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Some notes about the diagnosis and medical treatment monitoring of canine hyperadrenocorticism

Hyperadrenocorticism (HAC) is one of the most common endocrinopathies in the dog. The majority of cases (80-85%) are caused by an adrenocorticotrophic (ACTH)-secreting pituitary adenoma leading to pituitary-dependent-hyperadrenocorticism (PDH). Pituitary carcinomas also occur. Less common (15-20%) are cases associated with adrenocortical tumors (ACT) that secrete cortisol. These are most typically carcinomas. Rarely, PDH and ACT occur concurrently (1.1%). Rare reported causes for HAC in the dog are ectopic ACTH production, food-induced HAC, and non-cortisol secreting ACT (atypical HAC). Iatrogenic HAC due to excessive administration of glucocorticoids should also be considered in dogs with appropriate clinical signs.

The diagnosis of HAC is based on the dog's medical history and clinical signs, and compatible adrenal function tests. These tests can be divided in **screening and differentiating tests**.

Screening tests include the urine cortisol to urine creatinine ratio (UCCR), low-dose dexamethasone suppression test (LDDST), and ACTH stimulation test (ACTHst). The **differentiating tests** include the high-dose dexamethasone suppression test (HDDST), endogenous ACTH (eACTH), diagnostic imaging (pituitary, adrenal), and sometimes, the LDDST.

Drugs and adrenal function tests

Something to consider when performing and interpreting these tests is the potential effects of drugs on the tests' results. Recent administration of glucocorticoids, antiandrogens, progestogens, and ketoconazole may suppress the hypothalamic-pituitary-adrenal (HPA) axis. Suppression of the axis may yield false negative results for hyperadrenocorticism. Some glucocorticoids may cross-react with the cortisol assay. Our assay shows the greatest cross-reactivity with prednisolone (62%). Therefore, it is recommended to discontinue treatment and delay testing 24 h or longer (2-4 weeks or more for long acting steroids) prior to testing. If discontinuation of treatment is not possible, then the results should be interpreted cautiously. If the test is performed to rule out decreased function of the adrenal glands (caused by iatrogenic hyperadrenocorticism or by spontaneous hypoadrenocorticism), then testing can be pursued.

Screening tests

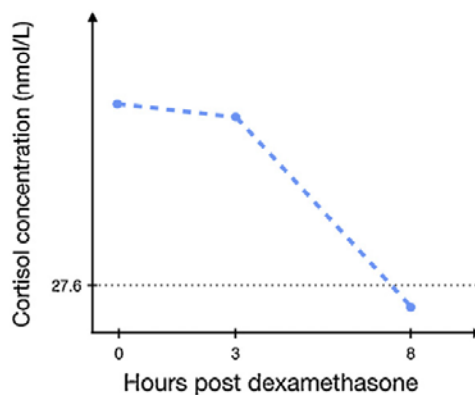
Urine cortisol to urine creatinine ratio (UCCR)

The UCCR is a sensitive test for HAC, but poorly specific. This means that it is a good test to use if you wish to rule out HAC, which you can, if the ratio is <10. However, when the ratio is >10, other more specific tests for HAC will need to be used to further support the diagnosis, such as the LDDST or ACTHst. Stress and non-adrenal illness can increase urine cortisol.

Low dose dexamethasone suppression test (LDDST)

Usually the test of choice for the diagnosis of HAC given its high sensitivity (90-95%). A negative result makes HAC unlikely. The LDDST is more specific than the UCCR, but less so than the ACTHst. This test is more affected by stress and non-adrenal illness than the ACTHst.

