was defined as the interval between the date of the surgery and the date on which the dog was last known to be alive or the date of its death due to any cause. The disease-free interval was calculated for the dogs in which remission of hyperadrenocorticism was obtained, and was defined as the interval between the date of surgery and the date on which the dog was last known to be free of signs of hyperadrenocorticism and to have UCCR $< 10 \times 10^{-6}$, or the date of recurrence of signs of hyperadrenocorticism and UCCR $\geq 10 \times 10^{-6}$. Dogs that had died from non-related causes and dogs that were still alive at the time of follow-up were counted as censored cases. Prognostic factors, expressed as hazard ratios, were first analyzed by univariate Cox's proportional-hazard analysis. The following variables were analyzed; age (n=181), gender including castration status (n=181), body weight (n=181), mean UCCR before surgery (n=180), UCCR after high-dose dexamethasone suppression (n=180), degree of dexamethasone suppression on UCCR (n=180), pituitary height (n=181), pituitary width (n=181), pituitary length (n=178), pituitary height-to-width ratio (n=181), pituitary height-to-length ratio (n=178), and pituitary width-to-length ratio (n=178), P/B ratio (n=180), pituitary volume (n=178), pituitary enlargement (n=180), thickness of the sphenoid bone (n=176), plasma concentrations of ACTH measured by RIA (n=59) and IRMA (n=112), plasma concentrations of cortisol (n=165), α-MSH (n=141), GH (n=103), PRL (n=78), LH (n=79), TSH (n=38) and T_4 (n=41). These indicators with a probability value less than 0.10 in the univariate analysis were entered into a stepwise multivariate Cox's proportional-hazard analysis with backward elimination using Newton Raphson algorithm.[19]

The UCCR in the time period 6-10 weeks after surgery was measured in 91 of the dogs that went into remission. These dogs were divided into three groups based on the postoperative UCCR; $< 1 \times 10^{-6}$ (37 dogs), 1 to $< 5 \times 10^{-6}$ (35 dogs), 5 to $< 10 \times 10^{-6}$ (19 dogs). Differences between Kaplan-Meier curves of these 3 groups were tested for significance ($P < 0.05$) by the Log Rank test. [19]

Further evaluation of the prognostic factors was performed with SPSS (SPSS Benelux BV, Gorinchem, The Netherlands) statistical package. Comparisons between two groups of dogs were performed with non-parametric tests for independent variables (Mann-Whitney test) and bivariate correlations. Chi-square analysis was used to compare height/width ratio in dogs with remission and residual disease. A P-value < 0.05 was considered significant. Bonferoni correction with factor 3 was applied in case of multiple comparisons. Box-plot graphs were made in Sigma.Plot version 9.0 (Systat Software GmbH, Erkrath, Germany).

Results

The median and range for preoperative variables are presented in Table 1. The median follow-up time was 636 days (range, 1-3002 days). Of the 181 dogs, there were 14 (7.7%) postopera-

tive mortalities, 12 (6.6%) dogs had residual disease and 155 (85.6%) went into remission. Of the dogs in remission, disease recurred in 36 cases (23%).

Table 1
Summary of preoperative variables*

Variable	No.	Median	Range
Age (years)	181	9	3 - 14
Body Weight (kg)	181	15	4 - 61
Pituitary height (mm)	181	5.4	2.1 - 15
Pituitary width (mm)	181	6.2	2.9 - 17.4
Pituitary length (mm)	178	6.0	2 - 18
Pituitary height-to-width ratio	181	0.86	0.48 – 1.7
Pituitary height-to-length ratio	178	0.97	0.48-2.3
Pituitary width-to-length ratio	178	1.1	0.57-2.4
P/B ratio (mm^{-1})	180	0.33×10^{-2}	$0.15\text{-}1.1 \times 10^{-2}$
Pituitary volume (mm^3)	178	89	10-2400
Sphenoid bone thickness (mm)	176	5.0	1.7-10.2
Basal plasma hormone concentrations			
ACTH(RIA) (pmol/l)	59	34	4.4-133
ACTH(IRMA) (pmol/l)	112	19	0.66-156
Cortisol (nmol/l)	165	211	2.4-1300
α-MSH (pmol/l)	141	12	1.4-560
GH (μg/l)	103	0.70	0.10-2.3
PRL (μg/l)	78	8.2	0.80-24
LH (μg/l)	79	5.8	1.0-66
TSH (μg/l)	38	0.14	0.0-1.4
T4 (nmol/l)	41	14	3.0-26

* No. = number of dogs; P/B ratio = pituitary height-to-brain area ratio; ACTH = adrenocorticotropic hormone; RIA = radioimmunoassay; IRMA = two-site immunoradiometric assay; α-MSH = α-melanocyte-stimulating hormone; GH = growth hormone; PRL = prolactin; LH = luteinizing hormone; TSH = thyroid stimulating hormone; T4 = thyroxin

The pituitary gland was not enlarged (P/B ≤ 0.31) in 78 cases, remission occurred in 68 cases (87%) of which disease recurred in 12 cases (18%). The pituitary gland was enlarged (P/B > 0.31) in 102 dogs, of which 86 (84%) went into remission and 24 (28%) had a recurrence.

