Canine Pituitary Dependent Hyperadrenocorticism (Cushing’s Disease) Overview and Diagnosis

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Epidemiology

In humans, pituitary adenomas (PAs) are common tumors with an overall prevalence in the general US population estimated at 16.7%.(1) Corticotroph adenomas, comprising functional and silent corticotroph adenomas, represent approximately 10%–15% of all PAs. Functional adrenocorticotropic hormone–secreting PAs (ACTH-PAs) secrete inappropriate amounts of ACTH, which results in disorderly and excessive production of cortisol by the adrenal gland. Functional ACTH-PAs (Cushing’s disease) are the most common cause of Cushing’s syndrome (hypercortisolemia from any source) and account for an estimated 70% of all cases. The prevalence of Cushing’s disease is estimated to be 1.2 – 2.4 per 1,000,000 people (approximately 12,000 people affected in the United States alone). This number, however, may be much higher, given that Cushing’s disease is frequently misdiagnosed and the diagnosis is often delayed (1).

Functional adrenocorticotropic hormone–secreting PA’s (pituitary dependent hyperadrenocorticism (PDH), Cushing’s disease) has a reported incidence of 0.2% of all dogs (1-2 cases/1000 dogs/year), with approximately 100,000 dogs affected yearly (2-3). PDH accounts for approximately 85-90% of cases of hypercortisolism with the remainder of cases being the result of functional adrenal tumors, meal or food induced cases, occult and atypical disease (3).

Current classification systems for PAs are based primarily on secretory characteristics of the tumor but are also classified on the basis of phenotypical characteristics, including tumor size, degree of invasiveness (e.g., Knosp scoring system), and immunohistological findings. The WHO classification system for PAs has been refined to include designations for benign adenoma, atypical adenoma, and pituitary carcinoma on the basis of p53 immunoreactivity, MIB-1 index, mitotic activity, and the absence/presence of metastases (1). More comprehensive molecular classification systems based on relevant gene expression have not been systematically used to further characterize pituitary tumors. Similar work to classify canine pituitary tumors both morphologically and functionally is currently underway.
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**Pathogenesis**

Two theories have been put forward to explain the development of an ACTH producing pituitary tumor (corticotrophinoma) (4). One is the hypothalamic theory. In this theory, the hypothalamus stimulates corticotrophs through enhanced secretion of corticotropin releasing hormone (CRH) and vasopressin (VP) (5). In addition, concurrent defects in the pituitary glucocorticoid receptor (GR) lead to greater stimulation of the corticotroph cells due to a lower inhibitory action of cortisol on CRH and ACTH synthesis (6). Karl et al. (7-8) and Lamberts (6) described a mutation in the gene that encodes the GR, with a reduction in the sites of DNA binding while maintaining affinity for cortisol. This de novo mutation promotes a general resistance to GC’s that precedes the formation of the corticotrophinoma. Recent studies by Teshima et al.(9-10), using triostane to decrease cortisol, demonstrated pituitary tumor growth as a consequence of a reduction in negative feedback and their studies on canine ACTH tumor cells reinforce that such events would first lead to corticotroph hyperplasia followed by a subsequent somatic mutation in the proteins that control the cell cycle leading to tumor development (5).

Other possibilities for a hypothalamic theory of PDH include dopaminergic neurodegeneration in aged individuals (11-13) or decreased expression of the D2 dopaminergic receptor in the corticotroph cells resulting in decreased dopaminergic inhibition resulting in hyperplasia (14-16). An adenoma would then evolve secondary to a somatic mutation in a hyperplastic cell. This theory is reinforced by the recurrence of ACTH producing tumors following surgery or cases where no tumor is found on MRI or when exploring the sella. Also lending support to this hypothesis, individuals with chronic stress with greater activation of the hypothalamic-pituitary-adrenal axis (HPA), show corticotroph hyperplasia (17).

The main evidence against the hypothalamic theory is the presence of tumor clonality in the majority of the adenomas studied in humans (5, 18). The monoclonal theory argues that the adenoma occurs in the pituitary outside of other influences and arises through the somatic mutation of a corticotroph cell resulting in a tumor clone. This mutation precedes the clonal expansion of the tumor (19).