The LDDST relies on the demonstration of decreased response of the HPA axis to negative glucocorticoid feedback (i.e., lack of suppression of cortisol secretion). The protocol consists on a pre-dexamethasone cortisol sample (baseline cortisol), followed by the intravenous administration of dexamethasone (0.010–0.015 mg/Kg). Additional samples are collected 4 h and 8 h post-dexamethasone. In healthy dogs, dexamethasone results in rapid and prolonged suppression (>24 h) of cortisol secretion. Lack of ACTH secretion leads to decreased cortisol within 2-3 h. An 8 h cortisol concentration above the laboratory cut-off, or less <50% suppression of baseline cortisol, are considered consistent with HAC (the cut-off value used in our laboratory is 40 nmol/L). Suppression of cortisol at 4 h and loss of suppression at 8 h is considered consistent with PDH. Historically considered as negative for HAC is the inverse pattern (Figure 1), which has been recently associated with PDH. Additional testing is recommended when this pattern is observed.



Inverse

Figure 1. Illustration of the inversed pattern in the LDDST. Although historically considered a negative result for HAC, the inverse pattern has recently been suggested as supportive of PDH in one report (Mueller et al., 2006. J Vet Intern Med, 159:489-91). Figure from Bennaim M., et al. 2019. Vet J, 252:1-11

ACTH stimulation test (ACTHst)

The ACTHst shows a wide range of sensitivity (0-95%) due to the varied control populations used for its evaluation. Overall, this test is less sensitive for HAC than the UCCR and LDDST. It is particularly less sensitive for ACT. A contributing factor to the low sensitivity of the ACTHst to ACT can be attributed to the occurrence of non-cortisol producing tumors. Some ACT may predominantly secrete hormones other than cortisol and a subnormal cortisol response to synthetic ACTH may occurred. Therefore, dogs exhibiting clinical signs of HAC without recent steroid administration, and with absent to weak stimulation of cortisol secretion could be interpreted as indicative of an ACT. The ACTHst is less affected by stress, leading to fewer false positives. Therefore, a negative result does not rule out HAC, and additional testing such as the LDDST, should be considered. A positive result is consistent with HAC. A diagnostic approach to consider is a LDDST as the initial screening test followed by an ACTHst for further confirmation.

The ACTHst permits the diagnosis of iatrogenic HAC (expect subnormal cortisol response to exogenous ACTH) and provides baseline data prior therapy and for monitoring therapeutic response.



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This test relies on the fact that dogs with either PDH or ACT have increased adrenocortical mass/volume. Thus, more than normal capacity to secrete cortisol, especially after stimulation. The protocol consists of a baseline cortisol sample (pre-ACTH), followed by IV or IM administration of exogenous ACTH (5 ug/Kg), and a final sample collected usually 60 minutes later. In healthy dogs, post-ACTH cortisol remains below the cut-off value (<550 nmol/L). Dogs with HAC may exhibit exaggerated responses (post-ACTH above cut-off value, >550 nmol/L) or a subnormal cortisol response (iatrogenic HAC and ACT). About half of dogs with cortisol-secreting ACT and about 80% of dogs with PDH show an exaggerated cortisol response to the ACTHst.

Types of ACTH

Synthetic and compounded (natural/gel) ACTH formulations can be used for the ACTHst. Synthetic ACTH is available in liquid, lyophilized or depot forms. The availability varies both geographically and temporally. With the exception of the depot formulations of ACTH, there does not appear to be a significant difference in the cortisol response. A post-ACTH sampling time of 180 min has been recommended for depot ACTH formulations. The use of compounded ACTH formulations is not advisable. This is because the concentration of ACTH may not be that indicated in the formulation, and due to lot-to-lot variability in the concentration of ACTH. The use of compounded ACTH has been associated with more variable cortisol responses that may lead to false positive or negative results.

Which screening test to perform

Choosing between the different screening tests to diagnose canine HAC can be confusing. The 2012 ACVIM consensus statement about the diagnosis of spontaneous canine HAC, postulated the LDDST as the screening test of choice for the diagnosis of HAC in the dog. Ultimately however, the screening test of choice will depend on what the clinician is looking for and the financial capabilities of the owner. Table 1 summarizes some of the features of the screening test for the diagnosis of HAC in the dog, which may help the veterinarian decide which test to perform.