The plasma α-MSH concentration was < 36 pmol/l in 122 dogs. Of the 19 dogs with elevated α-MSH concentration, 9 had a recurrence. These dogs were all among 11 dogs with preoperative α-MSH concentration > 120 pmol/l. Six of these 9 dogs had dexamethasone-resistant PDH and an enlarged pituitary gland.

The 1-year estimated survival rate was 86% (95% confidence interval [CI], 80 -91%). The 2-year estimated survival rate was 83% (CI, 76-88%), the 3-year estimated survival rate was 80% (CI, 73-86%). The 4-year estimated survival rate was 79% (CI, 70-85%) (Figure 3A). The 1-year estimated disease-free fraction was 90% (CI, 84-94%). The 2-year estimated disease-free fraction was 77% (CI, 68-84%). The 3-year estimated disease-free fraction was 72% (CI, 62-80%). The 4-year estimated disease-free fraction was 62% (CI, 49-72%) (Figure 3B).

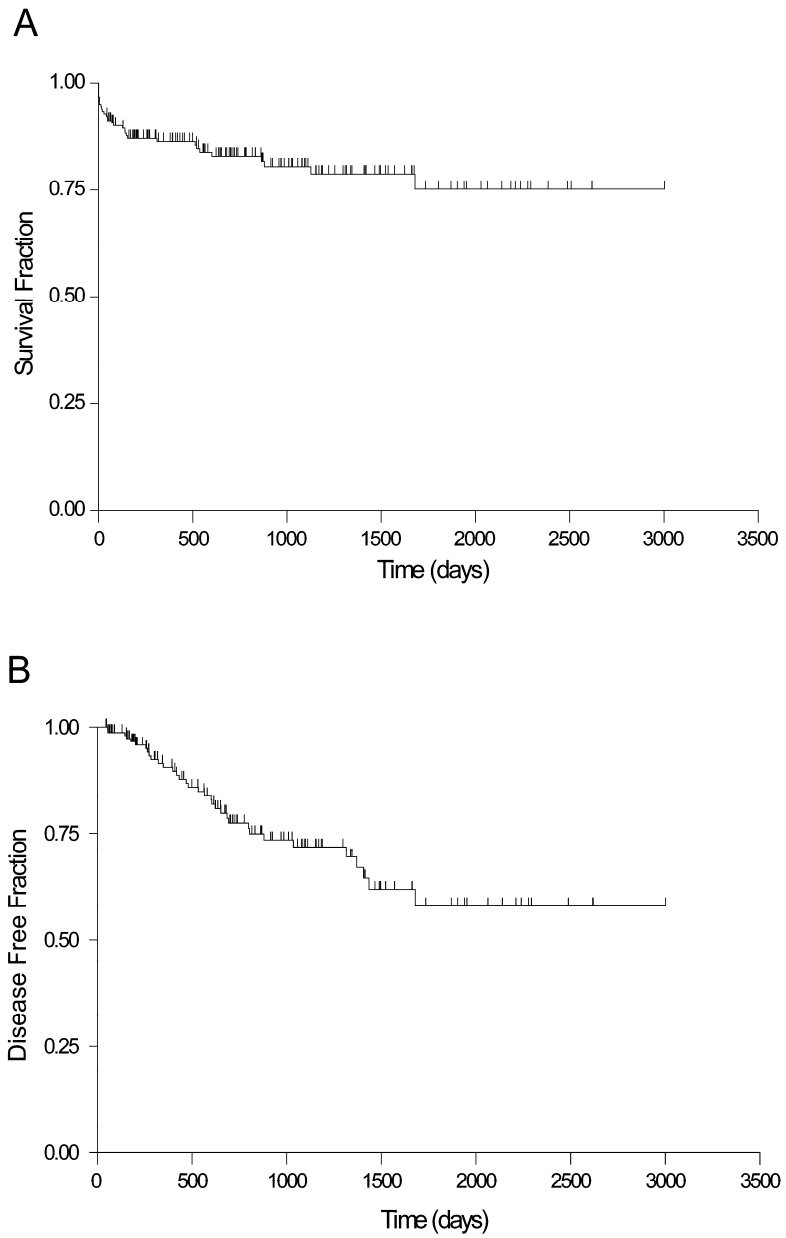

Figure 3. Survival curves calculated with Kaplan-Meijer estimate procedure for dogs after transsphenoidal surgery for the treatment of pituitary-dependent hyperadrenocorticism. (A) Survival time for the 181 dogs included in the study. (B) Disease-free period for 155 dogs with initial remission. Censored cases (still alive at the time of follow-up or died due to unrelated causes) are represented by vertical bars.