Unknown are which mutation(s) result in the development of the tumor. Taking into account microadenomas and macroadenomas (tumors extending beyond the sella), the existence of a variety of corticotrophinomas is suggested. Macroadenomas can display a variety of behaviors from limited growth and an indolent course, to more aggressive behavior as seen in human patients with Nelson’s syndrome where the pituitary tumor grows rapidly following bilateral adrenalectomy or suppressive medical therapy (20).
In man, 43 genes and 22 proteins have been identified as being overexpressed and 58 genes and 15 proteins as underexpressed in functional ACTH-PAs compared with normal pituitary tissue (1).

Of the genes, NEUROD1 and hPTTG1 were overexpressed in 3 different studies, whereas HIGD1B and HSD11B2 were overexpressed in 2 studies. Underexpression of CDKN1B, CDKN2A, and let-7 was shown in 4, 2, and 2 studies, respectively. Concerning the proteins, only c-myc was shown by IHC analysis to be overexpressed in more than 1 study. Underexpression of 2 proteins was shown multiple times: p27Kip1 in 4 studies and p16 in 2 studies.

In dogs, we are just beginning to learn more about genes and protein expression in patients with PDH. The recent demonstration of expression of somatostatin receptor subtypes (mainly sst2 with low levels of sst5) and dopamine receptor subtype 2 (D2) in canine corticotroph adenomas (15) offers the possibility for novel medical treatment of PDH with somatostatin analogs and dopamine agonists (See part 2 of this manuscript, Treatment of Canine Pituitary Dependent Hyperadrenocorticism).

Another possible origin of pituitary adenomas is found in cancer stem cells. In a recent study the expression of melanotroph specific transcription factor paired box protein 7 (Pax7) and stem cell marker and reprogramming factor sex determining region Y-box 2 (Sox2) was determined and correlated to clinical parameters. Pax7 expression was significantly higher in enlarged pituitaries, compared to non-enlarged pituitaries, but Pax7 or Sox2 immunopositivity did not correlate to other clinical parameters such as histological diagnosis, survival time or disease-free interval. Gene expression of Pax7 target genes, such as proconvertase 2 (PC2), pro-opiomelanocortin (POMC), and dopamine D2 receptor (DRD2), was significantly lower in the adenoma samples compared to normal tissue, indicating that Pax7 signaling was not activated in adenomas. It was suggested that Pax7 and Sox2 remain interesting targets for molecular investigations into their role in pituitary tumorigenesis, but were unsuitable as clinical prognosticators in dogs (21).

The ratio between pituitary height and the area of the brain (P/B) has been used to evaluate pituitary size. A P/B ratio > 0.31 indicates an enlarged pituitary, whereas a P/B ratio ≤ 0.31 indicates a nonenlarged pituitary. A recent study investigated the expression of proliferation markers Ki-67 and minichromosome maintenance-7 (MCM7) in canine corticotroph adenomas in enlarged and non-enlarged pituitaries and evaluated their relation to the size of canine pituitary corticotroph adenomas. Canine corticotroph adenomas in enlarged pituitaries show greater proliferation potential than do adenomas in nonenlarged pituitaries. MCM7 expression was significantly greater than Ki-67 expression in canine pituitary corticotroph adenomas. Thus, MCM7 may be superior to Ki-67 as a proliferation marker in pituitary tumors. (22)
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In the pituitary glands of humans and mice, the pro-opiomelanocortin (POMC)-expressing cell lineages, the corticotrophs and melanotrophs, have a specific marker in common, the T-box transcription factor Tpit (Tbx19), which is obligate for POMC expression. Tpit also regulates the late differentiation of the corticotrophs and melanotrophs, and therefore may contribute to the pathogenesis of corticotroph adenomas. A recent study examined the expression and mutation analysis of Tpit in normal canine pituitary and corticotroph adenomas. The distribution of the Tpit protein in the pituitary gland was studied with immunohistochemistry and the expression of the gene with RT-PCR. The coding region of Tpit cDNA from 14 dogs with PDH was screened for mutations. Tpit was expressed in corticotroph and melanotroph cells of the normal and adenomatous canine pituitary, and remained present in non-adenomatous corticotrophs of pituitaries from PDH dogs. No tumor-specific mutation in the Tpit cDNA from the corticotroph adenomas was found. However, a missense polymorphism in the highly conserved DNA-binding domain, the T-box, was discovered in one dog. It was concluded that Tpit can be used as a reliable marker for corticotroph and melanotroph cells in canine pituitary tissue and that mutations in the Tpit gene are unlikely to play a major role in the pathogenesis of canine corticotroph adenomas (23).

