

Lanreotide and Cabergoline treatment of Dogs with Cushing's Disease



Research Project Veterinary Medicine University Utrecht

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Abstract

Transsphenoidal selective adenomectomy is the first choice of treatment in humans with Cushing's disease (CD), even though recurrences are frequent. Therefore, it is important to find an effective and safe medical treatment for CD. Demonstration of expression of somatostatin and dopamine receptors on corticotroph adenomas of humans offers the possibility for medical treatment of CD with somatostatin analogues and dopamine agonists.

Because CD has a higher prevalence rate in dogs than in humans, research has been conducted on canine corticotroph adenoma tissue to look for similarities between humans and dogs. The results from this previous study revealed that canine corticotroph adenomas obtained after hypophysectomy provided an interesting model to study corticotroph cell physiology *in vitro*. In current study research has been conducted to see if dogs can be a suitable animal model *in vivo* for the medical treatment options of CD in human.

Five dogs were treated with lanreotide autogel for six months. One of these dogs also received Cabergoline for three months. Results showed that UCCR decreased after one month of treatment. One dog showed a decrease of plasma ACTH after six months of treatment with lanreotide. CT-scans showed an inhibition in tumor growth in Dog 1, 2 and 4 (Dog 5 has not yet finished the treatment period). In conclusion, lanreotide showed effects on UCCR, plasma hormones and tumor growth. The results until now were not statistically significant; however, the sample size until now was very small. Therefore, most probably, the results will be significant when an additional five dogs are included in the study.

1. Introduction

This study focuses on the treatment with a long-acting somatostatin analogue, and in one case also a dopamine agonist, of dogs with Cushing Disease (CD).

Cushing disease (CD) is a severe disorder characterized by chronic hypercortisolism due to an ACTH-secreting pituitary adenoma. {{Orth,D.N. 1995}} At this moment, the primary treatment of human patients with CD is transsphenoidal selective adenomectomy. {{Esposito,V. 2004; Porterfield,J.R. 2008}} Initial remission can be achieved for 55 to 80% of patients with CD. {{Atkinson,A.B. 2005; Fomekong,E. 2009}} The remission rate depends, among other things, on the anatomic location of the tumor, the size of the tumor, and the experience of the neurosurgeon. {{Yap,L.B. 2002}}

The recurrence rate after transsphenoidal selective adenomectomy ranges between 7 and 24%. {{Swearingen,B. 1999; Pereira,A.M. 2003; Esposito,V. 2004}} Consequently, an effective and safe medical treatment for CD is required for patients with recurrence. Because of the expression of somatostatin subtype receptors (sstrs) and dopamine receptor subtype 2 (DRD₂) in the majority of human corticotroph adenomas, these receptors may be targets for new forms of medical treatment of CD. {{der Hoek,Joost 2004; Pivonello,R. 2004; Missale,C. 1998}}

Somatostatin is a cyclopeptide and is secreted by hypothalamic neurons into the hypophyseal portal circulation. {{Patel,Y.C. 1999; Lamberts,S.W. 1988}} Important functions of somatostatin are the inhibition of anterior pituitary hormone secretion and suppression of cell proliferation. {{Lamberts,S.W. 1988; Patel,Y.C. 1999}} The actions of somatostatin are mediated by five different membrane-bound receptors, the somatostatin receptor subtypes (sstr 1-5). {{Patel,Y.C. 1999; Hoyer,D. 1995}} Targets for these sstrs are somatostatin analogues, which have gained much scientific interest as a potential medical treatment option for CD patients. {{Boscaro,M. 2009; Colao,A. 2007; de Bruin,C. 2008; de Bruin,C. 2009; der Hoek,Joost 2004; Hofland,L.J. 2008; Hofland,L.J. 2005; Batista,D.L. 2006}}

Octreotide is a somatostatin analogue which was first synthesized in 1982. {{Bauer,W. 1982}} It is widely used for the medical treatment of acromegaly. {{Lamberts,S.W. 1996}} Research on the *in vitro*

effects of the somatostatin analogue Octreotide, shows that the binding capacity of Octreotide is mainly restricted to sstr₂. {{Hofland,L.J. 2005}} Treatment with this analogue consists of two or three subcutaneous injections per day. To decrease the frequency of administration new formulations have been developed {{Ronchi,C.L. 2007; Cozzi,R. 2006}}, such as Octreotide long acting release, lanreotide slow release microparticles (20-30 mg every 7-14 days) {{Cozzi,R. 2006}}, and lanreotide Autogel (60, 90 or 120 mg every 28 days). {{Ronchi,C.L. 2007}} lanreotide slow release particles and lanreotide autogel have been used as first-line medical therapy of acromegaly {{Ayuk,J. 2002}}, and appears to cause a shrinkage of tumor size in up to 50% of cases. {{Bevan,J.S. 2005}} Due to the similarities between receptor expression in acromegaly and CD {{van der Hoek,J. 2007; Jaquet,P. 2000}}, somatostatin analogues are a potential option for the medical treatment of CD.

Dopamine is a catecholamine neurotransmitter and exerts its function by binding to dopamine receptors. {{Missale,C. 1998}} Binding to D₂-like receptors (DRDs) will inhibit cyclic AMP production {{Missale,C. 1998}} and will evoke an inhibitory effect. {{Missale,C. 1998}} Targets for DRDs are dopamine analogues, e.g. Cabergoline. In pituitary tumors dopamine agonists have shown to exert an anti-proliferative effect by inducing cell death. {{An,J.J. 2003}}

The expression of sstr₂ is lower compared to sstr₅ and DRDs. This is due to the negative feedback of corticosteroids on sstr₂ in human corticotroph adenoma. {{Hofland,L.J. 2008; de Bruin,C. 2009; van der Hoek,J. 2005; Hofland,L.J. 2008}}

CD is also a well-known endocrinopathy in dogs. {{Capen,C.C. 1975; Kempainen,R.J. 1994}} In dogs, the choice for hypophysectomy as treatment for CD depends on the size of the pituitary adenoma, the age of the dog, the urine corticoid-to-creatinine ratios (UCCRs), and thickness of the sphenoid bone. {{Hanson,J.M. 2007; Hanson,J.M. 2005}} CD in dogs can also be treated with a competitive inhibitor of 3 β -hydroxysteroid dehydrogenase (trilostane). {{Ruckstuhl,N.S. 2002}} Chemotherapy with the adrenocorticolytic drug mitotane can be used as an alternative therapy. {{Barker,E.N. 2005}}

CD occurs much more frequent in dogs than in humans. However, the exact prevalence of CD in the dog population is unknown, because there is no formal registration system. Estimations indicate that CD occurs in 1-2 dogs per 1,000 dogs per year. {{de Bruin,C. 2009}} In humans, the incidence of CD is 1.2-2.4 cases per million per year. {{Lindholm,J. 2001; Etxabe,J. 1994}} Kempainen (1994) mentioned dogs as a possible animal model for CD in humans. Dogs with CD usually have an anterior pituitary adenoma; this also occurs most frequently in humans with CD. {{Kempainen,R.J. 1994; Orth,D.N. 1995}} As a result of these similarities between dogs and humans, research has recently been conducted to compare receptor subtypes on primary corticotroph adenoma tissues. {{de Bruin,C. 2008}} In dogs sstr₂ was the predominant receptor subtype expressed on corticotroph adenoma tissue. {{de Bruin,C. 2008}} DRD₂ was modestly expressed and sstr₅ was expressed only at very low levels. {{de Bruin,C. 2008}} Although the receptor expression in humans and dogs are not completely comparable, the results in this study indicate that canine corticotroph adenoma is an interesting model to study corticotroph cell physiology *in vitro*. {{de Bruin,C. 2008}}

Given the model function of canine corticotroph adenoma tissue for human *in vitro*, this study focuses on the treatment with a long-acting somatostatin analogue (and in some cases a dopamine agonist) of dogs with CD. It is hypothesized that lanreotide autogel will reduce the tumor size and will achieve clinical improvement of dogs with CD.

The main study aim was to treat 9 dogs with pituitary dependent hypercortisolism with lanreotide Autogel for 6 months (and 3 months with Cabergoline if necessary). After the treatment period corticotroph adenomatous tissue was collected and characterized for the expression of the sstrs and DRDs of interest in the research of human CD.

The results of this study demonstrate that lanreotide has effects on dogs with CD. This suggests that administration of somatostatin analogues in combination with dopamine agonists will have beneficial effects on humans with CD, but further research is necessary.

2. Materials and methods

2.1. Study population and diagnosis of PDH

This study includes 10 dogs with pituitary-dependent hyperadrenocorticism (PDH). These dogs were of different breeds. Age ranged from 8 to 11 years and body weight ranged from 10.1 to 50.3 kg.

The diagnosis of hypercortisolism was based upon clinical signs, results of hematology and clinical biochemistry, and the UCCR in morning urine samples collected at home. {{Rijnberk,A. 1988; Stolp,R. 1983}} All 5 dogs had UCCRs (mean ratio $22.8 \pm 5.8 \times 10^{-6}$) exceeding the values in healthy dogs ($< 8.3 \times 10^{-6}$). {{van Vonderen,I.K. 1997}} A high dose dexamethasone suppression test (HDDST), consisting of administration of three oral doses of 0.1 mg dexamethasone/kg at 8-hour intervals after collection of the second urine sample, was performed in each dog. The next morning a third urine sample was collected at home. In 3 dogs the UCCR in the third urine sample was suppressed by more than 50% consistent with the diagnosis of PDH. {{Galac,S. 1997}} A suppression of less than 50% after dexamethasone was observed in 2 dogs. In these 2 dogs the diagnosis of dexamethasone-resistant PDH was confirmed by high plasma ACTH concentrations, ultrasonography of the adrenals, and pituitary imaging. Computed tomography (CT) was performed to visualize the dimensions of the pituitary gland and the surgical landmarks for hypophysectomy. {{van der Vlugt-Meijer,R.H. 2003}} The mean ratio between the pituitary height and the brain area (P/B ratio) of the 5 dogs was 0.5×10^{-2} and all were $> 0.31 \times 10^{-2} \text{ mm}^{-1}$ consistent with pituitary enlargement due to a pituitary adenoma. Further characteristics of the patients are listed in table 1.

2.2. Study design

Canine patients were treated in this study at the Department of Clinical Sciences of Companion Animals in Utrecht, the Netherlands. A CT scan was made as part of the diagnostic procedure for patients with CD. If the CT scan showed a pituitary macroadenoma, owners were asked whether their dog could participate in this study (see paragraph 2.5.).

The dogs were treated monthly, during a 6-month period, with a subcutaneous injection of lanreotide autogel. The dosage that was given was based on bodyweight (Table 1) and response to therapy (i.e. improvement of clinical symptoms and lowering of UCCR). Owners were asked to collect 2 morning urine samples before the treatment period and 1 every 7 days after the start of the treatment. Urine samples were collected at home to assure that they were collected in a stress free environment.

The dogs were evaluated each month, after which a new injection of lanreotide autogel was administered. During this evaluation 2 blood samples (12 ml) were taken from the jugular vein with an interval of 10-15 minutes. Blood samples were used for biochemical and plasma hormone analyses. Because of the pulsatile secretion of cortisol, ACTH, α -MSH and growth hormone (GH), the other blood sample was used for a second measurement of these hormones. Blood samples were collected in oxalate fluoride-coated tubes for the measurement of glucose concentrations. For the measurement of electrolytes and cortisol, samples were collected in heparin-coated tubes and in pre-cooled EDTA-coated tubes for the measurement of the plasma concentrations of ACTH, α -MSH, GH and insulin-like growth factor-I (IGF-1). All hormones were measured as described previously. {{Hanson,J.M. 2006}}

In addition, the severity and number of symptoms and signs of CD were assessed at the beginning of the study and at every monthly evaluation. The clinical parameters mentioned in Appendix 3 were assessed by interviewing the owners of the dogs and by clinical examination of the canine patients (performed by the same researcher every time). Symptoms and signs were graded as none (3), moderate (2) or severe (1).

During the evaluation moments, body fat was measured using bioelectrical impedance. Impedance was measured on the left side of the dog, behind the posterior ribs, 20 mm below the spine. Hairs were combed aside and a 15 cm line was wiped clean with ethanol. Then ethanol was applied again on hairs and skin in the region where the electrodes were placed to ensure good contact between the voltage electrodes and the skin. The body fat measurements were repeated 3 times with an interval of 10 seconds (Appendix 4).

The response on treatment was evaluated by the change in the UCCR and the changes in the clinical manifestations, two months after the beginning of the study. If the symptoms persisted and the UCCRs remained elevated, dogs were treated with a higher dose of lanreotide autogel. After a period of 4 months, a second assessment was performed. If not enough clinical improvement was noted, the dose of lanreotide was raised again.

In order to assess the effects of lanreotide autogel on pituitary size, the CT scan was repeated after 6 months of treatment. At the end of the experimental period, the dogs were treated according to normal treatment procedures. If hypophysectomy was performed, corticotroph adenomatous tissue was collected and investigated for the expression of sstrs and DRD's.

2.3. Surgical tissue

At the end of the experimental period, the dogs were treated according to normal treatment procedures. If the owner agreed on transsphenoidal hypophysectomy, the pituitary adenomatous tissue was surgically resected as described previously. {{Meij,B.P. 1997}} Additionally, corticotroph adenomatous tissue was collected and investigated for the expression of sstrs and DRDs. One part of the pituitary adenomatous tissue was fixated in 4% buffered paraformaldehyde and send for histopathology to evaluate ACTH, α -MSH and GH expression. The other part was used for cell isolation.

2.4. Cell isolation, distribution and culture

Cell isolation, distribution, culture and cytopspins were done as described previously {{de Bruin,C. 2008}} to be able to compare results with the results obtained by de Bruin *et al* (2008).

2.4. Statistical analysis

Since the number of dogs treated with lanreotide in this study is limited until now, there will be a case description for every dog individually. Besides these case descriptions, statistic analyses were done.

Data are expressed as mean \pm SEM or as median and range (as the data were not normally distributed). Differences between paired values were evaluated with the Wilcoxon signed rank test; because the data are non-parametric. To evaluate the effects of possible outliers for the UCCRs, a robust analysis was done for these parameters. A P value < 0.05 was considered statistical significant.

2.5. Ethics of experimentation

This study was approved by the Ethical Committee of the faculty of Veterinary Medicine, Utrecht University, The Netherlands. The written consent of the dog owner was obtained before participation in this study as described in appendix 5.

Table 1: Characteristics of patients

Dog	Breed	Gen- der	Age (yr)	BW (kg)	Pit Size ^a (mm)	P/B ratio ^b (x10 ⁻² mm)	UCCR ^c (x 10 ⁻⁶)	HDDST ^d (%)	ACTH ^e (pmol/l)	α-MSH ^f (pg/ml)	Cortisol ^g (nmol/l)
1	German Shepherd dog	M	10	50.3	8.7/12.4/10.9	0.46	25	42.3	96	37.5	136
2	English Cocker Spaniel	F	8	13.5	6.8/6.3/6.3	0.42	47.5	89.5	57	20.1	125
3	Border Terriër	M	10	10.1	8.3/10/10	0.48	45.4	49.3	85	23	128
4	Bouvier des Flandres	F	11	31.4	9.3/9.5/8	0.58	20.8	78.4	70		112
5	Border Collie	M	10	18.3	9.7/10/9.6	0.56	55.2	61.2	88.5		114.5

^a Pituitary size is measured on CT scan (height, width and length, mm)

^b P/B (mm⁻¹) ratio between the pituitary height (mm) and the brain area (mm²)

^c Pretreatment UCCR values are the mean of two morning urine samples (reference < 10 x 10⁻⁶)

^d Pretreatment degree of UCCR suppression after dexamethasone (percentage of reduction compared to the mean of the first two samples)

^{e,f,g} Pretreatment plasma ACTH (reference 1.1-18.7 pmol/L), α-MSH (reference <36 pg/ml) and cortisol (reference 11-136 nmol/L) values are a mean of two samples collected with an interval of 10-15 minutes.