Postoperative Mortality

The 14 dogs that died within 4 weeks postoperatively had a median P/B ratio of 0.36 (range, 0.21-0.70), and eight of these dogs had an enlarged pituitary. Two dogs developed hemorrhage from the arterial cerebral circle. Two dogs died within 6 h after surgery, and postmortem examination in one revealed thromboendocarditis of the right atrium, concentric myocardial hypertrophy of the left ventricle and lung edema due to circulatory failure. Four dogs in which surgery was uneventful died one day after surgery; 2 dogs became dyspnoeic, 1 dog had glucocorticoid associated myotonia as was diagnosed preoperatively by electromyography, and 1 dog developed hypernatremia due to insufficient oral fluid intake. Two dogs died 5 days after surgery; one due to accidental IV injection of oral potassium solution, and one, in which surgery and recovery were uneventful, died suddenly at home with unknown cause. Two dogs had a prolonged stay in the intensive care unit for 2 weeks because of severe hypernatremia and diabetic ketoacidosis, and were eventually euthanized. One dog developed severe bronchopneumonia, and died 4 weeks after surgery. One dog was euthanized 16 days after surgery because of perforative peritonitis caused by a foreign body.

Residual Disease

In the 12 dogs with residual disease after surgery the median P/B ratio was 0.40 (range, 0.20-0.76), and eight dogs had an enlarged pituitary. Of the dogs with residual disease, 5 were euthanized or died within 5 months after surgery for reasons associated with hyperadrenocorticism, 2 dogs were euthanized because of aggressive behavior and epileptic seizures 26 and 34 months after surgery, one dog died suddenly at home 2 months after surgery and 4 dogs were still alive at the time of assessment with survival times of 12, 16, 17 and 32 months. Three of the 12 dogs with residual disease were treated with mitotane 3 to 6 months after surgery. At the time of assessment, survival times of these 3 dogs were 17, 26, and 32 months. Bilateral adrenalectomy was performed in one case, and this dog survived for 34 months until it developed seizures and was euthanized. In two dogs, at 5 weeks and 6 months after pituitary surgery, medical treatment with trilostane, a 3 β–hydroxysteroid dehydrogenase inhibitor, was initiated and the survival times were 12 and 16 months.

Remission

Of the 155 dogs in remission, 119 (77%) remained in remission. The median P/B ratio of 118 of these 119 dogs was 0.32 (range, 0.15-1.1) and the pituitary was enlarged in 63 cases (53%). The P/B ratio was not available for one dog. Of the 119 dogs that remained in remission, UCCR was measured 6 to 10 weeks post surgery in 78 dogs. Median basal UCCR at this time was 0.8×10^{-6} (range, $0.2\text{-}9 \times 10^{-6}$). In 38 dogs the UCCR was $< 1 \times 10^{-6}$, in 31 dogs 1 to $< 5 \times 10^{-6}$, and in 9 dogs 5 to $< 10 \times 10^{-6}$. Over time, 69 of the 119 dogs in remission died (10 dogs) or were euthanized (59 dogs) for non-related causes after a median interval of 28 months (range, 1.5-100 months).

Recurrence

Of the 155 dogs in remission, disease recurred in 36 cases (23%) after a median of 16 months (range, 1.8-56 months). The median P/B ratio of these 36 dogs was 0.41 (range, 0.19-0.71) and the pituitary was enlarged in 24 cases (67%). The median UCCR measured 6 to

10 weeks post surgery (in 22 dogs) was 3.0 x 10^{-6} (range, 0.2-8.7 x 10^{-6}). The postoperative UCCR was < 1 x 10^{-6} in 5 cases, 1 to < 5 x 10^{-6} in 8 cases and 5 to < 10 x 10^{-6} in 9 cases. After recurrence of hyperadrenocorticism, 16 cases were treated with mitotane and 5 cases with trilostane. At the time of assessment, 16 dogs were euthanized or had died because of recurrent signs of hyperadrenocorticism, 13 dogs had died because of old age (1 dog), heart failure (1 dog), lung edema (1 dog), neurological signs (1 dog), hind limb weakness (1 dog), drowning (1 dog), chronic nasal bacterial infection (1 dog), thyroid carcinoma and sepsis (1 dog), gastrointestinal symptoms (1 dog), uncontrolled diabetes insipidus (1 dog) and unknown cause (3 dogs). Seven dogs were still alive at time of assessment.