Leukemia inhibitory factor (LIF) is a pleiotropic cytokine of the IL-6 family that activates the hypothalamic-pituitary-adrenal axis and promotes corticotroph differentiation during development. Using immunohistochemistry, immunofluorescence, and quantitative expression analysis, LIF and LIFR expression were studied in pituitary glands of control dogs and in specimens of corticotroph adenoma tissue collected through hypophysectomy in dogs with PDH. In the control pituitary tissues and corticotrope adenomas, there was a low magnitude of LIF expression. The LIFR, however, was highly expressed and co-localized with ACTH(1-24) expression. Cytoplasmic immunoreactivity of LIFR was preserved in corticotrope adenomas and adjacent nontumorous cells of pars intermedia. No mutation was found on mutation analysis of the complete LIFR cDNA. Surprisingly, nuclear to perinuclear immunoreactivity for LIFR was present in corticotrope adenomas and adjacent nontumorous cells of pars intermedia. No mutation was found on mutation analysis of the complete LIFR cDNA. Surprisingly, nuclear to perinuclear immunoreactivity for LIFR was present in nontumorous pituitary cells of the pars distalis in 10 of 12 tissue specimens from PDH dogs. These data demonstrated that LIFR is highly co-expressed with adrenocorticotropic hormone (ACTH) and alpha-melanocyte-stimulating hormone (alpha-MSH) in the canine pituitary gland and in corticotroph adenomas and that nuclear (rather than cytoplasmic immunoreactivity for LIFR in nontumorous cells of the pars distalis may indicate the presence of a corticotrope adenoma (24).

As mentioned earlier, a characteristic biochemical feature of corticotroph adenomas is their relative resistance to negative feedback by glucocorticoids. In a recent study, gene expression related to ACTH production and secretion, and the negative feedback by glucocorticoids in canine corticotroph adenoma was evaluated in pituitary tumors in 10 dogs with Cushing’s disease. In
order to investigate the alteration of gene expression between corticotroph adenoma and normal corticotrophic cells, ACTH-positive cells in the anterior lobe were microdissected using a laser-capture microdissection system, and mRNA levels of proopiomelanocortin (POMC), corticotropin releasing hormone receptor 1 (CRHR1), glucocorticoid receptor (GR), mineralocorticoid receptor (MR), and 11 beta hydroxysteroid dehydrogenase (11HSD) type 1 and type 2 were determined using real-time RT-PCR. POMC, CRHR1, and 11HSD2 mRNA levels in corticotroph adenoma were greater than those in normal corticotrophic cells (POMC, 5.5-fold; CRHR1, 4.9-fold; 11HSD2, 4.2-fold, P<0.01, respectively). MR and 11HSD1 mRNA levels in corticotroph adenoma were lower than those in normal corticotrophic cells (MR, 2.2-fold; 11HSD1, 2.9-fold, P<0.01, respectively). GR mRNA levels did not differ between corticotroph adenoma and normal corticotrophic cells. These results demonstrate increased ACTH production and resistance to negative feedback suppression by glucocorticoids in canine corticotroph adenomas. (10)