Table 1. Characteristics of the screening tests for the diagnosis of canine HAC

UCC ratio	LDDST*	ACTHst
High Sensitivity (good to r/o HAC)	High Sensitivity for both PDH and ATs	Good Sensitivity for PDH, poor for ATs
Low Specificity (21%)	Low Specificity (more affected by stress)	Reported highest Specificity, but studies contradict
Random single urine	Longer test (8 h)	Inexpensive and shorter (1-2 h)
No baseline for monitoring	No baseline for monitoring	Baseline data prior therapy and therapeutic response



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No useful for diagnosis of iatrogenic HAC	No useful for diagnosis of iatrogenic HAC	Diagnosis of iatrogenic HAC
Does not differentiate between PDH and ACT	May differentiate between PDH and ACT	Does not differentiate between PDH and ACT

*ACVIM 2012 consensus; screening test of choice, unless iatrogenic HAC is suspected

Clinical signs consistent with HAC but negative screening test

If the clinical signs are consistent with HAC, but the screening test is negative, the following can be considered:

- Follow-up with another screening test
- Non-cortisol secreting ACT (consider testing post-ACTH sex hormones)

If more than one screening test is negative, then consider:

- Possible negative for HAC
- Mild HAC (tests not yet positive) → retest in 1-2 months if the clinical signs progress.

Differentiating tests

Once the diagnosis of HAC has been made, distinction between the PDH and ACT should be pursued. This is important because treatment options and prognosis differ. Medical therapy is an option for ACT, however adrenalectomy is ideally performed. Otherwise, signs may develop due to local tumour effects. Surgery may be curative. Whereas, medical therapy of PDH by itself, may lead to clinical remission and prolonged survival.

High dose dexamethasone suppression test (HDDST)

The HDDST follows the same sampling protocol and interpretation that the LDDST but a higher dose of dexamethasone is used (0.1 mg/Kg, IV). Four- and 8-h post-dexamethasone cortisol values < 40 nmol/l or <50% of baseline cortisol indicate suppression. Dogs that show suppression at either or at both time points are consistent with PDH. Values >40 nmol/l or >50% of baseline cortisol indicate lack of suppression.

Although lack of suppression is found in all dogs with ACT, some dogs with PDH (approximately 25%) may show lack of suppression in a HDDST. Therefore, further evaluation with other diagnostic modalities (e.g., abdominal ultrasound, pituitary imaging) should be pursued to differentiate between PDH and ACT.

The HDDST should not be used as a screening test for HAC because the dose of dexamethasone is higher and dogs with PDH are more likely to have suppressed cortisol concentrations at 8 h. This would lead to a false negative diagnosis for HAC.



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Endogenous ACTH (eACTH)

Pituitary-dependent HAC is caused by excessive pituitary secretion of ACTH. Whereas the excess cortisol secretion by ACTs suppresses the secretion of ACTH. Therefore, plasma eACTH concentration is expected to be high in dogs with PDH but low in dogs with ACT. This test can be considered as the most accurate test to differentiate between PDH and ACT. The problem with this test however, is that eACTH is very labile; it suffers rapid degradation by plasma proteases. Thus, to avoid this degradation the samples need to be collected and handled in a way that is ultimately impractical. Thus, other methodologies for differentiation are more easily pursued.

A high eACTH concentration in the absence of a pituitary tumour and undetectable eACTH concentration in the absence of an ACT were also reported in a case of ectopic ACTH secretion and food-dependent HAC, respectively.

This test is not appropriate for the initial diagnosis of HAC. This is because ACTH secretion is pulsatile and its concentrations overlap between dogs with HAC and healthy dogs.

Adrenal imaging

In the canine, normal adrenal size varies with breed, body size and age. Measurement of the smaller adrenal gland appears to best differentiate dogs with ACT from dogs with PDH. A maximal thickness of the smaller adrenal gland <5 mm was associated with a sensitivity of 100% and a specificity of 96% to identify ACT. Other features that differentiate between hyperplasia and ACT is that adenomas and adenocarcinomas are more likely to be mineralized. Additionally, vascular invasion (phrenicoabdominal vein and/or caudal vena cava) may be observed with malignant tumors.