Prognostic Factors

In the univariate Cox's proportional-hazard analysis for survival time of the 181 dogs, the following factors had P-values < 0.10: age, pituitary height, width and length, maximum pituitary dimension, pituitary height-to-width ratio, P/B ratio, pituitary volume, and basal plasma concentration of ACTH(IRMA) (Table 2).

Table 2

Variables with P < 0.10 in univariate Cox's proportional-hazard analysis for survival and disease-free periods after transsphenoidal hypophysectomy for the treatment of pituitary-dependent hyperadrenocorticism in dogs*

Variable	No.	P	HR	95% CI
Survival period				
Age	181	0.030	1.211	1.018-1.440
Pituitary height	181	0.004	1.191	1.057-1.342
Pituitary width	181	0.008	1.163	1.040-1.300
Pituitary length	178	0.001	1.177	1.067-1.298
Max. pituitary dimension	178	0.001	1.182	1.067-1.310
Pituitary volume	178	0.005	1.001	1.000-1.002
Pituitary height-to-brain area ratio	180	0.009	12.82	1.912-85.95
Plasma ACTH (IRMA) conc.	112	0.014	1.003	1.001-1.005
Disease-free period				
Body weight	155	0.049	1.027	1.000-1.054
Body weight group		0.020	(Termwise Wald Test)	
Dogs > 30 kg	0.005	3.344	1.426-7.840	
Mean preoperative UCCR	155	<0.001	1.004	1.002-1.007
Preoperative UCCR (dex)	155	0.005	1.004	1.001-1.007
Pituitary height	155	<0.001	1.311	1.150-1.494
Pituitary width	155	<0.001	1.273	1.129-1.437
Pituitary length	152	<0.001	1.263	1.125-1.418
Max. pituitary dimension	152	<0.001	1.301	1.156-1.463
Pituitary height-to-width ratio	155	0.093	4.236	0.7851-22.86
Pituitary volume	152	<0.001	1.002	1.001-1.003
Pituitary height-to-brain area ratio	154	<0.001	45.62	5.930-351.0
Thickness of the sphenoid bone	150	0.002	1.360	1.123-1.647
Plasma α-MSH conc.	120	0.024	1.002	1.000-1.004

* No. = number of dogs; HR = Hazard Ratio; CI = Confidence Interval; Max = maximum; conc. = concentration; ACTH = adrenocorticotropic hormone; IRMA = two-site immunoradiometric assay; UCCR= Urinary corticoid-to-creatinine ratio; UCCR (dex) = UCCR after high-dose dexamethasone suppression; α-MSH = α-melanocyte-stimulating hormone (CI, 71-95%)

In the multivariate analysis, high age and increased pituitary length were associated with reduced survival time after hypophysectomy, when ACTH(IRMA) was not included. If ACTH(IRMA) was added in a new multivariate analysis, high age and high plasma ACTH (IRMA) concentration were associated with reduced survival time after hypophysectomy (Table 3).

Table 3
Multivariate Cox's proportional-hazard analysis (backward using Newton Raphson algorithm) for survival and disease-free periods after transsphenoidal hypophysectomy for the treatment of pituitary-dependent hyperadrenocorticism in dogs*

Time period	No.	No. (events)	Variables entered	Significant variables	P	HR	95% CI
Survival	178	32	age, pituitary length, P/B ratio	age pituitary length	0.0187 < 0.001	1.221 1.206	1.034-1.443 1.081-1.345
Survival	112	19	age, pituitary length, P/B ratio, plasma ACTH(IRMA) conc.	age Plasma ACTH (IRMA) conc.	0.088 0.039	1.205 1.003	0.9723-1.493 1.000-1.005
Disease-free	152	35	body weight, mean preop. UCCR, preop UCCR (dex), pituitary length, P/B ratio, sphenoid bone thick.	mean preop. UCCR sphenoid bone thick. P/B-ratio	0.017 0.032 0.025	1.004 1.241 17.22	1.001-1.007 1.018-1.512 1.422-208.4
Disease-free	117	27	body weight, mean preop. UCCR, preop. UCCR (dex), pituitary length, P/B ratio, sphenoid bone thickness plasma. α-MSH conc.	mean preop UCCR sphenoid bone thickness Plasma α-MSH conc.	0.070 0.022 0.030	1.004 1.309 1.002	0.9997-1.008 1.039-1.649 1.000-1.004