As tumors in dogs and man express the epidermal growth factor receptor (EGFR) we examined whether EGFR might provide a therapeutic target for Cushing’s disease. We demonstrated that in surgically resected human and canine corticotroph cultured tumors, blocking EGFR suppressed expression of proopiomelanocortin (POMC), the ACTH precursor. In mouse corticotroph EGFR transfectants, ACTH secretion was enhanced, and EGF increased POMC promoter activity. Blocking EGFR activity with gefitinib, an EGFR tyrosine kinase inhibitor, attenuated POMC expression, inhibited corticotroph tumor cell proliferation, and induced apoptosis. As predominantly nuclear EGFR expression was observed in canine and human corticotroph tumors, we preferentially targeted EGFR to mouse corticotroph cell nuclei, which resulted in higher POMC expression and ACTH secretion, both of which were inhibited by gefitinib. In athymic nude mice, EGFR overexpression enhanced the growth of explanted ACTH-secreting tumors and further elevated serum corticosterone levels. Gefitinib treatment decreased both tumor size and corticosterone levels; it also reversed signs of hypercortisolemia, including elevated glucose levels and excess omental fat. These results indicate that inhibiting EGFR signaling may be a novel strategy for treating Cushing disease (25).

Clinical Signs

The clinical signs and laboratory abnormalities seen in patients with Cushing’s disease are secondary to the effects of steroid excess, well recognized, and similar in scope to those seen with exogenous glucocorticoid supplementation (see Tables 1 and 2). The awareness of PDH has increased over time resulting in patients being presented with only mild clinical signs, clinical signs affecting only one organ system (e.g. polyuria and polydipsia or alopecia), absent clinical signs, or unusual isolated manifestations of the disease (Table 3).
Diagnostic Approach

Recently, ACVIM issued a consensus statement on the Diagnosis of Spontaneous Canine Hyperadrenocorticism: 2012. (26).


This provides a very thorough review on the diagnostic approach to patients with PDH and addresses many of the common clinical concerns veterinarians have when approaching these patients.

All patients being screened for PDH should have a thorough history and physical examination along with a complete routine database (CBC, chemistry panel, urinalysis, urine culture and blood pressure) prior to embarking on endocrine diagnostics. This is important as the clinical signs of PDH are rarely pathognomonic and the patients are generally older and may have comorbidities, which affect not only the endocrine function tests but also impact therapeutic options and prognosis.

Evaluation of the pituitary-adrenal axis is indicated in the following circumstances:

- Patients with compatible clinical signs and laboratory findings consistent with PDH and in whom non-adrenal illness has been ruled out and/or well controlled. If a patient has a concurrent serious illness it is advised to postpone testing until the illness or injury has resolved or been controlled to minimize false positive test results.
- Patients where an adrenal/pituitary mass or bilateral adrenal hyperplasia has been discovered in conjunction with compatible clinical signs.
- Patients with an incidentally discovered adrenal mass where adrenalectomy is being considered.
- A diabetic dog with insulin resistance.

Several screening tests are available to arrive at a diagnosis of hyperadrenocorticism (ACTH stimulation, low dose dexamethasone suppression (LDDS), urine cortisol to creatinine ratio (UCCR)), though additional tests may be required to differentiate PDH from other causes of hypercortisolemia (endogenous ACTH measurement and advanced imaging such as ultrasound CT or MRI). These tests are summarized in Table 4.

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Initial Screening Tests

The Low Dose Dexamethasone Suppression Test (LDDST)

Most consider the LDDST the screening test of choice unless iatrogenic HAC is suspected in which case the ACTH stimulation test is preferred. The LDDST is used to demonstrate decreased sensitivity to negative glucocorticoid feedback at the level of the pituitary via mechanisms previously discussed. Samples are obtained before, 4 and 8 hours after dexamethasone administration. A diagnosis of HAC is based on lack of suppression of the cortisol concentration 8 hours after dexamethasone administration. In veterinary medicine, the reported sensitivity and specificity of the LDDST range from 85 to 100% and from 44 to 73%, respectively (27-35).

The ACTH Stimulation Test

The ACTH stimulation test assesses adrenocortical reserve and is the gold standard for the diagnosis of iatrogenic hyperadrenocorticism and spontaneous Addison’s disease. Because of its low sensitivity, its diagnostic usefulness as a screening test for spontaneous HAC is inferior to the LDDST.

The sensitivity of the ACTH stimulation test for all forms of spontaneous canine HAC ranges between 57 and 95%. In dogs with PDH it is 80-83%. Specificity ranges between 59 and 93% (36-43).