Pituitary imaging

Pituitary imaging is typically done prior confirmation of a pituitary tumour by other methodologies for planning of radiotherapy or hypophysectomy, for prognosis, or if neurologic signs are present. Advanced imaging of the brain can be an option when the differentiating tests yield discordant results. It is important to note that a large proportion of dogs with PDH have microtumours that may be missed on evaluation.

Monitoring medical treatment

There are several medical options for the treatment of canine HAC, though trilostane and mitotane (less so) are the most widely used and will be briefly described here.

Trilostane

Trilostane is a competitive inhibitor of 3 β -Hydroxysteroid dehydrogenase (3 β -HSD), which enzymatic action is essential for the synthesis of cortisol and other steroids (Figure 2).

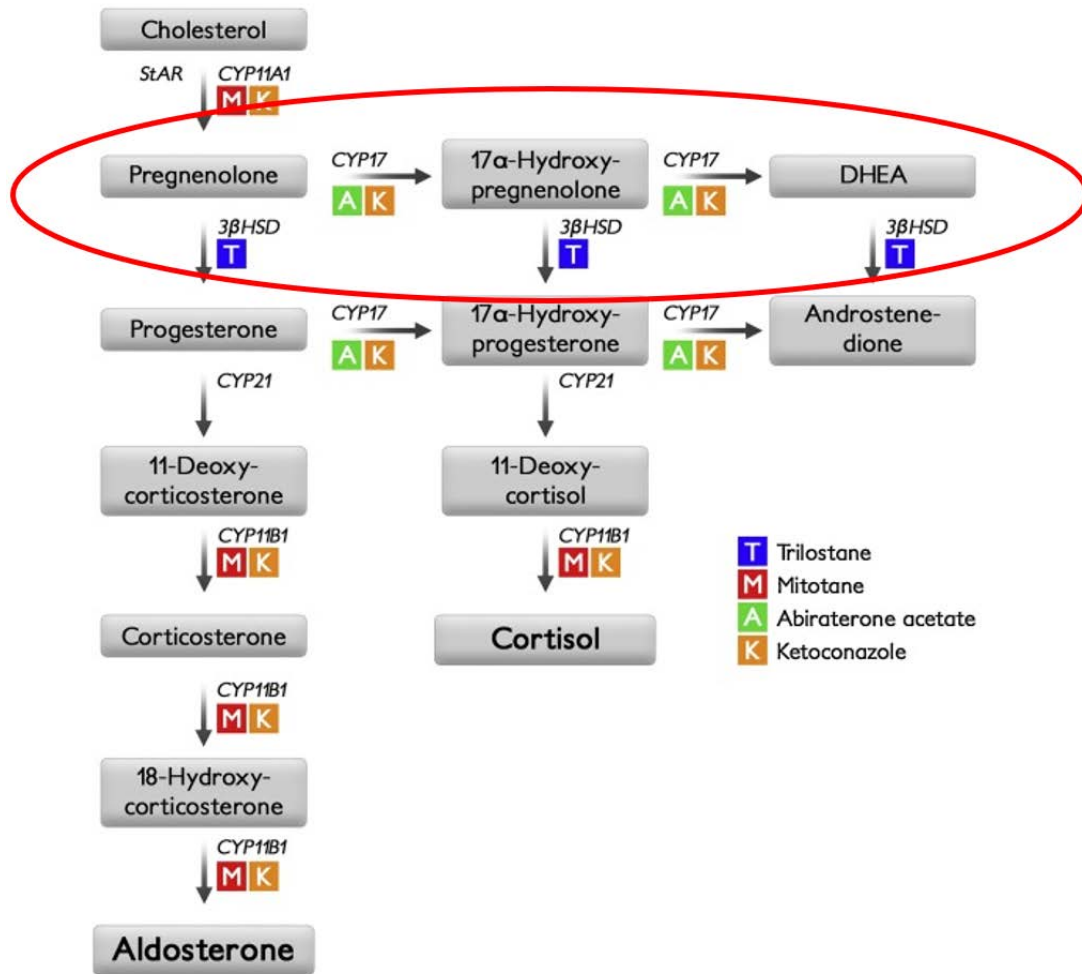


Figure 2. Schematic overview of the site of action of different inhibitors of steroidogenesis. The red circle highlights the site of action of trilostane. Modified from Sanders K., et al. 2018. Vet J, 241 :42-51