* No. = number of dogs; HR = hazard ratio; CI = confidence interval; P/B-ratio = pituitary height-to-brain area ratio; conc. = concentration; ACTH = adrenocorticotropic hormone; IRMA = two-site immunoradiometric assay; preop. = preoperative; UCCR= urinary corticoid-to-creatinine ratio; UCCR (dex) = UCCR after high-dose dexamethasone suppression; thick. = thickness; α-MSH = α-melanocyte-stimulating hormone

In the univariate Cox's proportional-hazard analysis for disease-free fraction in 155 dogs that went into remission, the following variables had a P-value < 0.10: body weight, body weight group, mean preoperative UCCR, UCCR after dexamethasone suppression, pituitary height, width and length, maximum pituitary dimension, pituitary volume, P/B ratio, thickness of the sphenoid bone, and basal plasma α-MSH concentration (Table 2). In the multivariate analysis the mean preoperative UCCR, P/B ratio and thickness of the sphenoid bone were associated with an increased risk for recurrence of hyperadrenocorticism when α-MSH was not included. If α-MSH was added to the multivariate analysis, the mean preoperative UCCR, thickness of the sphenoid bone and the plasma α-MSH concentration were associated with an increased risk for recurrence of hyperadrenocorticism (Table 3).

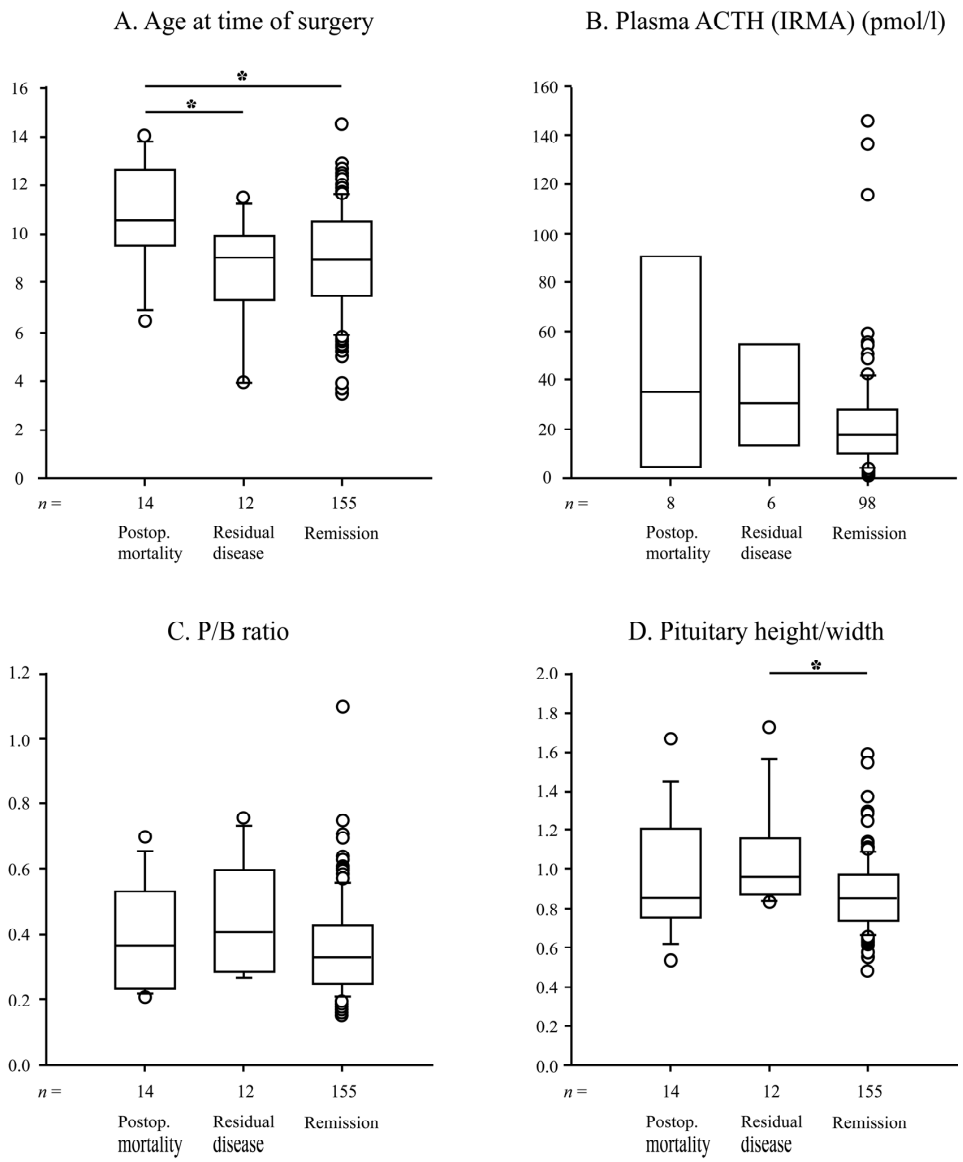

Figure 4. Box plot graphs over distribution of (A) age at time of surgery, (B) basal plasma ACTH concentration measured with a highly specific two-site immunoradiometric assay (IRMA), (C) pituitary height-to-brain area ratio (P/B ratio) and (D) pituitary height-to-width ratio in cases with early postoperative mortality (< 4 weeks), dogs with residual disease and dogs in remission. Significant differences between groups (connected with a line) are indicated with asterices. *P <0.05