Synthetic polypeptides containing the biologically active first 24 amino acids of ACTH are available, e.g., Cortrosyn (cosyntropin) or Synacthen (tetracosactrin). The potency of the preparations has not been compared.

After administration of Cortrosyn at 5 ug/kg or 250 ug/dog IV or IM, peak cortisol concentrations occur at 60–90 minutes. After 5 ug/kg IV, no difference was detected between 60 and 90 minute cortisol concentrations (38-41).

Using 4 compounded products (2.2 U/kg IM) in healthy dogs, cortisol concentrations at 60 minutes were similar to each other as well as to concentrations after Cortrosyn (5 ug/kg IV). However, at later times cortisol concentrations varied considerably (38). Because of greater purity and quality control, only use of synthetic ACTH is recommended and utilization of compounded ACTH is discouraged especially when monitoring patients on adrenolytic agents or adrenal enzyme blockers.

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Cosyntropin can be reconstituted and frozen in aliquots at -20°C in plastic syringes for 6 months (44). Whether Synacthen can be frozen has not been investigated; according to the manufacturer, it should be stored at 2–8°C.

**Urine Cortisol to Creatinine Ratio (UCCR)**

The UCCR provides an integrated reflection of cortisol production, adjusting for fluctuations in blood concentrations. Determination of basal UCCRs can be performed in tandem with a high-dose dexamethasone suppression test (see below). The combination has the advantage of potentially demonstrating both increased cortisol production and decreased sensitivity to glucocorticoid feedback.

When a single, random urine sample is collected in veterinary hospitals, the reported sensitivity and specificity of the UCCR for diagnosis of HAC ranges from 75–100% and 20–25%, respectively (46,47). However, using the protocol below, in dogs with physical and biochemical changes consistent with HAC, the sensitivity of finding 2 basal UCCRs above the cut-off level was 99% (95% confidence interval [CI], 94–100%) and the specificity was 77% (95% CI, 64–87%). In some dogs, considerable day-to-day variation exists in the UCCR (48).

**Differentiating PDH from ADH**

Given that PDH and ADH are the most common forms of HAC and that the treatment options and prognosis differ it is important to recommend additional testing to determine the exact etiology of HAC. Even if the clients decision regarding treatment options may not be affected by the ultimate diagnosis (e.g. surgery is not an option) important prognostic information can be obtained. Three tests are commonly used at differentiating tests.

**Endogenous ACTH**

Canine ACTH is secreted from the pituitary gland in an episodic, pulsatile fashion in healthy dogs and those with PDH (26). A circadian rhythm has not been convincingly demonstrated, although 1 study reported higher plasma cACTH concentrations in late afternoon than in the morning. Concentrations of cACTH do not differ between healthy dogs and those with PDH, and its measurement is not useful to screen for HAC. Measurement of cACTH is the most accurate stand-alone biochemical test for differentiating PDH from AT however the sensitivity of the assay differs with methodology. The most common problem with the cACTH assay is poor sensitivity. The
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The largest study of cACTH in dogs with HAC used a 2-site solid-phase chemiluminescent immunometric assay (Immulite ACTH kit and Immulite 2000 analyzer) and showed excellent discrimination between PDH and AT. Intra-assay and interassay variability (increased at lower cACTH concentrations), pulsatile ACTH secretion, and inappropriate sample handling allowing ACTH degradation increase the likelihood of a falsely low value in dogs with PDH (49-54).

Dexamethasone Suppression Testing

In normal dogs, dexamethasone administration causes rapid and prolonged suppression of cortisol secretion. In patients with an AT, dexamethasone at any dosage does not suppress cortisol secretion. In dogs with PDH, ACTH secretion is not appropriately suppressed by administration of a low dose of dexamethasone (0.01 mg/kg), but in 75% of dogs with PDH, cortisol concentrations decrease after administration of 0.1 mg/kg dexamethasone used in the high-dose dexamethasone suppression test. The other 25% of dogs with PDH do not demonstrate suppression even after receiving higher dexamethasone dosages (27). In dogs with PDH that do not suppress, a large pituitary tumor is more likely or the tumor may be arising from the pars intermedia(55).