3. Results

3.1. Follow-up of study population

3.1.1. UCCR

UCCR levels were decreased in 4 out of 5 dogs (80%). UCCRs of Dogs 1, 2, 4 and 5, treated with lanreotide autogel, were decreased after one month of treatment in comparison to the values before treatment (Fig. 1). Dog 1 had a decreased UCCR during the entire treatment period, except during week 7 (Fig 1 and Table 2). Dogs 2, 4 and 5 had a more variable response. Dog 3 had a minimal response during week 6 only. Overall, a decrease in UCCR could be observed during therapy (Dog 5 is still under treatment).

UCCRs are important indicators for improvement of dogs with CD. Dog 2 showed much variation in UCCRs and Dog 3 had missing values because this animal did not complete the treatment period. Therefore, a robust analysis was conducted as well to see if Dogs 2 and 3 had a significant influence on the statistical results of the UCCRs. As can be seen in Table 2, the results are not significantly affected when Dog 2 or 3 is excluded from the robust analysis. This supports the fact that the sample size is too small to have any statistical results until now.

Figure 1: UCCR of patient 1 to 5 during treatment period with lanreotide

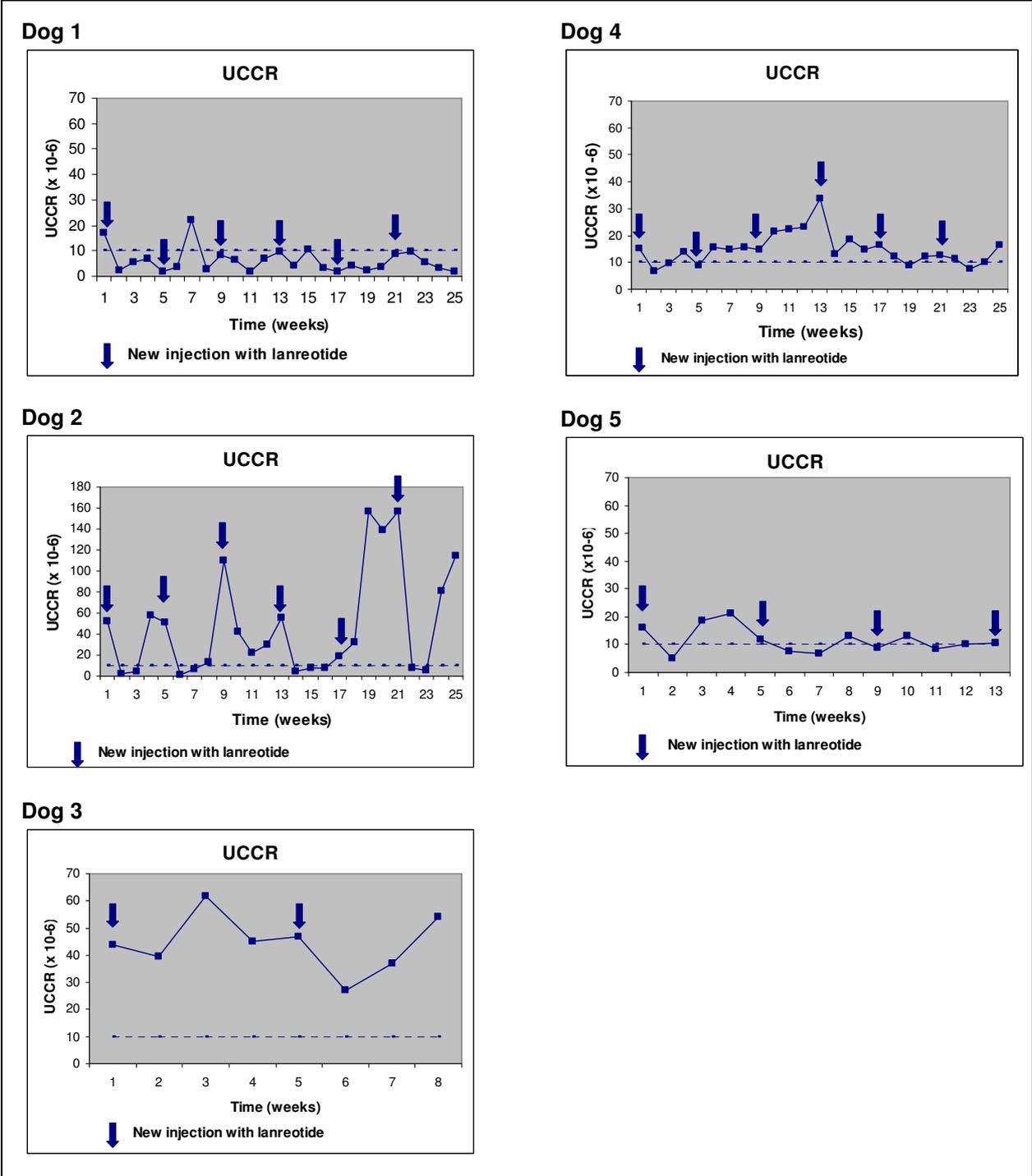


Table 2: Weekly urinary corticoid/creatinine ratio (UCCR)

	Start	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
UCCR	17 (12 to 52)	5 (2.1 to 39) ^{##}	9.5 (4.3 to 62)	21.1 (6.8 to 57.5)	11.8 (1.8 to 50.8)	7.6 (1.4 to 27)	14.9 (6.2 to 37)	13.4 (2.6 to 54)	11.8 (8.2 to 110)
UCCR - 2	16 (12 to 44)	5.9 (2.4 to 39)	14.1 (5.6 to 62)	17.5 (6.8 to 45)	10.3 (1.8 to 47)	11.7 (3.7 to 27)	18.5 (6.7 to 37)	14.5 (2.6 to 54)	8.7 (8.2 to 14.9)
UCCR - 3	16 (12 to 52)	3.7 (2.1 to 6.7)	7.6 (4.3 to 18.6)	17.5 (6.8 to 57.5)	10.3 (1.8 to 50.8)	5.7 (1.4 to 15.7)	10.8 (6.2 to 22.1)	23.3 (2.6 to 15.7)	11.8 (8.2 to 110)
	Week 9	Week 10	Week 11	Week 12	Week 13	Week 14	Week 15	Week 16	Week 17
UCCR	21.7 (6.4 to 42.5)	22 (2 to 22.3)	23.3 (7 to 30.2)	33.8 (9.9 to 55.8)	4.1 (4 to 13.2)	10.5 (7.4 to 18.5)	8.4 (3.3 to 14.6)	12.2 (1.8 to 18.5)	16.1 (4.2 to 31.8)
UCCR - 2	14.1 (6.4 to 21.7)	12.2 (2 to 22.3)	15.2 (7 to 23.3)	21.9 (9.9 to 33.8)	8.6 (4 to 13.2)	14.5 (11 to 18.5)	9.0 (3.3 to 14.6)	9.1 (1.8 to 16.4)	8.2 (4.2 to 12.2)
UCCR - 3	21.7 (6.4 to 42.5)	22 (2 to 22.3)	23.3 (7 to 30.2)	33.8 (9.9 to 55.8)	4.1 (4 to 13.2)	10.5 (7.4 to 18.5)	7.3 (3.3 to 14.6)	16.4 (1.8 to 18.5)	12.2 (4.2 to 31.8)
	Week 18	Week 19	Week 20	Week 21	Week 22	Week 23	Week 24		
UCCR	9 (2,4 to 157)	12.3 (3.5 to 139)	12.6 (8.6 to 157)	8.7 (7.6 to 9.8)	5.5 (5.3 to 5.7)	41.9 (3 to 80.7)	58.1 (1.8 to 114)		
UCCR - 2	5,7 (2,4 to 9)	7.9 (3.5 to 12.3)	10.6 (8.6 to 12.6)	NA	NA	NA	NA		
UCCR - 3	9 (2,4 to 157)	12.3 (3.5 to 139)	12.6 (8.6 to 157)	8.7 (7.6 to 9.8)	5.5 (5.3 to 5.7)	41.9 (3 to 80.7)	58.1 (1.8 to 114)		

Values are expressed as median and range. Urinary corticoid/creatinine ratio (UCCR n = 5).

UCCR - 2 = UCCRs minus values of Dog 2

UCCR - 3 = UCCRs minus values of Dog 3

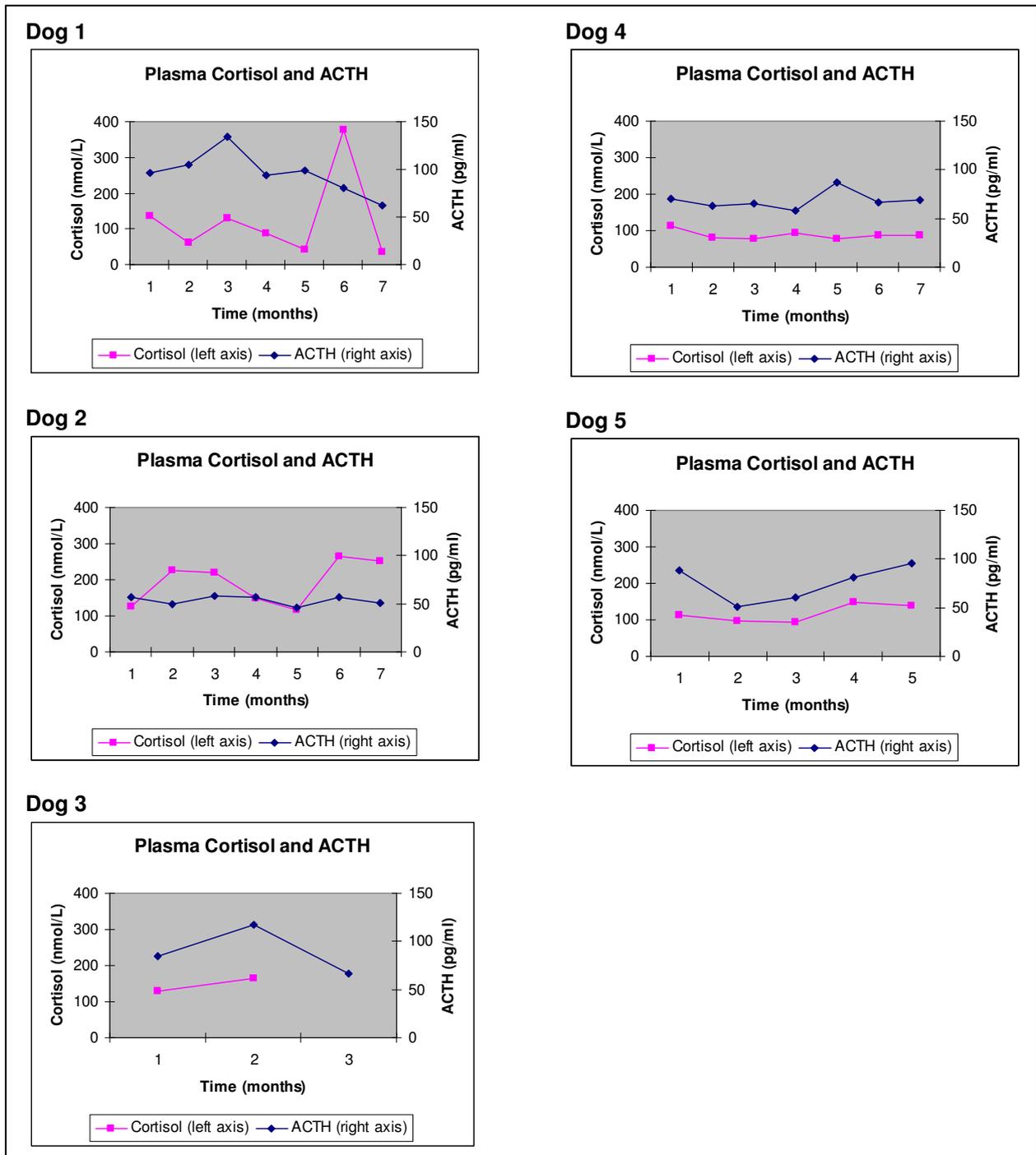
= statistical significant (P < 0.01)

= statistical significant (P < 0.05)

3.1.2. Plasma hormone concentrations

The hormones of main interest, ACTH and cortisol, are displayed in Figure 2. Of the dogs that finished the 6-month treatment (Dogs 1, 2 and 3), the plasma ACTH concentration was decreased during month 6 compared to the values at the beginning of the study. However, this decrease was not statistically significant. Plasma cortisol was more variable during the treatment period of Dogs 1-5. Table 3 shows the results of all the measured plasma hormones per month.

Figure 2: Plasma Cortisol and ACTH of patients 1-5 during treatment period with lanreotide



3.1.3. Clinical signs

When concerning all clinical parameters described in Table 4, endurance is the only clinical symptom which improved during the treatment period (Figure 3 and Table 4).

Dog 2 still had PU/PD during the treatment period. However, after 2 months of treatment, the owner noticed a remarkable difference in the dog's attitude; the dog was more active and was no longer focused on drinking the entire day.

Dog 4 had a response in hair growth on 2 places on the left and right thorax. An inflammatory reaction had occurred at these 2 places as a result of the subcutaneously injected dose of lanreotide (Appendix 8, Dog 4).