The dogs with postoperative mortality were significantly older than the dogs in remission ($P < 0.05$) and than the dogs with residual disease after surgery ($P < 0.05$) (Figure 4A). The P/B ratio and basal plasma ACTH(IRMA) concentration were not different in the dogs with postoperative mortality, residual disease or remission (Figure 4B,C). In the 12 cases with residual disease, the pituitary height-to-width ratio was significantly ($P < 0.05$) higher than in those that went into remission (Figure 4D).

Postoperative UCCR was measured 6 to 10 weeks after hypophysectomy in 91 dogs. In 37 of these 91 dogs the UCCR was $< 1 \times 10^{-6}$; and the estimated 1-year disease-free fraction was 97% (CI, 82-100%), the estimated 2-year disease-free fraction was 88% and the estimated 3-year disease-free fraction was 82% (CI, 62-93%). In 35 dogs the postoperative UCCR was 1 to $\leq 5.0 \times 10^{-6}$ and the estimated 1-year disease-free fraction was 97% (CI, 81-100%) and the estimated 2-year disease-free fraction was 78% (CI, 56-90%). In 19 dogs the postoperative UCCR was 5 to $\leq 10 \times 10^{-6}$ and the estimated 1-year disease-free fraction was 65% (CI, 34-84%), and the estimated 2-year disease-free fraction was 40% (CI, 15-65%).When these 3 groups were compared, the disease-free fractions were significantly lower in the group of dogs with a postoperative UCCR of 5 to $\leq 10 \times 10^{-6}$ than those in the groups with a postoperative UCCR of $< 5 \times 10^{-6}$ (Figure 5). In the univariate Cox's proportional-hazard analysis for disease-free fraction, dogs with a postoperative UCCR of 5 to 10×10^{-6} had a significantly (P=0.001) higher risk of having a recurrence (HR 7.088; CI, 2.361-21.280) than dogs with a postoperative UCCR of $< 1 \times 10^{-6}$.

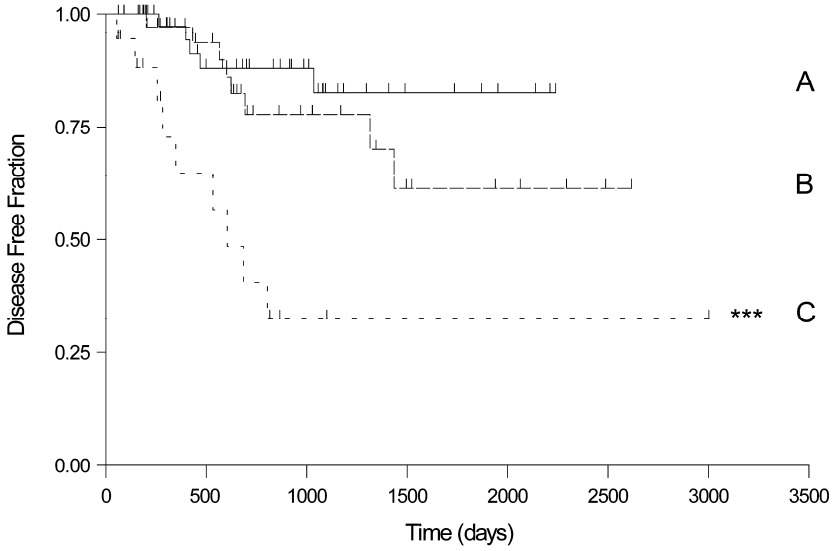

Figure 5. Survival curves calculated with Kaplan-Meijer estimate procedure for disease-free period in dogs with postoperative 6 to 10 week urinary corticoid-to-creatinine ratios (A) $< 1 \times 10^{-6}$, (B) 1 to $\leq 5.0\text{-x } 10^{-6}$, and (C) 5 to $\leq 10 \times 10^{-6}$ after transsphenoidal hypophysectomy for the treatment of pituitary-dependent hyperadrenocorticism in dogs. ***P < 0.001 compared with A and B