The largest study evaluating both suppression tests (LDDS and HDDS) included 181 dogs with PDH and 35 with AT. The criteria for identification of dogs with PDH using an LDDST are a 4-hour post dexamethasone cortisol concentration below the laboratory cut-off or <50% of the basal cortisol concentration or an 8-hour cortisol concentration <50% of the basal cortisol concentration, but greater than the laboratory cut-off (27).

The criteria for suppression on the HDDST are a 4 or 8 hour cortisol concentration or both below the laboratory cut-off or <50% of the basal cortisol concentration. Approximately 75% of dogs with PDH meet at least 1 criterion for suppression on either the LDDST or HDDST. Of dogs with PDH, 12% do not suppress on an LDDST but will on the HDDST. Therefore in dogs not showing suppression on the LDDS it is recommended to use an endogenous ACTH rather than a HDDS to differentiate PDH from ADH.

Dexamethasone resistance (i.e., no criteria were met) occurred in all dogs with AT and the remainder of the dogs with PDH. In 41 dogs with AT in another study, 28 LDDST and 30 HDDST were performed. No suppression was seen on any test (56).
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Therefore, suppression in response to dexamethasone supports a diagnosis of PDH, and a dog with dexamethasone resistance can have either AT or PDH.

Dexamethasone Suppression with UCCR. Decreased blood cortisol concentration after dexamethasone administration is reflected in decreased UCCR. After collection of a morning urine sample on 2 consecutive days at home, 3 doses of dexamethasone (0.1 mg/kg) are administered PO at 6 to 8 hour intervals, and a 3rd urine sample is collected the next morning. A decrease in the 3rd UCCR to <50% of the mean of the basal values is consistent with PDH. Lack of suppression does not confirm AT. In 160 dogs with HAC (49 AT and 111 PDH), the UCCR in 72% of dogs with PDH suppressed to <50% of the basal UCCR. The other 28% of dogs with PDH were dexamethasone-resistant. In dogs with AT, the maximum suppression was 44% of the baseline sample.

Advanced Imaging

While imaging can be very helpful in differentiating PDH from ADH it cannot be used to establish a diagnosis of HAC. Moreover, finding normal adrenal glands on imaging studies does not rule out HAC.

Radiography: Abdominal distension, good contrast because of abdominal fat deposition, hepatomegaly, and bladder distension may be seen as well as mineralization of the bronchi and pulmonary interstitium and of dermal and subcutaneous tissues in areas predisposed to calcinosis cutis. A small liver makes HAC unlikely. An AT may be visualized either because of mass effect or tumoral calcification.

Ultrasonographic Imaging: Adrenal gland width is the most informative parameter. Because the long axis of an adrenal gland often is misaligned with either the medial or dorsal plane of the body, cross-sectional images may lead to oblique views and miscalculation of glandular dimensions. Breed and body size-related differences also must be considered.

Ultrasonography can estimate AT size and possibly vascular or local soft tissue invasion. Symmetrical, normal sized, or enlarged adrenal glands are found in dogs with PDH, but mild asymmetry also may occur. Moderate asymmetry, contralateral adrenocortical atrophy (adrenal width <4 to 5 mm), destruction of normal tissue architecture, or some combination of these is
consistent with an AT (60-61). Distinguishing macronodular hyperplasia from AT can be difficult with ultrasonography. Although most AT are unilateral, bilateral tumors may occur and in these cases determination of endogenous ACTH concentrations should be employed.

When an AT has been confirmed, certain findings suggest malignancy. Possible metastases may be identified by thoracic radiography and abdominal ultrasonography. Metastasis can be confirmed by ultrasound-guided biopsy. Adrenal gland width >4 cm is highly correlated with malignancy. Invasion into the vena cava or adjacent tissues can be detected by ultrasonography, but CT and MRI are more sensitive techniques to identify vascular invasion and detect metastases (62). Therefore, abdominal ultrasonography ideally should be followed by CT or MRI before adrenalectomy. Differentiating benign from malignant AT often is difficult, even with histopathological examination. No dog should be sent to surgery for adrenalectomy without confirmation of the presence of an AT (and atrophy of contralateral adrenal gland) by abdominal ultrasound examination, CT, MRI, or some combination of these.