Figure 3: Change in the most important clinical symptoms in percentages of Dog 1-5

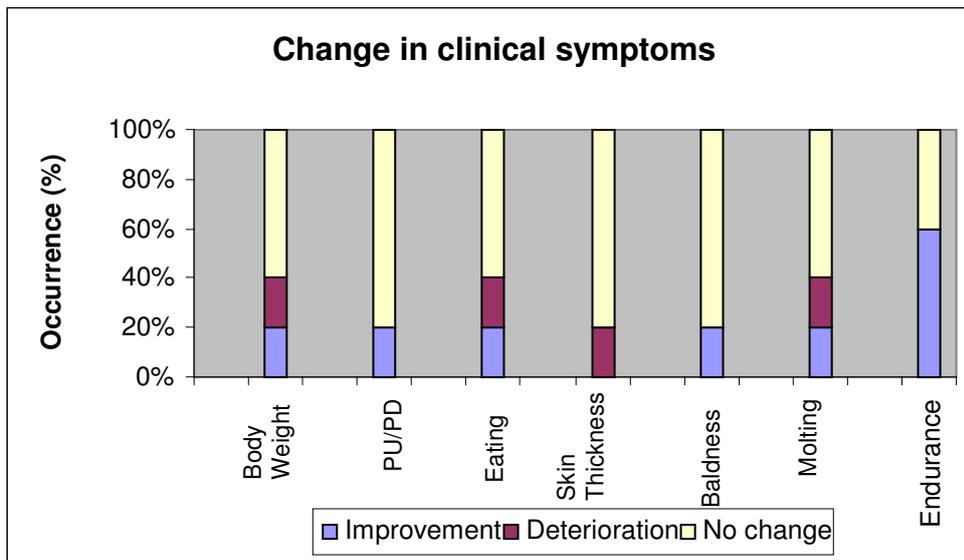


Table 3: Plasma hormone concentrations during 6 evaluation moments

Hormones	Start	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Cortisol (nmol/L)	119.9 ± 3.9	125,7 ± 30.4	130.5 ± 31.8	119.5 ± 16.5	78.3 ± 22	242.2 ± 84.7	144.5 ± 108
ACTH (pg/ml)	79.2 ± 7.0	83.6 ± 16.1	85.8 ± 24.4	75.8 ± 18.8	72.3 ± 26.8	68.5 ± 11.5	56.5 ± 5.5
TSH (ng/ml)	0.20 ± 0.05	0.26 ± 0.06	0.28 ± 0.07	0.38 ± 0.02	0.34 ± 0.03	0.24 ± 0.05	0.24 ± 0.10
T4 (nmol/l)	7.9 ± 3.3	10 ± 4.4	13.5 ± 4.3	9.3 ± 3.2	8.0 ± 5.0	8.0 ± 3.1	15.5 ± 3.5
GH (ng/ml)	0.56 ± 0.06	0.58 ± 0.09	0.47 ± 0.09	NA	NA	NA	NA
Alfa-MSH	NA	NA	NA	NA	NA	NA	NA
IGF-1 (ng/ml)	NA	NA	NA	NA	NA	NA	NA

Values are expressed as mean ± S.E.M. Adrenocorticotropin (ACTH n = 5), thyroid stimulating hormone (TSH n = 5), Tyroxine (T4 n = 5), growth hormone (GH n = 4), insulin-like growth factor (IGF-1 n = 5)

NA = not yet available

= statistical significant (P < 0.01)

= statistical significant (P < 0.05)

Table 4: Clinical symptoms of patient 1 to 5 per month

Dog	1						2						3		4						5						
Evaluation moment	0	1	2	3	4	5	6	0	1	2	3	4	5	6	0	1	0	1	2	3	4	5	6	0	1	2	3
weight loss	3	3	3	3	3	3	3	3	3	3	3	2	2	3	3	3	2	3	3	3	3	3	3	3	3	3	
Behavioural chance	3	2	3	3	3	3	3	2	3	3	3	2	3	3	2	2	2	2	3	2	3	3	2	2	3	2	
Neurological symptoms	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	
Eating	3	3	3	3	3	3	3	2	3	3	3	2	3	3	2	2	3	3	3	3	3	3	2	2	2	2	
Drinking	1	1	1	1	3*	1	1	1	1	1	1	2	2	1	1	1	1	1	1	1	1	1	2**	2	2	2	
skin thickness	?	3	3	3	3	3	3	2	2	2	2	2	2	2	1	1	2	2	2	2	2	2	3	2	2	2	
Turgor	3	2	3	2	2	2	3	2	2	2	2	2	2	2	2	2	3	3	3	2	3	2	3	3	3	3	
baldness	3	3	3	3	3	3	3	2	3	3	3	3	3	3	2	2	1	1	1	1	1	1	2	2	2	2	
Molting	3	3	3	3	3	3	3	3	3	3	3	2	2	2	1	1	1	1	1	2	1	1	1	1	1	1	
endurance	2	2	2	3	3	3	3	2	2	2	3	3	3	3	2	2	3	3	3	3	3	3	2	3	3	3	

* During this month the dog was treated with Minrin. The response strongly suggests diabetes insipidus due to the large pituitary adenoma.

** Dog 5 was continuously treated with Minrin for PU/PD. The dog became category 1 after 1 day without Minrin suggesting diabetes insipidus due to the large pituitary adenoma.

3.1.4. Body Fat

Monthly body fat measurements provide an overview of abdominal fat during the experimental period. As can be seen in Figure 4, body fat measurements show much variety during the treatment period. Table 5 shows that changes in body fat during the treatment period were not statistically significant.

Table 5: Abdominal Fat measurements during evaluation moments

	Start	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Abdominal Fat	36.3 ± 1.9	37.3 ± 0.9	35.4 ± 2.7	37.8 ± 1.3	35.9 ± 1.9	38.9 ± 1.2	NA

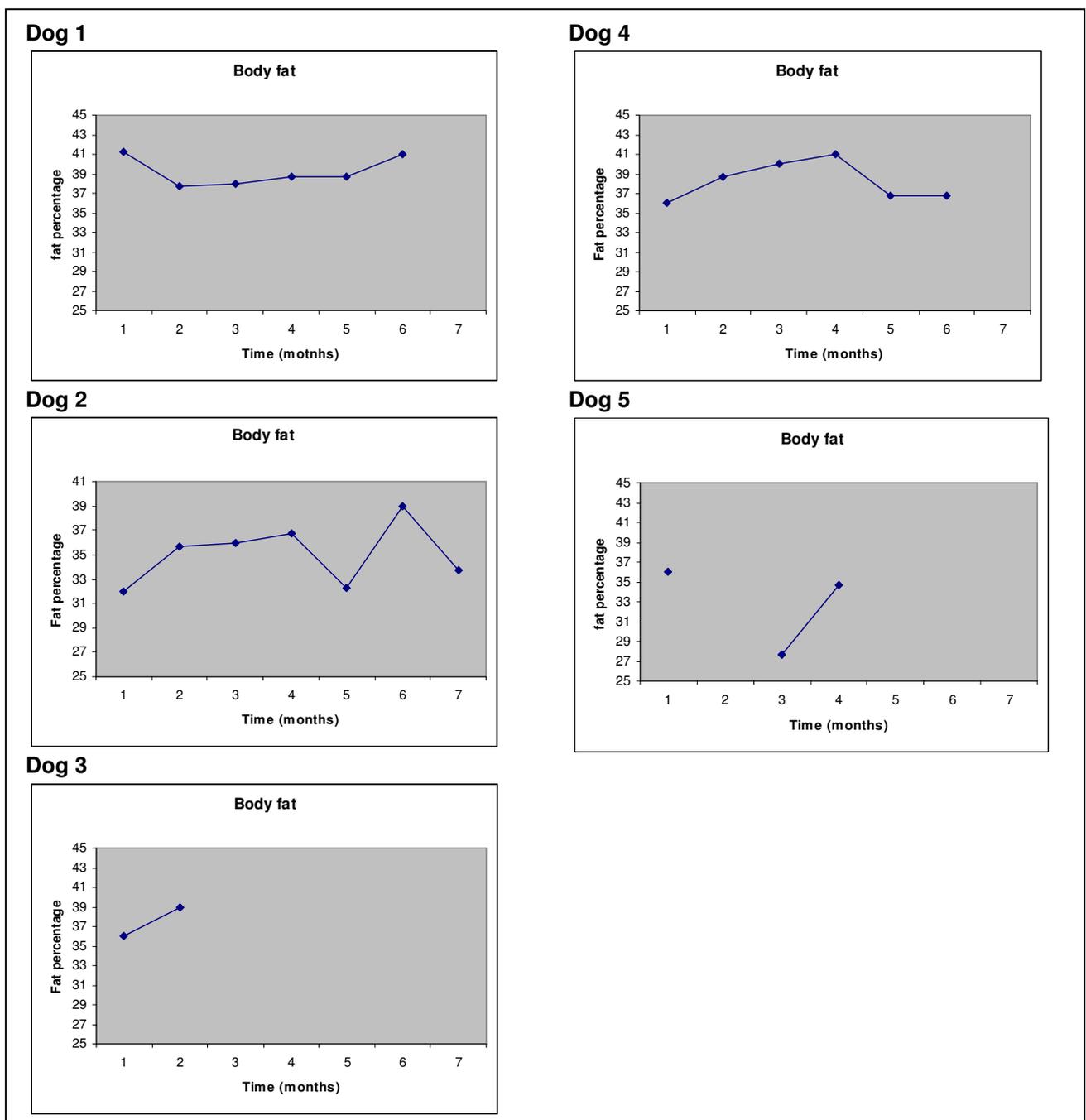
Values are expressed as mean ± S.E.M. Abdominal Fat (n=4)

NA = not yet available

= statistical significant (P < 0.01)

= statistical significant (P < 0.05)

Figure 4: Body fat of patients during experimental period



3.1.5. CT scans and P/B-ratios

CT scans were used to measure the 3-D dimensions of the pituitary and the P/B ratios. Figures 5, 6 and 7 show P/B ratios before and after treatment. The P/B-ratio of Dog 1 did not change after the treatment period, whereas the ratio of Dog 2 was decreased with 0.05 mm and the ratio of Dog 4 increased 0.04 (Figures 6 and 8). A change under 0.10 mm does not confirm growth or reduction of tumor size has actually taken place.

Figure 5: CT scans dog 1

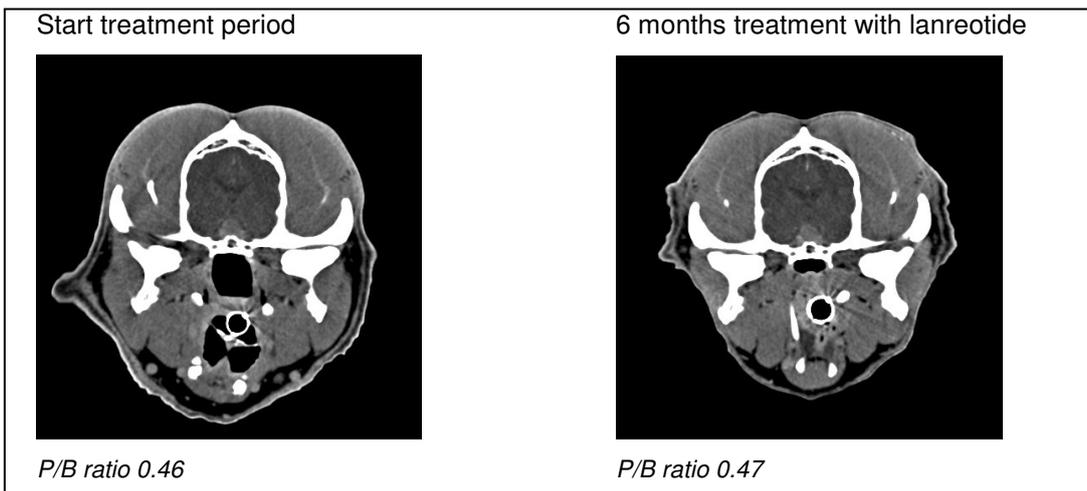


Figure 6: CT scans dog 2

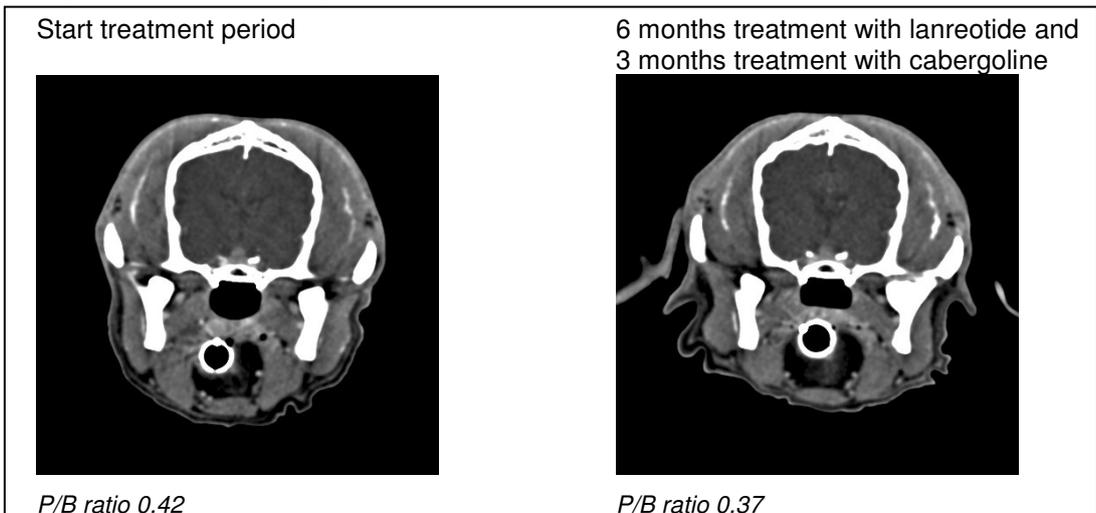


Figure 7: CT scans dog 4

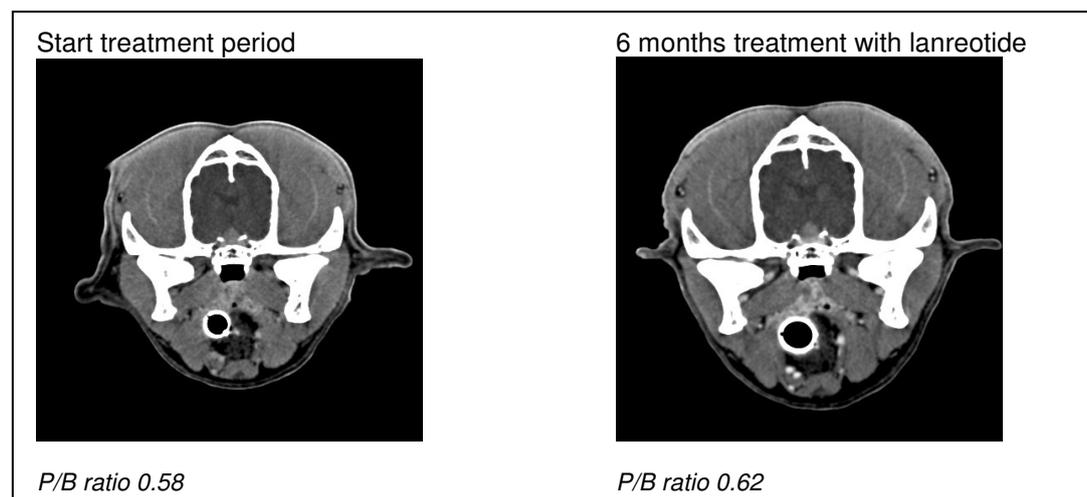
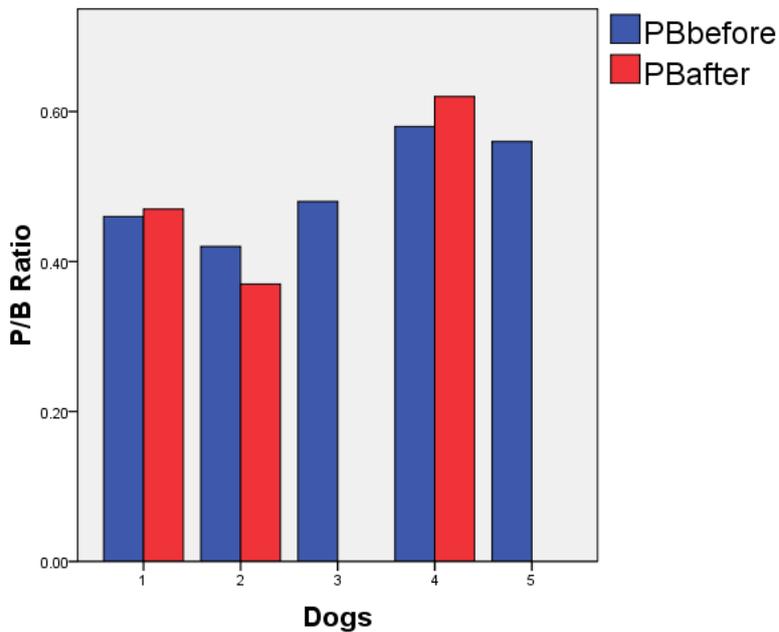


Figure 8: P/B ratios before and after a six-month treatment period



3.1.6. Tolerability and side effects

The side effects that occur in human CD-patients during lanreotide treatment are mostly gastrointestinal {{Ronchi,C.L. 2007; Lucas,T. 2006}} and have sporadically been observed in treated dogs. Dog 3 had an inflammatory reaction to the subcutaneous injection of lanreotide and suffered from worsening of the clinical symptoms. This dog was excluded from the study and treated with Vetoryl[®]. The inflammatory reactions were treated with antibiotics (Amoxicillin Clavulanate, Synulox[®], 20 mg/kg 2dd) and wound refreshment.