Post-ACTHst serum cortisol concentration

Monitoring treatment is typically done by assessment of the clinical response and serum cortisol concentrations post-ACTHst, though an official consensus does not exist. Additionally, the proposed optimal target cortisol concentrations for dogs undergoing treatment are varied. The target cortisol concentrations proposed here are per the manufacturer recommendations (Dechra®). The first monitoring occurs between days 10-14 of treatment consisting of a clinical exam, chemistry panel, CBC, and ACTHst 4 h post-trilostane. Trilostane should be given with food (routinely and on days of recheck) to enhance absorption (fasting invalidates the test results). Depending on the clinical response and post-ACTH cortisol concentration, doses are adjusted (return to day 1 of treatment) or treatment is continued at the current dose for later rechecking. If no dosing adjustment and clinically well, then rechecking should be at 1 to 3 months, at 3 months, and then every 3 to 6 months (Figure 3).

Monitoring treatment – post-ACTHst [cortisol]

*Modified from "Treatment and Monitoring of Hyperadrenocorticism" flowchart published by Dechra Ltd., 2009

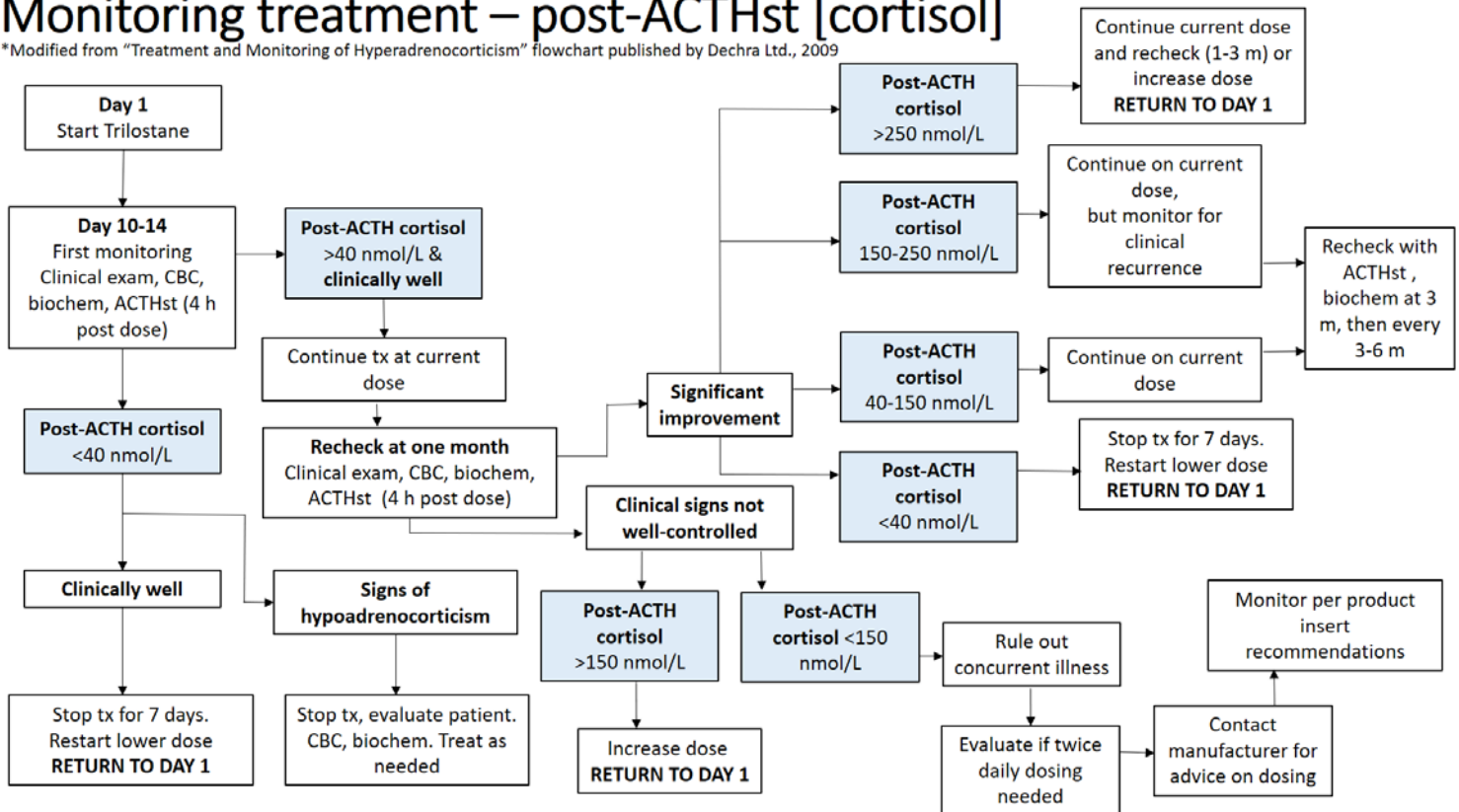


Figure 3. Flowchart of proposed monitoring protocol of dogs undergoing treatment for HAC with trilostane (once or twice daily dosing) based on clinical response and post-ACTH serum cortisol concentration.

Pre-trilostane serum cortisol concentration