Pituitary Imaging: Pituitary imaging provides valuable information regarding treatment options and prognosis. Pituitary lesions range from small nests of hyperplastic cells to large tumors. The absence of neurological abnormalities does not exclude a pituitary macrotumor (i.e., a tumor that is either >1 cm diameter, extends above the sella turcica, or has a pituitary/brain ratio of >0.31). Because pituitary lesions may be quite small, contrast-enhanced CT and MRI may identify a normal sized pituitary gland in dogs with PDH. The blood supply of the posterior pituitary gland is direct(arterial), whereas that of the anterior pituitary gland is mainly indirect via the pituitary portal system; dynamic contrast-enhanced CT takes advantage of this difference. In a dog with a normal pituitary gland, after IV administration of contrast medium, the posterior pituitary gland can be identified first. This phase is called the “pituitary flush,” and its absence indicates atrophy of the posterior pituitary gland because of compression by a pituitary tumor. Displacement or distortion of the flush can be used to identify and localize anterior pituitary microtumors. Dorsal displacement and decreased signal intensity of the posterior lobe on T1-weighted MRI also indicates the presence of a microtumor (64,65).

Pituitary imaging should be considered for all dogs at the time of PDH diagnosis especially if hypophysectomy or radiation therapy is being considered. If clinical features suggest a pituitary macrotumor, confirmation requires imaging.
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Summary

Canine PDH is a common endocrine disorder in older dogs. The pathogenesis of ACTH producing pituitary tumors is becoming more evident based on ongoing studies looking at gene and protein expression, which will aid our understanding of tumorigenesis and point us towards targeted specific therapies. The diagnosis of PDH requires incorporating information from the history, physical examination, and routine laboratory tests. Specific endocrine tests and imaging modalities are available to diagnose HAC and distinguish between the various causes of hypercortisolism. No single test is perfect and if the initial screening test is negative, and high clinical suspicion of HAC exists, additional tests should be performed to rule in or rule out HAC. Endocrine evaluation of patients with HAC and non-adrenal illness can be difficult and it is important to eliminate or manage the concurrent illness before undertaking adrenal function tests. ■
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References


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<table>
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<th>Table 1: Common Clinical Signs of PDH in Dogs</th>
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<tr>
<td>■ Polyuria and polydipsia</td>
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<td>■ Polyphagia</td>
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<td>■ Abdominal distention</td>
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<td>■ Bilaterally symmetric endocrine alopecia</td>
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<td>■ Panting</td>
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<td>■ Hypertension</td>
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<td>■ Urinary tract infections</td>
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<td>■ Additional dermatologic signs:</td>
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<td>Thin skin</td>
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<tr>
<td>Pyoderma</td>
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<td>Calcinosis cutis</td>
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Table 2: Common Laboratory Findings of PDH in Dogs

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<th>Hematologic Abnormalities</th>
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<tr>
<td>“Stress” leukogram</td>
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<td>Neutrophilic leukocytosis</td>
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<td>Lymphopenia</td>
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<td>Eosinopenia</td>
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<td>Mild thrombocytosis</td>
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<td>Mild erythrocytosis</td>
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<th>Serum Biochemical Abnormalities</th>
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<td>Increased serum alkaline phosphatase</td>
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<td>Milder increased in alanine aminotransferase</td>
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<td>Hypercholesterolemia</td>
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<td>Hypertriglyceridemia</td>
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<th>Urinalysis</th>
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<td>Decreased urine specific gravity &lt; 1.018</td>
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<td>Proteinuria</td>
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<td>Urinary tract infection (even in the absence of pyuria and bacteriuria)</td>
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<td>Table 3: Atypical Presentations of PDH in Dogs</td>
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<tr>
<td>■ Thromboembolic disease</td>
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<td>■ Myotonia</td>
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<td>■ Pancreatitis</td>
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<td>■ Cranial cruciate ligament injury</td>
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<td>■ Facial nerve paralysis</td>
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<td>■ Gall bladder mucocoele</td>
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<td>■ Reproductive abnormalities</td>
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Table 4: Diagnostic Tests in the Evaluation of Patients with Suspected PDH

### SCREENING TESTS

**General Principles**

A. Ideally endocrine testing should be postponed in patients with significant non-adrenal illness until the illness has resolved or been controlled.