Dog 4 experienced gastrointestinal problems after the second injection with lanreotide during a period of 2 days. After the third injection this dog vomited once. Also this dog showed 2 inflammatory reactions with a diameter of approximately 5 cm on both the left and right thorax (Appendix 8, Dog 4). These inflammatory reactions were treated with antibiotics (Amoxicillin Clavulanate, Clavubactin[®], 20 mg/kg 2dd).

Dogs 2 and 3 experienced a period of vomiting of 2 days after extending the therapy with Cabergoline. By slowly raising the dose of Cabergoline this problem was resolved. Continuation of the Cabergoline therapy in Dog 2 caused a period of decreased appetite. This problem resolved after 1 month.

3.2. Effects of dexamethasone on sst mRNA expression

Regulation of receptor subtype expression by glucocorticoids was investigated by the effects of dexamethasone on sstr mRNA expression in primary corticotroph cultures with enough cells to allow for additional experiments.

4. Discussion

Somatostatin analogues can be used to show *in vitro* effects on human and canine pituitary adenomatous tissue {{de Bruin,C. 2008; 4 de Bruin,C. 2008}} This thesis studies the *in vivo* effects of somatostatin analogues in dogs to confirm that dogs with CD are a valid animal model for this disease in humans. Moreover, the complete results of this study indicate whether administration of somatostatin analogues will be a future treatment option for dogs with PDH.

The observed responses of UCCR and plasma hormones to the treatment with lanreotide and Cabergoline varied per dog. Dogs 1, 2, 4 and 5 showed intermittent reductions of UCCR values to baseline values ($< 8.3 \times 10^{-6}$) during the experimental period. Plasma ACTH concentration in Dog 1 showed a 35% reduction, whereas the plasma ACTH concentrations in Dog 2 showed no clear response. Intermittent responses of ACTH were noted in Dogs 4 and 5. Apart from polyuria and polydipsia (PU/PD), Dog 1 showed no clinical problems at the end of the study. The clinical results of Dogs 2, 4 and 5 were less evident. Because of the small sample size, no statistically significant results have been obtained until now, with the exception of the UCCRs of week 1 (Table 2). However, the results have not been completed yet and another 5 dogs will be included in this study.

The difference in experimental dosages may explain why the response of Dog 4 was less clear than the response of Dog 1. The initial research protocol described a dose of 1.5 mg/kg lanreotide each month (Appendix 1). Because of the importance of detecting the effects of lanreotide, Dog 1 received 2.3 mg/kg (1 syringe) lanreotide per month. Subsequent dogs were treated with a maximum dose of 60 mg each (half a syringe). As a result, Dog 4 did not receive as much lanreotide as Dog 1 (1.9 mg/kg). These results may indicate that a higher dosage is needed to maintain the effects of lanreotide. The protein-binding capacity of Octreotide in humans is approximately 65% {{Boscaro,M. 2009}}; this may be higher in dogs. In addition, it is also possible that lanreotide has a higher turnover rate in dogs than humans. This is highlighted by the results obtained from Dog 2. Figure 1 (Dog 2) shows decreased UCCRs in the first 2 weeks after lanreotide autogel administration. During weeks 3 and 4, most of the UCCRs had increased above reference values again. The response of the UCCRs of Dog 2 may indicate that the treatment interval was too short to achieve a 6-month therapeutic level of lanreotide. Moreover, the duration of treatment can also contribute to the obtained results. As can be seen in Figure 1 (Dog 1), the ACTH levels are within reference rate (5-85 pg/ml) {{Javadi,S. 2006}} during months 5 and 6 only. Therefore it is possible that a longer treatment period is necessary to have a sustained period in which plasma ACTH is normalized and a more significant response on the lanreotide therapy will occur.

Dogs with PDH in which a macroadenoma has been detected usually exhibit high UCCRs which are not suppressed by the HDDST. {{Kooistra,H.S. 1997}} The dogs included in this study did not exhibit extremely high UCCRs at the beginning of the study; therefore, the decreases in UCCRs after administering lanreotide were not excessive. In order to have an equally divided study population, it is more accurate to include dogs with high UCCRs (e.g. > 50) during the remainder of the study.

Previous studies showed that the effects of both transsphenoidal surgery in humans and hypophysectomy in dogs depend on pituitary size. {{Hanson,J.M. 2007; Yap,L.B. 2002}} If lanreotide autogel can induce tumor reduction, it would improve the surgical outcome. Therefore, the influence of lanreotide (and cabergoline) on tumor size is an important factor.

The P/B ratio of Dog 1 showed a negligible rise after 6 months of treatment with lanreotide autogel (P/B ratio from 0.46 to 0.47 mm). This indicates an inhibition of adenoma tissue growth during lanreotide treatment. However, no reduction of pituitary adenoma tissue was detected, which may be explained by the duration of treatment. A treatment period of 6 months can be too short to detect tumor size reduction in dogs with PDH. Dog 2 showed a reduction in P/B-ratio from 0.42 to 0.37 mm. However, a reduction in P/B ratio of 0.05 mm does not confirm that a reduction in tumor size has actually occurred. However, the P/B ratios observed in this dog also suggest that lanreotide autogel inhibited further tumor growth. Dog 2 received a dose of lanreotide of 4.3 mg/kg. Besides lanreotide, this dog also received cabergoline after 3 months of treatment. The inhibitory effect on tumor growth in this dog may be due to the high dose of lanreotide administered and the combined therapy with cabergoline.

In humans with acromegaly administration of somatostatin analogs exhibit tumor shrinkage effects. {{Lucas,T. 2003}} A positive hormone response was a predictive factor of significant reduction in tumor size. {{Lucas,T. 2003}} Although Dog 1 displayed clear decreases in both UCCRs and plasma ACTH, no reduction in P/B ratio was observed in this dog. However, the CT-scan of Dog 1 showed a reduction in tumor width of 2.5 mm (12.4 mm before treatment period and 9.9 mm after treatment period). The width of the tumor is a parameter which is not expressed in the P/B ratio. As described previously {{Kooistra,H.S. 1997}}, the height of the pituitary is the most sensitive indicator of pituitary gland enlargement. Therefore, the P/B ratio is calculated as the quotient of pituitary height and the brain area. It should be taken into account that a possible change in pituitary proportions occurred, which was not expressed in the P/B ratio.

Measurements of computed tomography images may suffer from observer objectivity. {{van der Vlugt-Meijer,R.H. 2006}} This potential bias is partly undermined by allowing 2 different persons to measure the P/B ratios.

Each dog currently included in this study had a pituitary macroadenoma. A resistance to dexamethasone was observed in 2 dogs. It should be taken into account that dogs with a moderately enlarged pituitary gland may develop a more sufficient response to the lanreotide treatment and will have more defined improvements of their clinical symptoms. However, in this study the effect on the pituitary size is an important factor of investigation and this will be less clear if pituitary tumors are smaller. {{van der Vlugt-Meijer,R.H. 2003}} Therefore the remainder of the dogs which will be included in this study will also be selected on the presence of pituitary macroadenomas.

The most severe adverse effects observed were inflammatory reactions due to the subcutaneous injection of lanreotide. Skin atrophy is common in both dogs and humans with CD. {{Fisher,L.B. 1971; Rokowski,R.J. 1981}} Consequently, the skin is easily damaged; therefore, it should be taken into consideration that human patients with CD may develop inflammatory reactions due to deep subcutaneous injections, similar to the observations in Dogs 2 and 4 in this study. Although there is a delayed wound healing in humans and dogs suffering from CD {{Alrich,E.M. 1951}}, Dogs 2 and 4 showed a good response to the antibiotic therapy; within 1 month the infections had healed and new skin had grown on these places (Appendix 8, Dog 2 and 4). These observations suggest a better wound healing due to the lanreotide treatment.

As mentioned in previous research, there are some differences between dogs and humans in the expression of somatostatin receptor subtypes. {{de Bruin,C. 2008}} In humans sst₅ is expressed to a

higher extent and sst_2 to a lower extent compared to dogs. {{de Bruin,C. 2008}} A different somatostatin analogue SOM230 has a 40-times higher binding capacity to sst_5 and 2.5-times lower binding capacity to sst_2 compared to octreotide. {{der Hoek,Joost 2004; Hofland,L.J. 2005}} Therefore, administration of SOM230 may be a promising therapy for humans, diagnosed with CD. A multicenter phase II trial is now performed to test SOM230 in humans. {{Boscaro,M. 2009}} Because of the expression of sstrs and DA receptors in corticotroph adenomas and the potential synergistically working mechanism of these receptors {{Rocheville,M. 2000}}, the combination therapy of SOM230 with cabergoline is very promising for future medical use. {{Colao,A. 2007; de Bruin,C. 2009}} Concerning the negative feedback of corticosteroids on sst_2 {{Stalla,G.K. 1994; de Bruin,C. 2009}} therapy can be focused on reducing corticosteroids through $sstr_5$ and DRD_2 with the use of SOM230 and cabergoline. If sst_2 receptors up-regulate in reaction to this therapy, focus will also be on this receptor in the medical therapy of CD. {{de Bruin,C. 2009; van der Hoek,J. 2007}} Sst_2 receptors and, to a lower extent, DRDs are also expressed in dogs with CD. {{de Bruin,C. 2008}} Consequently, dogs still are a promising animal model for human CD when considering this new approach.

In conclusion, the results in this study demonstrate that lanreotide has effects on dogs with CD. This suggests that administration of somatostatin analogues in combination with dopamine agonists will have beneficial effects on humans with CD. Although the results until now are variable and not statistically significant, there is a reasonable chance they will be when more dogs are included in this study. The research protocol will be further defined concerning forthcoming dogs that will enroll in the study. Dogs will be treated with an increasing, high and sustained dose of lanreotide only (without addition of cabergoline) in order to assure that the effects noticed are more clearly related to lanreotide treatment. Addition of the therapy with dopamine receptor agonists will be a point of interest in further research. (Appendix 7)

5. References

- Alrich, E. M., J. P. Carter, and E. P. Lehman. "The Effect of ACTH and Cortisone on Wound Healing; an Experimental Study." Annals of Surgery 133.6 (1951): 783-9.
- An, J. J., et al. "Anti-Proliferative Effects and Cell Death Mediated by Two Isoforms of Dopamine D2 Receptors in Pituitary Tumor Cells." Molecular and cellular endocrinology 206.1-2 (2003): 49-62.
- Atkinson, A. B., et al. "Long-Term Remission Rates After Pituitary Surgery for Cushing's Disease: The Need for Long-Term Surveillance." Clinical endocrinology 63.5 (2005): 549-59.
- Ayuk, J., et al. "Long-Term Safety and Efficacy of Depot Long-Acting Somatostatin Analogs for the Treatment of Acromegaly." The Journal of clinical endocrinology and metabolism 87.9 (2002): 4142-6.
- Barker, E. N., et al. "A Comparison of the Survival Times of Dogs Treated with Mitotane Or Trilostane for Pituitary-Dependent Hyperadrenocorticism." Journal of veterinary internal medicine / American College of Veterinary Internal Medicine 19.6 (2005): 810-5.
- Batista, D. L., et al. "The Effects of SOM230 on Cell Proliferation and Adrenocorticotropin Secretion in Human Corticotroph Pituitary Adenomas." The Journal of clinical endocrinology and metabolism 91.11 (2006): 4482-8.
- Bauer, W., et al. "SMS 201-995: A very Potent and Selective Octapeptide Analogue of Somatostatin with Prolonged Action." Life Sciences 31.11 (1982): 1133-40.
- Bevan, J. S. "Clinical Review: The Antitumoral Effects of Somatostatin Analog Therapy in Acromegaly." The Journal of clinical endocrinology and metabolism 90.3 (2005): 1856-63.
- Boscaro, M., et al. "Treatment of Pituitary-Dependent Cushing's Disease with the Multireceptor Ligand Somatostatin Analog Pasireotide (SOM230): A Multicenter, Phase II Trial." The Journal of clinical endocrinology and metabolism 94.1 (2009): 115-22.
- Bosje, J. T., et al. "Plasma Concentrations of ACTH Precursors Correlate with Pituitary Size and Resistance to Dexamethasone in Dogs with Pituitary-Dependent Hyperadrenocorticism." Domestic animal endocrinology 22.4 (2002): 201-10.
- Brazeau, P., et al. "Hypothalamic Polypeptide that Inhibits the Secretion of Immunoreactive Pituitary Growth Hormone." Science (New York, N.Y.) 179.68 (1973): 77-9.
- Capen, C. C., and S. L. Martin. "Animal Model: Hyperadrenocorticism (Cushing's-Like Syndrome and Disease in Dogs)." The American journal of pathology 81.2 (1975): 459-62.

- Colao, A., et al. "Combined Therapy of Somatostatin Analogues and Dopamine Agonists in the Treatment of Pituitary Tumours." European journal of endocrinology / European Federation of Endocrine Societies 156 Suppl 1 (2007): S57-63.
- Colao, A., and L. J. Hofland. "The Role of Somatostatin and Dopamine Receptors as Molecular Targets for the Treatment of Patients with Pituitary Adenomas." European journal of endocrinology / European Federation of Endocrine Societies 156 Suppl 1 (2007): S1.
- Cozzi, R., et al. "Primary Treatment of Acromegaly with Octreotide LAR: A Long-Term (Up to Nine Years) Prospective Study of its Efficacy in the Control of Disease Activity and Tumor Shrinkage." The Journal of clinical endocrinology and metabolism 91.4 (2006): 1397-403.
- de Bruin, C., et al. "Somatostatin and Dopamine Receptors as Targets for Medical Treatment of Cushing's Syndrome." Reviews in endocrine & metabolic disorders (2008).
- de Bruin, C., et al. "Differential Regulation of Human Dopamine D2 and Somatostatin Receptor Subtype Expression by Glucocorticoids in Vitro." Journal of Molecular Endocrinology 42.1 (2009): 47-56.
- de Bruin, C., et al. "Expression and Functional Analysis of Dopamine Receptor Subtype 2 and Somatostatin Receptor Subtypes in Canine Cushing's Disease." Endocrinology 149.9 (2008): 4357-66.
- de Bruin, C., et al. "Cushing's Disease in Dogs and Humans." Hormone research 71 Suppl 1 (2009): 140-3.
- de Bruin, C., et al. "Co-Expression of Dopamine and Somatostatin Receptor Subtypes in Corticotroph Adenomas." The Journal of clinical endocrinology and metabolism (2009).
- der Hoek, Joost, Steven Lamberts, and Leo Hofland. "The Role of Somatostatin Analogs in Cushing's Disease." Pituitary 7.4 (2004): 257-64.
- Esposito, V., et al. "Transsphenoidal Adenectomy for GH-, PRL- and ACTH-Secreting Pituitary Tumours: Outcome Analysis in a Series of 125 Patients." Neurological sciences : official journal of the Italian Neurological Society and of the Italian Society of Clinical Neurophysiology 25.5 (2004): 251-6.
- Etxabe, J., and J. A. Vazquez. "Morbidity and Mortality in Cushing's Disease: An Epidemiological Approach." Clinical endocrinology 40.4 (1994): 479-84.
- Fisher, L. B., and H. I. Maibach. "The Effect of Corticosteroids on Human Epidermal Mitotic Activity." Archives of Dermatology 103.1 (1971): 39-44.
- Fomekong, E., et al. "Outcome of Transsphenoidal Surgery for Cushing's Disease: A High Remission Rate in ACTH-Secreting Macroadenomas." Clinical neurology and neurosurgery (2009).