Monitoring treatment via the pre-trilostane cortisol concentration can be considered if monitoring treatment via the ACTHst is not possible due to either financial constraints or unavailability of exogenous ACTH. It is important to note however, that the scientific evidence to justify the use of this monitoring protocol is sparse. So far only two studies have evaluated this approach, both of which were funded by Dechra®, the manufacturer of the drug Vetoryl®.

Monitoring treatment using the pre-trilostane cortisol relies heavily on the clinical response as reported by the owners and clinical findings by the veterinarian. Using the pre-trilostane cortisol concentration for monitoring should be reserved for dogs that are clinically well. Dogs that are exhibiting signs of cortisol deficiency should have a complete evaluation, including an ACTHst, performed. The dog is first rechecked at day 10, and if clinically well, maintained on the current dose until day 28. At day 28, the dog is clinically evaluated and the pre-trilostane serum cortisol determined. Clinical signs of HAC and pre-trilostane cortisol above cut-off value warrant dose adjustment and the process starts again (return to day 1). Otherwise, if the dog is well-controlled, the treatment is continued at the current dose and rechecking every 3 months. If at any point the dog is clinically unwell (clinical signs of hypoadrenocorticism), treatment should be stopped, and a standard ACTHst and electrolyte determination are indicated. The dose is adjusted and symptomatic treatment is given as necessary.

Monitoring treatment – pre-Trilostane [cortisol]

*Modified from "Pre-Veteryl Cortisol: an improved monitoring protocol" flowchart published by Dechra Ltd., 2018