Discussion

The results of this study demonstrate that increased age, pituitary size, and basal plasma ACTH concentration are risk factors for shorter postoperative survival times, and that the thickness of the sphenoid bone, mean preoperative UCCR, pituitary size and plasma α-MSH concentration are risk factors for recurrence after transsphenoidal hypophysectomy for the treatment of PDH in dogs. Hypophysectomy in dogs has a higher postoperative mortality (8%), but remission (86%) and recurrence rates (23%) are comparable with those reported for humans with Cushing's disease.[2,7,41,45,49] The total success-rate in this study is 67% which is comparable to what has been reported in humans after pituitary surgery when long-term recurrences are considered.[2] The study-period of 12 years equals approximately 70-80 years in humans. Thus, we could include late recurrences. Recurrences despite the aim of complete hypophysectomy probably has to be ascribed to remnant islets of pituitary cells, as previously shown by Meij and co-workers.[36] These remnant cells may be of neoplastic origin or representants of unaffected pituitary cells. Regrowth of remnant adenomatous corticotrope cells most probably is responsible for part of the recurrences reported in the present study.[23] However, the very late recurrences (> 3-4 years after surgery) may very well be the result of de novo formation of adenomatous tissue from remnant corticotropes.

Prognosticators for Survival Time

The survival time after surgery is influenced by external factors, for example decision of the owners for either medical treatment or euthanasia in case of recurrence. The analysis of survival time also includes cases with postoperative mortality and residual disease, which are excluded from the analysis of disease-free period due to the absence of initial remission. This explains the initially surprising finding that age was a prognosticator for survival time. The Kaplan-Meijer analysis compensates for age by censoring deaths that are not related to the disease, therefore, the prognosticator age had to be related to PDH or to exert its effect in the early postoperative period (within 4 weeks after surgery) in which all deaths were defined as (disease-related) events. In line with the latter explanation, dogs that died during the first 4 weeks after surgery were significantly older than the dogs with residual disease or the dogs that achieved remission. Subsequently, the Cox's proportional-hazard analysis of survival time identified age as a risk factor for early mortality, which could be ascribed to the higher age among the dogs with immediate postoperative mortality. Whether the higher postoperative mortality in older dogs is caused by the presence of concurrent unrelated disease or a reflection of higher risk of complications due to a longer preoperative exposure to hypercortisolism remains to be investigated. For example, human patients with Cushing's disease comprise a high-risk group for pre- and postoperative morbidity and mortality of cardiovascular events and thromboembolism.[9,33,49]

In agreement with our previous study[24] and reports on transsphenoidal surgery for the treatment of Cushing's disease in humans,[11,13,41,55,60] it was expected to find pituitary size as a prognosticator for survival time. The present study provides calculated hazard ratios of the influence of the pituitary size on surgical outcome, which can be applied for individual risk calculations. There was no difference in pituitary size among dogs with postoperative mortality, residual disease or remission. Thus, in contrast to age, the pituitary size influences the survival time beyond the postoperative 4-week period. Human patients with Cushing's disease and suprasellar extension of the pituitary tumor[22] and tumor invasion into the

cavernous sinus [6,11] have higher risk of residual disease. In this study dogs with residual disease had higher pituitary height-to-width ratio than dogs that went into remission.

Interestingly, preoperative plasma concentration of ACTH(IRMA) was a significant prognosticator for survival time, and replaced the pituitary length in the final equation of the multivariate analysis. High preoperative basal plasma ACTH concentrations have been reported in humans with residual disease after transsphenoidal surgery,[11,49] and this has been ascribed to the large tumor size in this subgroup. However, low plasma ACTH concentrations have also been reported in humans with large pituitary tumors.[26] There was no significant correlation between pituitary size and the basal plasma ACTH(IRMA) concentration in the present series of dogs. However, in previous reports on dogs,[10,21] the basal plasma ACTH concentration correlated with pituitary size, but in this case the ACTH was determined by the RIA that also measures ACTH-precursor peptides. It is therefore concluded that plasma ACTH concentration, besides being related to adenoma size, may also give additional information on intrinsic characteristics of the corticotrope associated with poorer surgical outcome.