- Galac, S., et al. "Urinary corticoid/creatinine Ratios in the Differentiation between Pituitary-Dependent Hyperadrenocorticism and Hyperadrenocorticism due to Adrenocortical Tumour in the Dog." The Veterinary quarterly 19.1 (1997): 17-20.
- Hammer, G. D., et al. "Transsphenoidal Microsurgery for Cushing's Disease: Initial Outcome and Long-Term Results." The Journal of clinical endocrinology and metabolism 89.12 (2004): 6348-57.
- Hanson, J. M., et al. "Plasma Profiles of Adrenocorticotrophic Hormone, Cortisol, Alpha-Melanocyte-Stimulating Hormone, and Growth Hormone in Dogs with Pituitary-Dependent Hyperadrenocorticism before and After Hypophysectomy." The Journal of endocrinology 190.3 (2006): 601-9.
- Hanson, J. M., et al. "Prognostic Factors for Outcome After Transsphenoidal Hypophysectomy in Dogs with Pituitary-Dependent Hyperadrenocorticism." Journal of neurosurgery 107.4 (2007): 830-40.
- Hanson, J. M., et al. "Efficacy of Transsphenoidal Hypophysectomy in Treatment of Dogs with Pituitary-Dependent Hyperadrenocorticism." Journal of veterinary internal medicine / American College of Veterinary Internal Medicine 19.5 (2005): 687-94.
- Hofland, L. J., et al. "The Multi-Ligand Somatostatin Analogue SOM230 Inhibits ACTH Secretion by Cultured Human Corticotroph Adenomas Via Somatostatin Receptor Type 5." European journal of endocrinology / European Federation of Endocrine Societies 152.4 (2005): 645-54.
- Hofland, L. J. "Somatostatin and Somatostatin Receptors in Cushing's Disease." Molecular and cellular endocrinology 286.1-2 (2008): 199-205.
- Hoyer, D., et al. "Classification and Nomenclature of Somatostatin Receptors." Trends in pharmacological sciences 16.3 (1995): 86-8.
- Jaquet, P., et al. "Human Somatostatin Receptor Subtypes in Acromegaly: Distinct Patterns of Messenger Ribonucleic Acid Expression and Hormone Suppression Identify Different Tumoral Phenotypes." The Journal of clinical endocrinology and metabolism 85.2 (2000): 781-92.
- Javadi, S., et al. "Aldosterone-to-Renin and Cortisol-to-Adrenocorticotrophic Hormone Ratios in Healthy Dogs and Dogs with Primary Hypoadrenocorticism." Journal of veterinary internal medicine / American College of Veterinary Internal Medicine 20.3 (2006): 556-61.
- Kanasaki, H., et al. "Involvement of p38 Mitogen-Activated Protein Kinase Activation in Bromocriptine-Induced Apoptosis in Rat Pituitary GH3 Cells." Biology of reproduction 62.6 (2000): 1486-94.
- Kemppainen, R. J., and M. E Peterson. "Animal Models of Cushing's Disease." Trends in endocrinology and metabolism: TEM 5.1 (1994): 21-8.

- Kooistra, H. S., et al. "Correlation between Impairment of Glucocorticoid Feedback and the Size of the Pituitary Gland in Dogs with Pituitary-Dependent Hyperadrenocorticism." The Journal of endocrinology 152.3 (1997): 387-94.
- Lamberts, S. W., et al. "Octreotide." The New England journal of medicine 334.4 (1996): 246-54.
- Lamberts, S. W. "The Role of Somatostatin in the Regulation of Anterior Pituitary Hormone Secretion and the use of its Analogs in the Treatment of Human Pituitary Tumors." Endocrine reviews 9.4 (1988): 417-36.
- Lindholm, J., et al. "Incidence and Late Prognosis of Cushing's Syndrome: A Population-Based Study." The Journal of clinical endocrinology and metabolism 86.1 (2001): 117-23.
- Lucas, T., et al. "Preoperative Lanreotide Treatment for GH-Secreting Pituitary Adenomas: Effect on Tumour Volume and Predictive Factors of Significant Tumour Shrinkage." Clinical endocrinology 58.4 (2003): 471-81.
- Lucas, T., R. Astorga, and Spanish-Portuguese Multicentre Autogel Study Group on Acromegaly. "Efficacy of Lanreotide Autogel Administered Every 4-8 Weeks in Patients with Acromegaly Previously Responsive to Lanreotide Microparticles 30 mg: A Phase III Trial." Clinical endocrinology 65.3 (2006): 320-6.
- Meij, B. P., et al. "Transsphenoidal Hypophysectomy in Beagle Dogs: Evaluation of a Microsurgical Technique." Veterinary surgery : VS : the official journal of the American College of Veterinary Surgeons 26.4 (1997): 295-309.
- Missale, C., et al. "Dopamine Receptors: From Structure to Function." Physiological Reviews 78.1 (1998): 189-225.
- Orth, D. N. "Cushing's Syndrome." The New England journal of medicine 332.12 (1995): 791-803.
- Patel, Y. C. "Somatostatin and its Receptor Family." Frontiers in neuroendocrinology 20.3 (1999): 157-98.
- Pereira, A. M., et al. "Long-Term Predictive Value of Postsurgical Cortisol Concentrations for Cure and Risk of Recurrence in Cushing's Disease." The Journal of clinical endocrinology and metabolism 88.12 (2003): 5858-64.
- Pivonello, R., et al. "Dopamine Receptor Expression and Function in Corticotroph Pituitary Tumors." The Journal of clinical endocrinology and metabolism 89.5 (2004): 2452-62.
- Porterfield, J. R., et al. "Surgery for Cushing's Syndrome: An Historical Review and Recent Ten-Year Experience." World journal of surgery 32.5 (2008): 659-77.

- Rijnberk, A., et al. "Effects of Bromocriptine on Corticotrophin, Melanotrophin and Corticosteroid Secretion in Dogs with Pituitary-Dependent Hyperadrenocorticism." The Journal of endocrinology 118.2 (1988): 271-7.
- Rijnberk, A., and J. A. Mol. "Adrenocortical Function." Clinical Biochemistry of Domestic Animals. Academic Press, 1997. 553.
- Rijnberk, A., A. van Wees, and J. A. Mol. "Assessment of Two Tests for the Diagnosis of Canine Hyperadrenocorticism." The Veterinary record 122.8 (1988): 178-80.
- Rocheville, M., et al. "Receptors for Dopamine and Somatostatin: Formation of Hetero-Oligomers with Enhanced Functional Activity." Science (New York, N.Y.) 288.5463 (2000): 154-7.
- Rokowski, R. J., J. Sheehy, and K. R. Cutroneo. "Glucocorticoid-Mediated Selective Reduction of Functioning Collagen Messenger Ribonucleic Acid." Archives of Biochemistry and Biophysics 210.1 (1981): 74-81.
- Ronchi, C. L., et al. "Efficacy of a Slow-Release Formulation of Lanreotide (Autogel) 120 mg in Patients with Acromegaly Previously Treated with Octreotide Long Acting Release (LAR): An Open, Multicentre Longitudinal Study." Clinical endocrinology 67.4 (2007): 512-9.
- Ruckstuhl, N. S., C. S. Nett, and C. E. Reusch. "Results of Clinical Examinations, Laboratory Tests, and Ultrasonography in Dogs with Pituitary-Dependent Hyperadrenocorticism Treated with Trilostane." American Journal of Veterinary Research 63.4 (2002): 506-12.
- Stalla, G. K., et al. "Octreotide Exerts Different Effects in Vivo and in Vitro in Cushing's Disease." European journal of endocrinology / European Federation of Endocrine Societies 130.2 (1994): 125-31.
- Stewart, P. M., et al. "Depot Long-Acting Somatostatin Analog (Sandostatin-LAR) is an Effective Treatment for Acromegaly." The Journal of clinical endocrinology and metabolism 80.11 (1995): 3267-72.
- Stolp, R., et al. "Urinary Corticoids in the Diagnosis of Canine Hyperadrenocorticism." Research in veterinary science 34.2 (1983): 141-4.
- Swearingen, B., et al. "Long-Term Mortality After Transsphenoidal Surgery for Cushing Disease." Annals of Internal Medicine 130.10 (1999): 821-4.
- van der Hoek, J., S. W. Lamberts, and L. J. Hofland. "Preclinical and Clinical Experiences with the Role of Somatostatin Receptors in the Treatment of Pituitary Adenomas." European journal of endocrinology / European Federation of Endocrine Societies 156 Suppl 1 (2007): S45-51.

- van der Hoek, J., et al. "Distinct Functional Properties of Native Somatostatin Receptor Subtype 5 Compared with Subtype 2 in the Regulation of ACTH Release by Corticotroph Tumor Cells." American journal of physiology. Endocrinology and metabolism 289.2 (2005): E278-87.
- van der Vlugt-Meijer, R. H., et al. "Dynamic Computed Tomography of the Pituitary Gland in Dogs with Pituitary-Dependent Hyperadrenocorticism." Journal of veterinary internal medicine / American College of Veterinary Internal Medicine 17.6 (2003): 773-80.
- van der Vlugt-Meijer, R. H., B. P. Meij, and G. Voorhout. "Intraobserver and Interobserver Agreement, Reproducibility, and Accuracy of Computed Tomographic Measurements of Pituitary Gland Dimensions in Healthy Dogs." American Journal of Veterinary Research 67.10 (2006): 1750-5.
- van Vonderen, I. K., H. S. Kooistra, and A. Rijnberk. "Intra- and Interindividual Variation in Urine Osmolality and Urine Specific Gravity in Healthy Pet Dogs of various Ages." Journal of veterinary internal medicine / American College of Veterinary Internal Medicine 11.1 (1997): 30-5.
- Voorhout, G., et al. "Nephrotomography and Ultrasonography for the Localization of Hyperfunctioning Adrenocortical Tumors in Dogs." American Journal of Veterinary Research 51.8 (1990): 1280-5.
- Willeberg, P., and W. A. Priester. "Epidemiological Aspects of Clinical Hyperadrenocorticism in Dogs (Canine Cushing's Syndrome)." Journal of the American Animal Hospital Association 18 (1982): 717-24.
- Yap, L. B., et al. "Undetectable Postoperative Cortisol does Not always Predict Long-Term Remission in Cushing's Disease: A Single Centre Audit." Clinical endocrinology 56.1 (2002): 25-31.

Appendix 1: Research Protocol

Research protocol

Lanreotide and cabergoline treatment of dogs with Cushing's disease

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Introduction

Cushing's disease (CD) is a severe disorder characterized by chronic hypercortisolism due to an ACTH-secreting pituitary adenoma. Transsphenoidal selective adenomectomy is the first choice of treatment in humans with CD but recurrences occur frequently. Finding an effective and safe medical treatment for CD may improve long term clinical outcome. The recent demonstration of expression of somatostatin and dopamine receptors in human corticotroph adenomas¹ offers the possibility for medical treatment of CD with somatostatin analogues and dopamine agonists.

Investigation of the effects of somatostatin analogues and dopamine agonists in humans with CD is hampered by the low incidence of CD in humans. In this respect, the dog deserves attention as model for CD in humans. CD due to a corticotroph adenoma in dogs is a frequent endocrinological disorder with striking similarities with the disease in humans. Recent studies show that canine corticotroph adenomas also express somatostatin and dopamine receptors. In dogs the somatostatin subtype 2 receptor is the predominant receptor subtype expressed, dopamine 2 receptor is expressed modestly, and somatostatin subtype 5 receptor is expressed only at very low levels.² In addition it was shown that *in vitro* both the somatostatin analogue octreotide and the dopamine agonist cabergoline resulted in a significant decrease in (CRH-induced) ACTH secretion by canine corticotroph adenoma cells.²

These results suggest that long-acting somatostatin analogues (in combination with cabergoline) may be used as medical treatment for CD in dogs. The aim of this study is to investigate the effects of lanreotide and the combination of lanreotide and cabergoline in dogs with CD. A successful effect of the combination of a somatostatin analogue and a dopamine D2 agonist may also indicate that dogs with CD may be used as model to study the effects of the novel chimeric molecule dopastatin that combines the effect of a somatostatin analogue and a dopamine D2 receptor agonist.

Methods

At least 9 dogs with CD will be treated monthly by subcutaneous injection with 1.5 mg lanreotide (autogel) per kg body weight during a period of six months. The owners will be asked to collect morning urine samples before treatment and every 2 weeks after the start of the treatment (at home, so the urine samples will be collected in a stress-free environment) (Table 1). The urine samples will be used for determination of the urinary corticoid/creatinine ratio (UCCR) and the urine density.

Every month blood samples will be taken for routine biochemical analysis (including plasma concentrations of glucose, fructosamine, electrolytes, etc.) and for determination of the plasma concentrations of ACTH, cortisol, α -MSH, growth hormone and insulin-like growth factor-1. In addition, clinical parameters such as the presence of polydipsia, polyphagia, the size of the abdomen, body weight, and condition of skin and hairs will be evaluated every month (Table 1).

Two months after the start of the treatment, the results of lanreotide will be assessed by the change in the UCCR and the clinical signs. In cases in which the symptoms do not

disappear and the urinary corticoid/creatinine ratio remains elevated, the dogs will be treated orally with 10 µg cabergoline per kg body weight once daily, in addition to the monthly lanreotide injections (Table 1).

At 4 months in the study there will be a second evaluation. If there is no clinical improvement after 4 months, the experimental treatment will be stopped.

Computed tomography (CT) will be performed before the dogs enroll in the study (as a part of the diagnostic work-up of pituitary-dependent hypercortisolism) and is repeated prior to hypophysectomy (HX) (Table 1). This will enable the assessment of the effect of medication on pituitary size.

After 6 months of treatment there will be a third clinical evaluation. At the end of the experimental treatment (after 4 or 6 months) the dogs will be treated in the routine manner. If the dogs will be treated by hypophysectomy, corticotroph adenoma tissue will be collected and investigated for the expression of somatostatin receptor subtypes and dopamine receptors.

Table 1: overview of lanreotide and cabergoline treatment of dogs with CD

Month		1	1	1	1	2	2	2	2	3	3	3	3	4	4	4	4	5	5	5	5	6	6	6	6	7
Weeks		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Lanreotide																										
Cabergoline										*																
UCCR	diagn																									
Clin exam	diagn																									
Blood sample	diagn																									
Evaluation 1										E1																
Evaluation 2																		E2								
Evaluation 3																										E3
CT	diagn																									CT before HX

* Cabergoline is added if the symptoms do not disappear and the urinary corticoid/creatinine ratio (UCCR) does not decline after 2 months of lanreotide treatment.