B. Since dogs do not exhibit circadian cortisol secretion it is assumed that time of day does not affect the LDDST or ACTH stimulation test. The effect of feeding on these test results is unknown, however, feeding during these tests is not recommended. Fasting before testing is not necessary unless lipemia affects results of the cortisol assay used (check with your particular laboratory for specific details.)

C. In veterinary medicine, the ability of glucocorticoids of any form, progestagens and ketoconazole to suppress cortisol secretion is known. No effect on the LDDST, ACTH stimulation test or UCCR has been documented in dogs treated with phenobarbital (35,45).

**LDDS**

The LDDST should be performed using 0.01 (dexamethasone) or 0.015 (dexamethasone sodium phosphate) mg/kg.

Samples are obtained before and 4 and 8 hours after dexamethasone administration.

**ACTH Stimulation Test**

Cortrosyn, Cosyntropin Injection, and Synacthen can be used interchangeably. Perform the test using 5 ug/kg IV or IM of the preferred compound with blood samples drawn before and 60 minutes after administration.

**UCCR**

To avoid the influence of stress, urine for UCCR should be collected at home at least 2 days after a visit to a veterinary clinic. Although a UCCR sample can be collected at any time of day, morning urine may be preferred because it usually represents several hours of urine production. In addition, the frequency of false positive results may be reduced by having the owners collect the first morning voided urine sample on 2-3 consecutive mornings, pool the samples and an aliquot of the pooled sample is submitted to the diagnostic laboratory.

High UCCRs in dogs without a high degree of clinical suspicion of HAC should be interpreted cautiously.

### DIFFERENTIATING TESTS

**Dexamethasone Suppression Testing**

Lack of suppression after dexamethasone administration on either the LDDST or HDDST does not confirm an AT because approximately 25% of dogs with PDH fail to suppress.

Suppression to <50% of baseline on an LDDST (using criteria outlined above) in a dog with HAC confirms the disease as pituitary-dependent.

The HDDST should be performed as described for the LDDST except that the dosage of dexamethasone is 0.1 mg/kg IV. The free alcohol form should be avoided.

If there is no suppression on an LDDST, measurement of cACTH or abdominal ultrasound is recommended. If these tests are not available, the HDDST is an alternative but it will only provide differentiation in approximately 12% additional PDH cases.

Results of either the LDDST or HDDST cannot be considered 100% absolute and when imaging data and endocrine conflict the endocrine results should be given preference.

**Endogenous ACTH**

Plasma proteases degrade cACTH rapidly if samples are not cooled appropriately. Blood should be collected into chilled, silicon-coated glass or plastic tubes containing EDTA, centrifuged within 15 minutes (ideally in a cooled centrifuge), and the plasma transferred to plastic tubes and frozen immediately. Samples must stay frozen until analysis; if a courier is used for quick transport to a reference laboratory, ice may be sufficient. If samples are shipped, they should be sent overnight packed in dry ice.

However, addition of the protease inhibitor aprotinin (Trasylol) prevents ACTH degradation by plasma proteases and greatly facilitates sample handling. Check with your specific laboratory regarding suitability as with some assays (Immulite), aprotinin introduces an artifactual decrease and is not recommended.
Dr. Bruyette received his Doctor of Veterinary Medicine degree from the University of Missouri in 1984. He completed an internship at Purdue University and a residency program in internal medicine at the University of California Davis. He was a staff internist at the West Los Angeles Veterinary Medical Group, as well as a member of the Department of Comparative Medicine at Stanford University, an Assistant Professor and Head of Internal Medicine at Kansas State University, and Director of the Analytical Chemistry Laboratory at Kansas State.

In addition to his duties as Medical Director, Dr. Bruyette practices internal medicine and specializes in the hormonal system and its diseases. His interests also include adrenal disease, diabetes and thyroid disorders. Dr. Bruyette joined VCA West Los Angeles Animal Hospital in 1996.