Prognosticators for Disease-Free Period (Recurrences)

It may be hypothesized that prognostic factors for recurrences are associated with increased risks of leaving remnant pituitary adenoma tissue in the pituitary fossa. In the dog, there is a large difference in skull size between breeds. For example, the smallest dog operated on in this study was a Yorkshire Terrier with a body weight of 4 kg and the largest dog was an Alaskan Malamute with a body weight of 61 kg. The differences in skull sizes and shape affect the distance between the surgeon and the pituitary fossa and consequently the visibility of the surgical field. An even more important factor for the accessibility of the surgical field may be the thickness of the sphenoid bone. In the future, this risk factor may be better addressed with the use of image-guided endoscopy which has gained popularity in pituitary surgery in human.[27,42] Using a rigid endoscope to explore the pituitary fossa for pituitary remnants after hypophysectomy, the recurrence rate may be reduced in the long term.

The pituitary size has frequently been reported to influence surgical outcome,[13,24,41,55] and it may be hypothesized that the larger the tumor, the higher the risk of remnant adenoma cells in the fossa. However, when added to the multivariate analysis, the basal plasma α-MSH concentration replaced the P/B ratio in the multivariate equation. This finding can partly be explained by the correlation between basal α-MSH concentration and pituitary size, as previously published for dogs.[10] Additionally, the basal α-MSH concentration may reflect an aggressive behavior of the pituitary tumor, similarly to what has been described for high plasma pro-opiomelanocortin concentrations in humans with aggressive corticotrope cell tumors.[48] In the dog, the plasma α-MSH concentration correlates significantly with plasma ACTH precursor concentrations.[10] Interestingly, disease recurred in most of dogs with high α-MSH concentrations. These dogs usually had dexamethasone resistant PDH and enlarged pituitaries, most of them probably of pars intermedia origin.[51,52]

Independent of pituitary size and the plasma α-MSH concentration, a high preoperative UCCR was associated with increased risk of recurrence in dogs, which is in agreement with a study in humans where a high urinary cortisol excretion was a risk factor for recurrence.[57] Measuring cortisol in morning urine has the advantage that it mirrors the integrated production over a period of about 8 h, and thereby adjusts for the wide and rapid fluctuations in plasma cortisol levels. It has been speculated that the degree of resistance to suppression of

the cortisol secretion by dexamethasone correlates with the risk of recurrences.[11] In the present study, after high-dose dexamethasone administration, the relative suppression of pre-operative UCCR was not a prognosticator for disease-free period, but the absolute suppressed UCCR-value was.

Within the group of corticotrope cell tumors there is a wide heterogeneity with regard to cellular characteristics. The results of this study indicate that measurements of the plasma α-MSH and ACTH concentrations and urinary cortisol may contribute to pre-operative characterization of the pituitary lesion.

Postoperative UCCR

One may hypothesize that dogs with postoperative UCCRs in the upper normal range have a higher risk of recurrence or even can be considered to have residual disease. In the present study the postoperative UCCRs sampled 6-10 weeks post surgery were analyzed. In this time period remnant islets of pituitary tissue may release ACTH in response to CRH stimulation.[37] Indeed, as confirmed in the present study, there is a considerable difference in the remission rate between dogs with a low UCCR ($< 5 \times 10^{-6}$) 6 to 10 weeks post surgery compared to dogs with UCCR between 5 and 10×10^{-6}. However, a urinary cortisol below detection limits ($UCCR < 1 \times 10^{-6}$) is no guarantee for lifelong remission, whereas dogs with cortisol secretion in the upper normal range do not all develop a recurrence. These findings further illustrate the difficulty of distinguishing remnant normal corticotropes from remnant adenoma cells, as described previously for dogs.[36,37] The same holds true for selective pituitary adenomectomy in humans with Cushing's disease.[2,12,17,45,49,54,66] It should be noted that the dogs in the present study were on a physiological dose of hydrocortisone, which was withheld for 12 h prior to urine sampling. In humans, the best predictive values for surgical outcome were achieved when no cortisol was administered, until symptoms of hypocortisolism arose combined with early postoperative cortisol measurement and dexamethasone suppression test.[28,54]

Conclusions

We have identified several risk factors that can be addressed to further improve surgical outcome after hypophysectomy in the treatment of PDH in dogs. Old age, large pituitary size and high basal plasma ACTH concentration are significant prognosticators for postoperative survival. Pituitary enlargement, elevated plasma α-MSH concentration, thick sphenoid bone and mean preoperatively UCCR are significant risk factors for recurrence. In parallel with findings in human studies, postoperative UCCR measured at 6 to 10 weeks after surgery can be used as guidance for predicting surgical outcome, albeit it is insufficient to predict every case of recurrence.

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