References

1. Hofland L.J., van der Hoek J., Feelders R., van Aken M.O., van Koetsveld P.M., Waaijers M., Sprij-Mooij D., Bruns C., Weckbecker G., de Herder W.W., Beckers A., Lamberts S.W. The multi-ligand somatostatin analogue SOM230 inhibits ACTH secretion by cultured human corticotroph adenomas via somatostatin receptor type 5. *European Journal of Endocrinology* 2005; 152: 645-654.
2. Bruin, de C., J.A. Hanson, B.P. Meij, H.S. Kooistra, A.M. Waaijers, P. Uiterlinden, S.W.J. Lamberts, L.J. Hofland. Somatostatin Subtype 2 Receptor Predominance in Canine Corticotroph adenomas. *Endocrine reviews*, 2007.

Appendix 2: Research Information for Dog Owner

INFORMATIE LANREOTIDE BEHANDELING HONDEN MET ZIEKTE VAN CUSHING

Deze folder geeft informatie over het onderzoekstraject waar u hond momenteel in is opgenomen.

Bij uw hond is de ziekte van Cushing vastgesteld. Dit betekent dat de symptomen van uw hond worden veroorzaakt door een goedaardige tumor in de hypofyse. De hypofyse is een klein kliertje, onder aan de hersenen, dat onder andere hormonen afgeeft die de bijnier stimuleren. Doordat er in de hypofyse een goedaardige tumor zit, wordt de bijnier te veel gestimuleerd en gaat de bijnier teveel hormonen afgeven. Dit veroorzaakt de ziekteverschijnselen bij uw hond.

Wanneer een hond deze ziekte heeft dan zijn er verschillende behandelingsmogelijkheden. De hypofyse (inclusief de tumor) kan operatief verwijderd worden of de hond kan medicijnen (Vetoryl[®]) krijgen die ervoor zorgen dat er minder hormonen door de bijnier aangemaakt worden.

De ziekte van Cushing komt ook bij mensen voor. Ook bij mensen wordt als therapie vaak het operatief verwijderen van de hypofysetumor toegepast. Tegenwoordig kunnen bij mensen met de ziekte van Cushing ook medicijnen gegeven worden die op het tumorweefsel in de hypofyse werken. Hierdoor hoopt men niet alleen de ziekteverschijnselen te verminderen, maar tevens dat de grootte van de tumor in omvang afneemt.

Nu is er de mogelijkheid om een beperkt aantal honden met de ziekte van Cushing te gaan behandelen met de medicijnen die voor mensen worden gebruikt. De medicijnen zijn erg kostbaar maar in het kader van een onderzoek kunnen wij een beperkt aantal honden zonder medicijnkosten behandelen voor een periode van zes maanden. Omdat er al eerder onderzoek is gedaan naar receptoren in de hypofyse van honden¹ en deze voor een groot deel overeen komen met die van de mens, wordt verwacht dat uw hond gebaat zal zijn bij het gebruik van deze medicijnen. We verwachten dat de ziekteverschijnselen minder zullen worden.

Uw hond zal een injectie onder de huid krijgen met het medicijn (lanreotide). Dit werkt voor een periode van een maand. Na toediening van lanreotide willen we graag dat u wekelijks urine op gaat vangen van uw hond. Deze urine kunt u in aparte potjes (deze geven we u mee) gekoeld bewaren. Aan de hand van de urine kunnen we kijken of het hormoon dat de meeste problemen veroorzaakt bij uw hond al aan het dalen is.

Na een maand willen we u vragen om langs te komen voor controle, zodat we kunnen kijken (onder andere via bloedonderzoek) of het beter gaat met uw hond. Tijdens dit bezoek zullen we tevens een nieuwe injectie met lanreotide geven. Op de ochtend voorafgaand aan het bezoek willen we u nog een keer vragen om thuis urine op te vangen en dit (samen met de andere verzamelde urinemonsters) mee te nemen bij het bezoek.

Deze procedure zullen we voor een periode van drie maanden herhalen (zie onderstaande tabel).

Na drie maanden gaan we voor de derde keer kijken hoe het met uw hond gaat. Wanneer we kunnen constateren dat het beter gaat met uw hond en de symptomen verminderd zijn, dan gaan we nog drie maanden behandelen met lanreotide. Als na drie maanden blijkt dat uw hond onvoldoende vooruitgang heeft geboekt, dan zullen we de drie erop volgende maanden naast lanreotide nog een ander medicijn voorschrijven (cabergoline). Dit medicijn werkt tevens op receptoren in de hypofyse waarvan uit onderzoek¹ blijkt dat deze ook bij honden met ziekte van Cushing voorkomen.

Na zes maanden is het onderzoek beëindigd. Dan zullen we bij uw hond gaan kijken of de hypofyse in omvang is afgenomen door middel van een CT scan en gaan we uw hond definitief behandelen.

Als er nog vragen zijn of als er problemen zijn, neemt u dan gerust contact met ons op.

Onderzoekers:

Lona van der Duijn Schouten, Jenny Buijtelts, Sara Galac, Björn Meij en Hans Kooistra

Bereikbaar via: 030-2534779 of H.S.Kooistra@uu.nl

¹ Zie bijgevoegd artikel

OVERZICHT BELANGRIJKE DATA

Wanneer	Wat	Datum
1 week na 1 ^e gift lanreotide	Urine thuis opvangen en gekoeld bewaren.	
2 weken na 1 ^e gift lanreotide	Urine thuis opvangen en gekoeld bewaren.	
3 weken na 1 ^e gift lanreotide	Urine thuis opvangen en gekoeld bewaren.	
Maand na 1 ^e gift lanreotide	-Urine thuis opvangen en meenemen samen met andere drie urinemonsters -Langs komen voor controle -Tweede gift lanreotide	
1 week na 2 ^e gift lanreotide	Urine thuis opvangen en gekoeld bewaren	
2 weken na 2 ^e gift lanreotide	Urine thuis opvangen en gekoeld bewaren.	
3 weken na 2 ^e gift lanreotide	Urine thuis opvangen en gekoeld bewaren.	
Maand na 2 ^e gift lanreotide	-Urine thuis opvangen en meenemen samen met andere drie urinemonsters -Langs komen voor controle -Derde gift lanreotide	
1 week na 3 ^e gift lanreotide	Urine thuis opvangen en gekoeld bewaren.	
2 weken na 3 ^e gift lanreotide	Urine thuis opvangen en gekoeld bewaren.	
3 weken na 3 ^e gift lanreotide	Urine thuis opvangen en gekoeld bewaren.	
Maand na 3 ^e gift lanreotide	-Urine thuis opvangen en meenemen samen met andere drie urinemonsters -Langs komen voor controle -Evaluatie voortgang en planning komende drie maanden	

Appendix 3: Manual Monthly Evaluation

HANDLEIDING EVALUATIEMOMENT LANREOTIDE BEHANDELING HONDEN MET HAH

Datum.....

Evaluatiemoment 1 / 2 / 3

Gegevens eigenaar

Naam.....
 Adres.....
 Telefoon.....

Signalement hond

Naam + patiëntnummer.....
 Ras.....
 Geslacht.....
 Intact of niet.....
 Geboortedatum.....
 Gewicht.....
 Bijzonderheden.....

Vragen naar urinemonsters van afgelopen drie weken

Anamnese

Vragen	Classificatie
Algemeen	
Is uw hond nuchter?	
Uiterlijk	
Vindt u uw hond vermagerd? (1=duidelijk, 2=licht, 3=niet)	
Vindt u de buik in omvang afgenomen? (1=duidelijk, 2=licht, 3=niet)	
Vindt u uw hond dikker geworden? (1=duidelijk, 2=licht, 3=niet)	
Vindt u de buik in omvang toegenomen? (1=duidelijk, 2=licht, 3=niet)	
Gedrag	
Vindt u uw hond veranderd qua gedrag? (1=apathisch, 2=sloom, 3=actief)	
Vindt u uw hond sneller moe? (1=vaak en al snel, 2=af en toe na lange wandeling, 3=niet)	
Neurologisch	
Ziet u uw hond wel eens doelloos cirkels lopen? (1=vaak, 2=zelden, 3=nooit)	
Ligt uw hond wel eens bewegingloos op de grond en is de hond dan niet aanspreekbaar? (1=vaak, 2=zelden, 3=nooit)	
Heeft uw hond last van dringen? (1=vaak, 2=zelden, 3=nooit)	
Heeft uw hond wel eens last van ongecoördineerde bewegingen? (1=vaak, 2=zelden, 3=nooit)	
Voedsel- en wateropname	
Eet uw hond meer? Hoeveel meer?	
Bedelt uw hond meer? (1=veel meer, 2=meer, 3=niet meer)	
Eet uw hond minder? Hoeveel minder? (1=veel	

minder, 2= niet veranderd, 3= minder)	
Drinkt u hond veel? 1=>200ml/kg/dg, 2=>100ml/kg/dg, 3=< 100 ml/kg/dg	
Zijn er andere huisdieren aanwezig? (kunnen deze bij voedsel/water van betreffende hond komen?)	
Huid en beharing	
Kale plekken? Symmetrisch? (1=geheel kaal, 2=enkele kale plekken, 3=geen kale plekken)	
Dorre, droge vacht (1=erg droog, 2=matig droog, 3=glanzend, normaal)	
Verhaart uw hond? 1=niet, 2=normaal, 3=veel	
Is de huid ontstoken? (1=ernstig, 2=matig, 3=niet)	
Vindt u de huid van uw hond dunner geworden? (1=veel dunner, 2=dunner, 3=niet veranderd)	
Skelet en spieren	
Vindt u dat uw hond veel hijgt? (1=erg veel, 2=meer dan normaal, 3=normaal)	
Zakt uw hond sneller door de poten? (1=veel vaker, 2=soms, 3=nooit)	
Vindt u de stand van de poten veranderd? (1=steunt op hakken, 2=zakt beetje door de hakken, 3=normale stand)	
Medicatie	
Heeft u reacties opgemerkt op de injectieplek na toediening van lanreotide?	
Heeft u bijwerkingen opgemerkt na toediening van lanreotide? (braken, diarree, misselijk, winderigheid, anorexie)	
Heeft u bijwerkingen opgemerkt na toediening van de cabergoline? (Sufheid, anorexie, braken)	

Algemene indruk

Gedrag (1=erg lethargisch 2=lethargisch 3=alert)	
Houding en gang	
Verzorgingstoestand	
Voedingstoestand	
Gewicht in kg	
toe- of afgenomen	
BCS	

Algemeen onderzoek

Ademhaling	
Pols	
Temperatuur	
Slijmvliezen Kleur CRT	
Lymfeknopen	
Vacht	
Verharing	
Verkleuringen	
Kale plekken (1=geheel kaal, 2=enkele kale plekken, 3=geen kale plekken) Symmetrisch of niet	
Dor (1=erg droog, 2=matig droog, 3=glanzend, normaal)	
Huid	
Turgor (1=slecht, 2=matig, 3=goed)	
Schilfers (1=veel schilfers, 2=geringe schilfering, 3= geen schilfering)	
Dikte buikhuid (1=veel dunner, 2=dunner, 3=niet veranderd) (meetpunt: ventrale buikwand handbreedte achter laatste rib)	
Pigmentatie	
Pyodermie (1=ernstig, 2=gering, 3=niet)	
Sprake van calcinosis cutis (1=erg, 2=matig, 3=niet)	
Buikomvang	
Buikbelijning (1=erg afhankelijk, 2=licht afhankelijk, 3=opgetrokken)	
Lever palpabel of niet (1=ruim buiten ribboog, 2=net buiten ribboog, 3=niet)	
Aantal cm omvang (referentiepunt 5 cm achter borstbeen)	
Skelet en spieren	
Hijgt (ademhalingsspieren) (1=hijgt continu, 2=hijgt veel, 3=hijgt niet)	
Zakt door poten (1=ernstig, 2=gering, 3=niet)	

Buik-vet-meter

Uitslag buik-vet-meter Op de rug net achter ribboog, 2 cm onder rugwervels stukje geschoren → daar 3 x gemeten.	
--	--

Dosis Lanreotide

Hoeveelheid toegediend lanreotide	
Bepalingen bloed (12 ml) EDTA op ijs + Heparine + NaF	
Ureum	

Kreatinine	
Glucose	
Fructosamine	
Natrium	
Kalium	
Calcium	
AF (+ AF 65)	
Galzuren	
Cortisol	
T4	
TSH	
ACTH	
GH	
Insulineachtige groeifactor-1	
α -MSH	

Tweede bloedafname T=15 min. (12 ml) EDTA op IJS + Heparine

Cortisol	
TSH	
ACTH	
GH	
α -MSH	
Invriezen	
- EDTA plasma	
- Heparine plasma	

Bepalingen urinemonsters

	C/C ratio	Soortelijk gewicht
Week 1		
Week 2		
Week 3		
Week 4		

	Doorbellen resultaten en volgende afspraak vastleggen
--	---

Foto's

- Zijaanzicht (l en r), achteraanzicht, bovenaanzicht, specifiek van vacht- en of huidafwijkingen

Appendix 4: Measuring abdominal fat using bioelectrical impedance



cleaning skin with ethanol



combing hairs aside 20 mm under spine and behind posterior ribs



hairs and skin applied with ethanol



pushing electrode against region



holding firm against region



repeat measurement three times

Pictures from: http://kaden.watch.impress.co.jp/cda/parts/image_for_link/

Appendix 5: Contest of Agreement of Dog Owner

Overeenkomst gebruik lanreotide autogel bij honden met hypofyse afhankelijk hypercortisolisme

- 1.** Hierbij geeft:
- Eigenaar van:

toestemming om zijn/haar hond het hierna genoemde geneesmiddel toe te dienen voor een periode van zes maanden bij de hierboven genoemde hond. Het geneesmiddel wordt toegediend conform de bijsluiter.

- 2.** Naam geneesmiddel: Somatuline® (lanreotide autogel)

- 3.** Het geneesmiddel wordt verstrekt op voorschrift van:

- naam en telefoonnummer behandelend dierenarts:

.....

- 4.** Beschrijving van de ziekte of aandoening waarvoor de toediening van het geneesmiddel nodig is:

Hypofyse afhankelijk hypercortisolisme

- 5.** Het geneesmiddel dient te worden verstrekt voor een periode van zes maanden.

- 6.** Dosering: 1,5 mg/kg lichaamsgewicht

- 7.** Plaats en datum

Plaats: Universiteit Utrecht, Faculteit Diergeneeskunde (Yalelaan 108)

Datum: maandelijkse toediening op afspraak

- 8.** Wijze van toediening: subcutaan (onder de huid)

- 9.** Het geneesmiddel zal worden toegediend door: Drs. J. Buijtels, Drs. S. Galac, Dr. H.S. Kooistra, Dr. B.P. Meij of Drs. A. van der Duijn Schouten.

- 10.** Het geneesmiddel wordt bewaard in de apotheek diergeneeskunde (Yalelaan 106)

- 11.** Evaluatiedatum: drie maanden na start behandeling

- 12.** Bovenstaande persoon stemt toe de behandeling niet voortijdig te stoppen wanneer hiervoor geen indicatie is.

- 13.** Wanneer na drie maanden blijkt dat de hond onvoldoende vooruitgang boekt, dan gaat bovenstaande persoon ermee akkoord dat de behandeling uitgebreid wordt met het hierna genoemde geneesmiddel.

- 14.** Naam geneesmiddel: Galastop® (cabergoline)

- 15.** Het geneesmiddel wordt verstrekt op voorschrift van

- naam en telefoonnummer behandelend dierenarts:

.....

16. Het geneesmiddel dient te worden verstrekt vanaf drie maanden dat het onderzoek is gestart tot het einde van het onderzoek (drie maanden in totaal).
17. Dosering: 10 µg/kg lichaamsgewicht
18. Plaats en datum
 Plaats: wordt thuis toegediend
 Datum: dagelijkse toediening door eigenaar 1 maal per dag
19. Wijze van toediening: oraal (via de bek)
20. Het geneesmiddel zal worden toegediend door: de eigenaar zelf.
21. Het geneesmiddel wordt bewaard bij kamertemperatuur (15-25°C) en in het donker.
22. Evaluatiedatum: zes maanden na de start van de behandeling
23. Door ondertekening van dit formulier gaat de eigenaar akkoord met het aanleveren van de urine stalen en afname van bloedmonsters tijdens de evaluatie momenten zoals bepaald in het onderzoeksprotocol.
24. Indien de hond de totale duur van het onderzoek heeft doorlopen dan gaat de eigenaar akkoord met het herhalen van het beeldvormend onderzoek (CT scan) alvorens de definitieve behandeling wordt gestart.

Voor akkoord,

Plaats en datum:

Naam eigenaar:

Handtekening eigenaar:

Plaats en datum:

Naam onderzoeker:

Handtekening onderzoeker:

Appendix 6: Interim Evaluation

Interim evaluation

Lanreotide and cabergoline treatment of dogs with Cushing's disease

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² Department of Internal Medicine, Faculty of Medicine, Erasmus Medical Center, Erasmus University, Rotterdam, The Netherlands

1. Introduction

Treatment of the first dog started in July 2008. At this moment (March 2009) two dogs have completed the somatostatin analogue therapy. One of these dogs is waiting for hypophysectomy. Two dogs are currently undergoing treatment, while one dog had to be taken out of the study as his clinical symptoms worsened.

Dogs of different breeds with pituitary-dependent hypercortisolism (PDH) were included in the study. The mean age of the dogs was 9.8 (± 0.49) years, median body weight was 18.3 (10.1-50.3) kg. Table 1 lists further characteristics of the patients.

The diagnosis of hypercortisolism was based upon clinical signs, results of hematology and clinical biochemistry and the urine corticoid-to-creatinine ratio (UCCR) in morning urine samples collected at home. All five dogs had a UCCR (mean ratio $22.8 \pm 5.8 \times 10^{-6}$) exceeding the values in healthy dogs ($< 10 \times 10^{-6}$). A high dose dexamethasone suppression test (HDDST), consisting of administration of three oral doses of 0.1 mg dexamethasone/kg at 8-hour intervals after collection of the second urine sample, was performed in each dog. The next morning a third urine sample was collected at home. In 3 dogs the UCCR in the third urine sample was suppressed by more than 50%, consistent with the diagnosis of PDH. Two dogs had $< 50\%$ suppression after dexamethasone. In these two dogs the diagnosis of dexamethasone-resistant PDH was confirmed by high plasma ACTH concentrations, ultrasonography of the adrenals and pituitary imaging. Computed tomography (CT) was performed to visualize the dimensions of the pituitary gland and the surgical landmarks for hypophysectomy. The ratios between the pituitary height and the brain area (P/B mm) of the 5 dogs were 0.46×10^{-2} , 0.42×10^{-2} , 0.48×10^{-2} , 0.58×10^{-2} and 0.56×10^{-2} (reference range $< 0.31 \times 10^{-2}$), consistent with pituitary enlargement due to a pituitary adenoma.

Each month the dogs were monitored as mentioned in the research protocol (see appendix). The results of the UCCR, plasma ACTH and clinical symptoms of each dog are presented and discussed in the following *results* and *discussion*.

Table 1: Characteristics of the dogs with PDH

	Breed	Gen-der	Age (yr)	BW (kg)	P/B^a (mm⁻¹)	UCCR^b (x 10⁻⁶)	HDDST^c (%)	ACTH^d (pg/ml)	α-MSH^e (pg/ml)	Cortisol^f (nmol/l)
1	German Shepherd dog	M	10	50.3	0.46	17	42.3	96	37.5	136
2	English Cocker Spaniel	F	8	13.5	0.42	26	89.5	57	20.1	125
3	Border Terrier	M	10	10.1	0.48	44	49.3	85	23	128
4	Bouvier des Flandres	F	11	31.4	0.58	15	78.4	70		112
5	Border Collie	M	10	18.3	0.56	12	61.2	88.5		114.5

^aP/B (mm⁻¹) ratio between the pituitary height (mm) and the brain area (mm²)

^b Pretreatment UCCR values are the mean of two morning urine samples (reference < 10 x 10⁻⁶)

^c Pretreatment degree of UCCR suppression after dexamethasone (percentage of reduction compared to the mean of the first two samples)

^{d,e,f} Pretreatment plasma ACTH (reference 1.1-18.7 pmol/L), α-MSH (reference <36 pg/ml) and cortisol (reference 11-136 nmol/L) values are a mean of two samples with an interval of 10-15 minutes.

2. Results

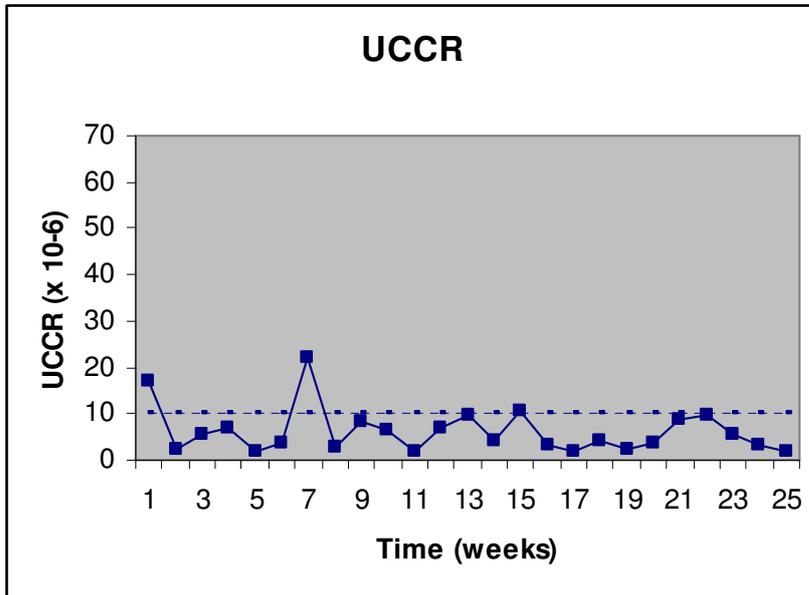
2.1 UCCR and plasma hormone values per patient

UCCR and plasma hormone concentrations will be presented in figures below. UCCR was determined once every week and plasma cortisol and ACTH once every month.

Patient 1

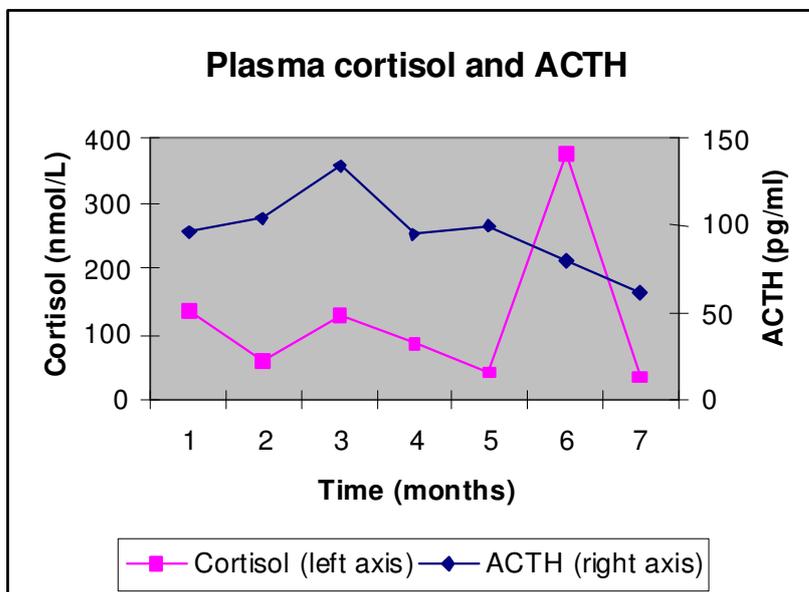
Dog 1 was treated with 120 mg lanreotide (2.4 mg/kg) per month.

Figure 1: UCCR in morning urine samples



Urine samples were collected weekly by the owner in a stress free environment.

Figure 2: Mean plasma ACTH and cortisol concentrations

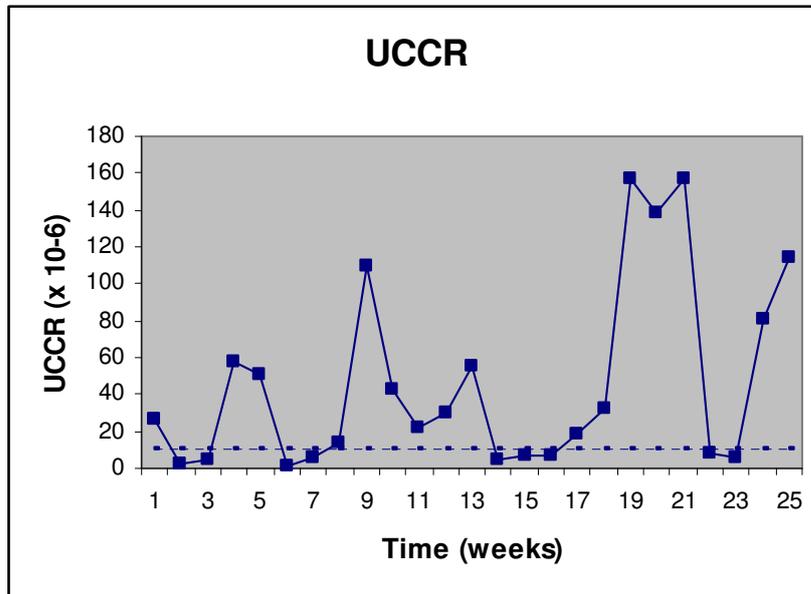


Plasma ACTH and cortisol concentration was measured as the mean of 2 blood samples taken with an interval of 10-15 minutes.

Patient 2

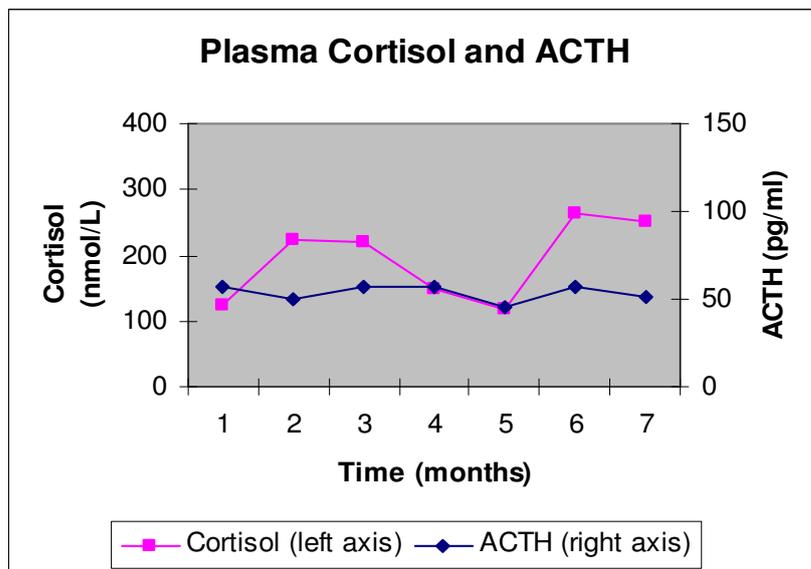
Dog 2 was treated with 60 mg lanreotide (4.3 mg/kg) per month. After 3 months of treatment this dog also received cabergoline (5 µg/kg week 1 and 10 µg/kg the following weeks) during the remainder of the experimental period.

Figure 3: UCCR in morning urine samples



Urine samples were collected weekly by the owner in a stress free environment.

Figure 4: Mean plasma ACTH and cortisol concentrations

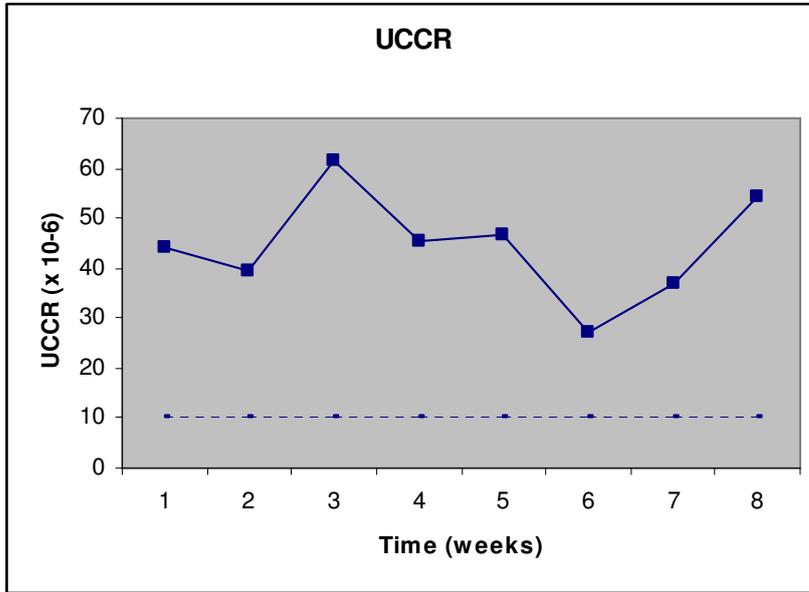


Plasma ACTH and cortisol concentration was measured as the mean of 2 blood samples taken with an interval of 10-15 minutes.

Patient 3

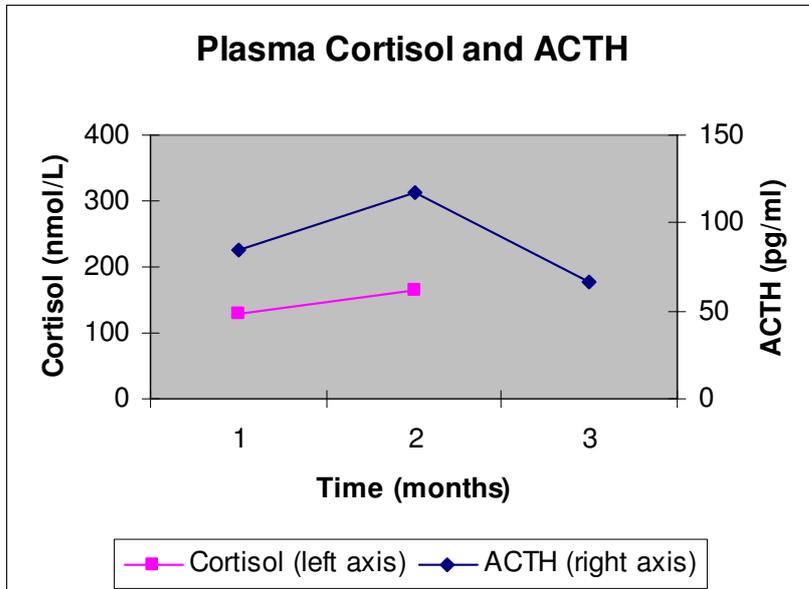
Dog 3 was treated with 40 mg lanreotide (4 mg/kg) during month 1. During month 2 this dog received 80 mg lanreotide (8 mg/kg) together with cabergoline (5 µg/kg week 1 and 10 µg/kg the following weeks).

Figure 5: UCCR in morning urine samples



Urine samples were collected weekly by the owner in a stress free environment.

Figure 6: Mean plasma ACTH and cortisol concentrations

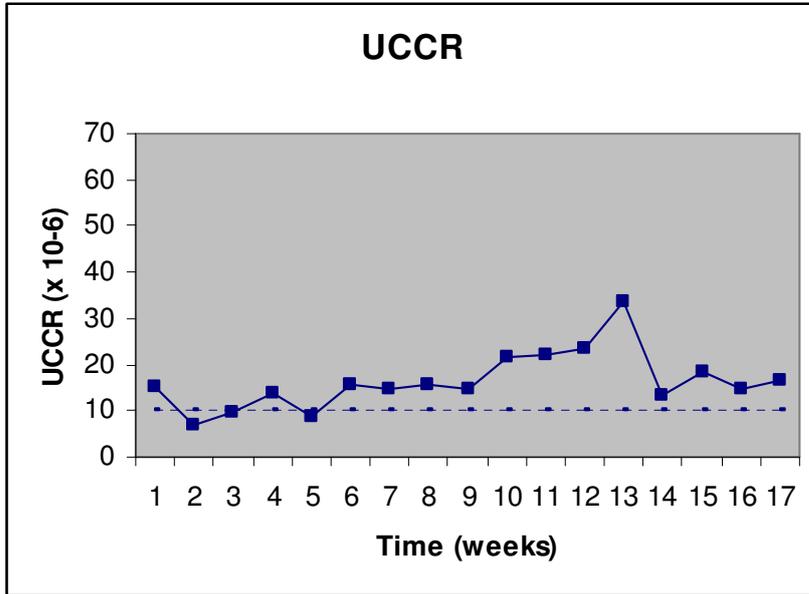


Plasma ACTH and cortisol concentration was measured as the mean of 2 blood samples taken with an interval of 10-15 minutes.

Patient 4

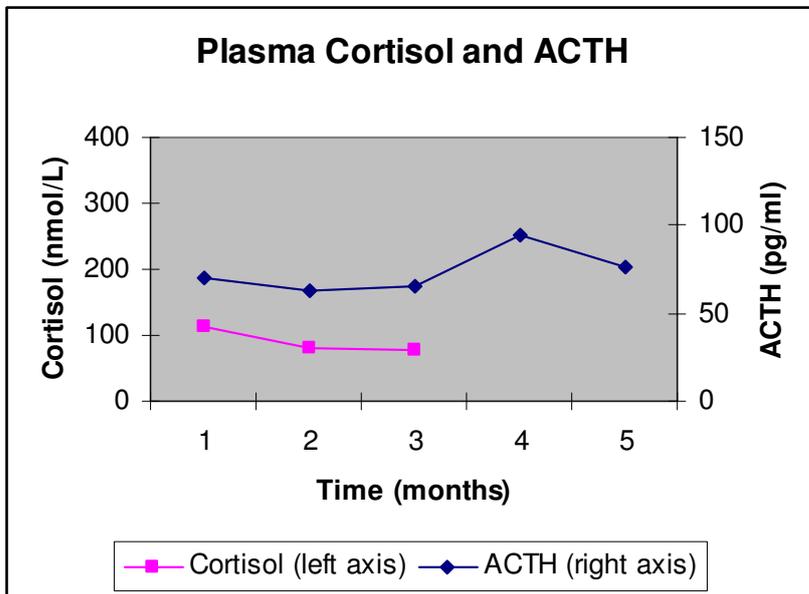
Dog 4 was treated with 60 mg lanreotide (1.9 mg/kg) per month.

Figure 7: UCCR in morning urine samples



Urine samples were collected weekly by the owner in a stress free environment.

Figure 8: Mean plasma ACTH and cortisol concentrations



Plasma ACTH and cortisol concentration was measured as the mean of 2 blood samples taken with an interval of 10-15 minutes.

2.2. Combination with dopamine 2-agonist

After 3 months of treatment, dog 2 in the study was treated orally with cabergoline (10 mg/kg) together with the lanreotide injections. Dog 3 was started on treatment with cabergoline after 1 month of treatment with lanreotide.

2.3. CT scans

Both dogs that finished the 6-month therapy underwent a CT scan. The CT scans of dog 1 showed P/B-ratios of 0.46 before therapy and 0.47 after six months of treatment. The CT scans of dog 2 showed a P/B-ratio of 0.42 and 0.37, respectively.

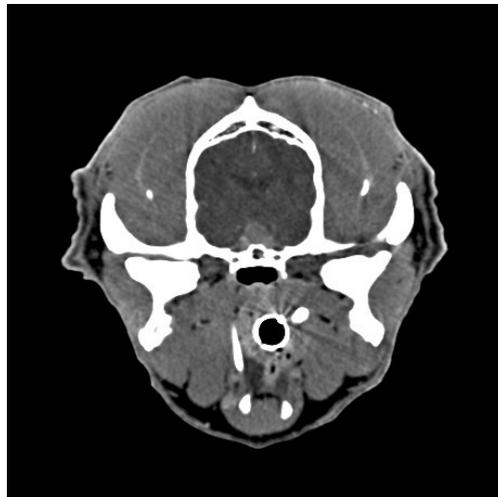
Patient 1

Start treatment period



P/B ratio 0.46

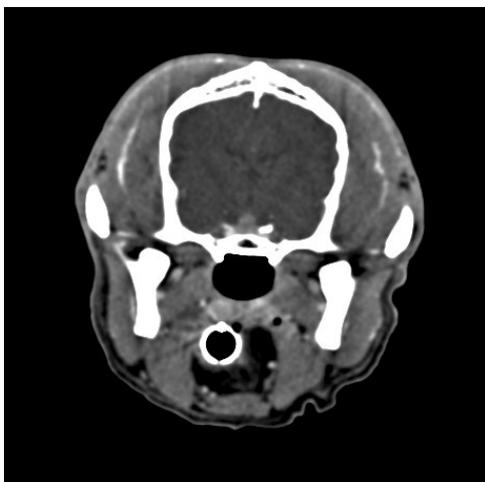
After 6 months therapy with lanreotide



P/B ratio 0.47

Patient 2

Start treatment period



P/B ratio 0.42

After 6 months therapy with lanreotide and 3 months therapy with cabergoline



P/B ratio 0.37

2.4. Clinical symptoms

The clinical symptoms are listed in Table 2. The scale ranges between 1 and 3 (1 not good, 3 good, 2 in between).

Table 2: Clinical symptoms per month

Dog	1							2						3		4				5		
Evaluation moment	0	1	2	3	4	5	6	0	1	2	3	4	5	6	0	1	0	1	2	3	0	1
weight loss	3	3	3	3	3	3	3	3	3	3	3	2	2	3	3	3	2	3	3	3	3	3
Behavioural chance	3	2	3	3	3	3	3	2	3	3	3	2	3	3	2	2	2	2	3	2	2	2
Neurological symptoms	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Eating	3	3	3	3	3	3	3	2	3	3	3	2	3	3	2	2	3	3	3	3	2	2
Drinking	1	1	1	1	1	1	1	1	1	1	1	2	2	1	1	1	1	1	1	1	2**	2
skin thickness	?	3	3	3	3	3	3	2	2	2	2	2	2	2	1	1	2	2	2	2	3	2
Turgor	3	2	3	2	2	2	3	2	2	2	2	2	2	2	2	2	3	3	3	2	3	3
baldness	3	3	3	3	3	3	3	2	3	3	3	3	3	3	2	2	1	1	1	1	2	2
Molting	3	3	3	3	3	3	3	3	3	3	3	2	2	2	1	1	1	1	1	2	1	1
endurance	2	2	2	3	3	3	3	2	2	2	3	3	3	3	2	2	3	3	3	3	2	3

* During this month the dog was treated with Minrin. The response strongly suggests diabetes insipidus due to the large pituitary adenoma.

** Dog 5 was continuously treated with Minrin for PU/PD. The dog became category 1 after 1 day without Minrin suggesting diabetes insipidus due to the large pituitary adenoma.

2.5. Side effects of lanreotide

The side effects mentioned to occur in humans patients have sporadically been observed in the treated dogs. Dog 3 had worsening of the clinical symptoms and an inflammatory reaction to the subcutaneous injection of lanreotide. This dog was taken out of the study and treated with Vetoryl. The inflammation reactions were treated with antibiotics and wound refreshment.

Dog 4 had a period of 2 days of gastrointestinal problems after the second injection with lanreotide. This dog also had two inflammatory reactions with a diameter of circa 7 cm on the left and the right thorax. These inflammatory reactions were treated with antibiotics.

Dog 2 and 3 experienced a period of vomiting of 2 days after extending the therapy with cabergoline. Slowly raising the dose of cabergoline solved this problem. Continuation of the therapy with cabergoline in dog 2 caused a period of decreased appetite. After a month this problem resolved.

3. Discussion

Responses of UCCR and plasma hormone variables to treatment with lanreotide and cabergoline varied per dog. Dog 1 and 2 showed intermittent reduction of UCCR values to baseline values ($< 8.3 \times 10^{-6}$) during the experimental period. Plasma ACTH concentration in dog 1 showed a 35% reduction whereas the plasma concentrations in the other dogs showed no clear response. Table 2 shows that dog 1 had no clinical problems at the end of the study, apart from polyuria and polydipsia (PU/PD). The results in dogs 2 and 4 were less clear.

The experimental dosage may explain why the response in dog 4 was not as clear as in dog 1. The research protocol prescribed a dose of 1.5 mg lanreotide autogel per kg body weight. Because of the importance of detecting effects of lanreotide, dog 1 received 2.3 mg lanreotide per kg bodyweight (one syringe) per month during 6 months. Subsequent dogs were given a maximum dose of 60 mg per dog (half a syringe). As a result dog 4 did not receive as much lanreotide as dog 1 (1.9 mg/kg).

It turned out to be impossible to use the dosages described in the protocol. The syringes containing the lanreotide autogel only allowed for a maximum of two usages. This is due to the construction of the syringe which is for single use only in humans. As a result most dogs have been given a higher dose than envisaged in the research protocol. In particular this applies to dog 1, which received a whole syringe per month. This dog showed the most prominent reduction in UCCR and plasma ACTH. It may be possible that lanreotide autogel is not equally distributed in the syringe (because in humans it is for single use only). This may be the reason that the others dogs in the study did not show as much response as dog 1. In figure 3, the UCCR was above the reference values during weeks 10-13 and weeks 18-21. In figure 7 the UCCR remained high during weeks 6-9 and 10-13. This may be due to the fact that part of the syringe used during that time did not contain enough lanreotide.

In addition it may be possible that lanreotide has a higher turnover rate in dogs than in humans. This is highlighted by the results of dog 2 in the study. Figure 3 showed a decreased UCCR in week 1 and 2 after administering lanreotide autogel. In week 3 and 4 after most of the injections with lanreotide the UCCR was again above reference values. The response of the UCCR of dog 2 may indicate that the interval of treatment was probably too short to achieve a 6 month therapeutic level of lanreotide.

This study was undertaken to assess the *in vivo* efficacy of lanreotide autogel. Therefore the exact dosages used were not, at this moment, our special point of interest. Because of the usefulness of detecting an effect of lanreotide it is important using enough lanreotide autogel. Therefore, we would like to have more syringes to continue this study successfully. Especially syringes with a lower dose (60 and 90 mg syringes) are recommended to exclude further variables during treatment (e.g. distribution of lanreotide in syringes which are now used twice).

The P/B ratio of dog 1 showed a negligible rise after six months of treatment with lanreotide autogel (PB ratio from 0.46 to 0.47). This may indicate possible inhibition of adenoma tissue growth during treatment with lanreotide. A possible explanation for the absence of shrinkage of pituitary adenoma may be the duration of treatment. A treatment period of 6 months may be too short to see shrinkage of the tumor in dogs with PDH.

Dog 2 showed a reduction in P/B ratio of 0.42 to 0.37. However, a reduction in P/B ratio of 0.05 does not confirm that a reduction in tumor size has actually taken place. However, the P/B ratios observed in this dog may suggest that lanreotide autogel administered for six months at least inhibited further tumor growth. Dog 2 received a dose of lanreotide of 4.3 mg/kg. Besides lanreotide, this dog also received cabergoline after three months of treatment. The inhibitory effect on tumor growth in this dog may be due to the high dose used in this dog and the combination therapy of cabergoline together with lanreotide.

In conclusion, the results until now are promising. Lanreotide seems to have effects on the UCCR and plasma ACTH concentration, and possibly inhibits tumor growth. In our opinion it is important to finish this study with at least another 4 dogs as mentioned in the research protocol. To achieve this it will be helpful to receive another 18 syringes of 90 mg and 12 of 60 mg instead of 120 mg.

Appendix 7: Revised Research Protocol

Revised research protocol

Lanreotide Treatment of Dogs with Cushing's Disease

(Van der Duijn Schouten A, Kooistra HS, Meij BP, Hofland LJ, Lamberts SWJ)

In the interim evaluation of our project (*Lanreotide and Cabergoline Treatment of Dogs with Cushing's Disease*) various possible explanations for the observed interindividual differences in the effects of lanreotide autogel were discussed. To sort out these variables, there will be a change in the original research protocol.

The dogs that will enroll in the study during the upcoming months will receive an increasing dose of lanreotide depending on their body weight and response on therapy.

At the start of the treatment period, the dogs will be divided into classes depending on their body weight as mentioned in Table 1. When there is insufficient response after 1 month of treatment with lanreotide (which will be clear within 2 weeks after the 2nd injection), the 3rd dose will be higher in comparison with the 1st and the 2nd dose. When the higher dose is followed by a good response, this dose will be sustained during the next months. However, when the 3rd dose still does not result in an acceptable response, the dose of the 5th injection with lanreotide will be increased again (see Table 1).

Table 1: Dose of lanreotide used in different classes of bodyweight

Class	Body weight (kg)	Starting dose	2nd Evaluation (3rd injection)	4th Evaluation (5th injection)
I	5-15	60 mg	NR*: 90 mg	NR*: 120 mg
II	18-35	90 mg	NR*: 120 mg	90 / 120 mg**
III	> 35	120 mg	120 mg	120 mg

* NR = no response / insufficient response: when there is no response or insufficient response after the first dose of Lanreotide (which will be clear within 2 weeks after the 2nd injection) the 3rd dose of lanreotide will be increased as described in this table. This procedure will be repeated during the 4th and 5th injection with lanreotide.

** Depending on the results after the 1st, 2nd and 3rd evaluation and the change in dose during the 2nd evaluation.

Secondly, extension of the therapy with cabergoline is no longer pursued in this study. As a result, the occurring drug effects will be related to the lanreotide treatment only. Still, should the results of the lanreotide treatment be sufficient, it would be interesting to investigate the effects of the combination therapy of lanreotide and cabergoline in future research.

To be able to finish this study as described in this revised research protocol, it would be helpful if syringes with varying doses of lanreotide would be available. Table 2 shows the exact amounts and doses.

Table 2: Syringes necessary to continue research project

Dose lanreotide (mg)	Number of syringes
60	18
90	18
120	10

If these syringes will become available, 5 other dogs suffering from CD could be treated with an increasing (if necessary), sustained, high dose of lanreotide autogel.

Appendix 8: Photographs

Dog 1



Before treatment period



After 6 months treatment

Dog 2



Before treatment period



After 6 months treatment

Dog 3



Before treatment period



After 1 month treatment

Dog 4



Before treatment period



After 6 months treatment



Close up hair growth on injection place

Dog 5



Before treatment period



After 4 months treatment