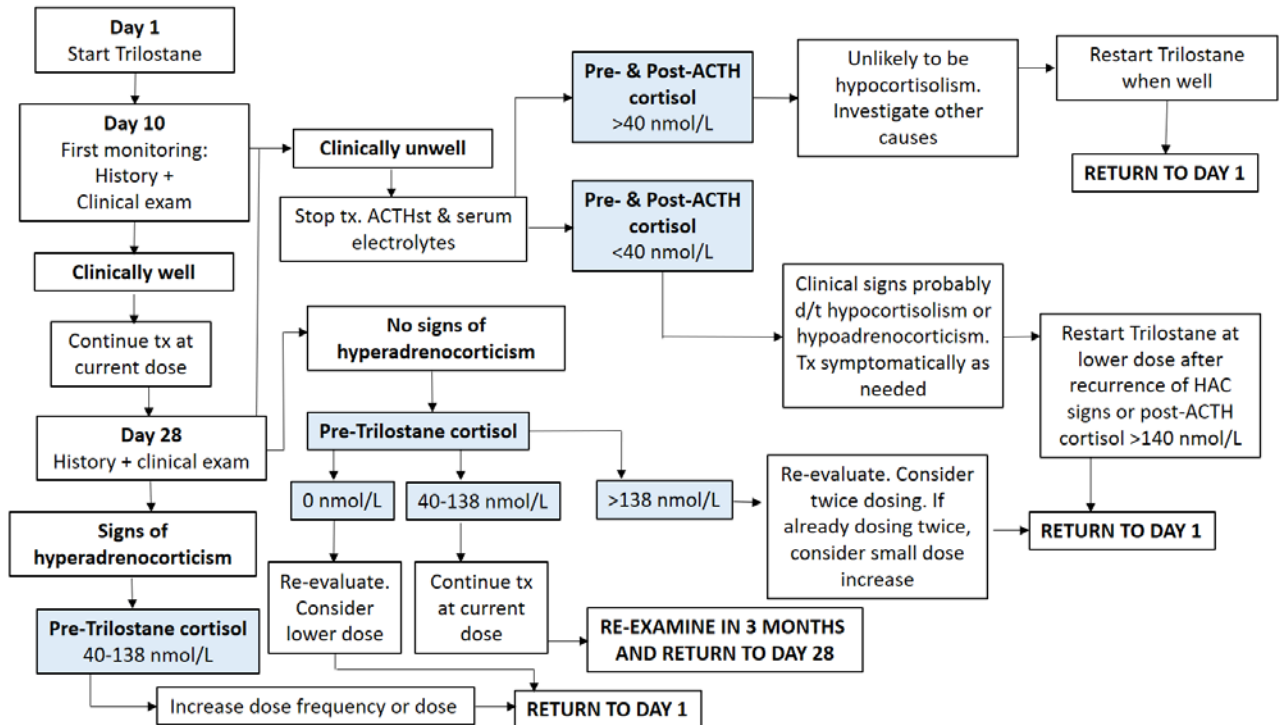


Figure 4. Flowchart of proposed monitoring protocol of dogs undergoing treatment for HAC based on clinical response and pre-trilostane (once or twice daily dosing) serum cortisol concentration.

Mitotane (o,p'-DDD)

Mitotane is a potent adrenocorticolytic agent derived from the insecticide DDT that leads to progressive adrenocortical necrosis and atrophy. It inhibits steroidogenic enzymes cytochrome P450 cholesterol side-chain cleavage enzyme (CYP11A1) and CYP11B1 (Figure 2). This contributes to inhibition of cortisol synthesis. Mitotane enhances the metabolic clearance of glucocorticoids. The use of mitotane for the treatment of canine PDH has largely been replaced by that of trilostane (trilostane is just as effective, safer to handle, and has fewer adverse effects). However, mitotane remains a good option for the treatment of ACT, because it has the added advantage that it can destroy ACT cells (treatment protocol aimed at complete adrenocortical destruction).

The systemic availability of mitotane can be improved by administration with food as it is fat soluble. Treatment with mitotane is characterized by two phases; the loading phase and the maintenance phase. The loading phase is characterized by the rapid destruction of the adrenocortical tissue. Once the loading phase is completed, the patient is switched to the maintenance dosage. During the loading phase, owners should monitor water and food intake, for clinical signs of hypoadrenocorticism (vomiting, diarrhea, or lethargy), or adverse effects. If there are no new clinical signs, the first recheck is usually 5-10 days into the loading phase, when an ACTHst is performed (36-48 h after mitotane dosing). If at the first recheck, the post-ACTHst cortisol is above the cut-off value, the loading dosage is continued for another 5-10 days, when the dog is rechecked as described above. If there is a good response (water intake <60-100 mL/Kg/d and post-ACTHst cortisol 25-125 nmol/L) maintenance dosing is started. Unless adverse reactions occur, this is followed by a recheck at 1 month, 3 months,

and then every 3-6 months. If at any point during the maintenance dosage, post-ACTH cortisol is >125 nmol/L, weekly dosage is increased or the loading dosage is repeated for 5-10 days. Monitor as above, then increase weekly maintenance dose. If at any point the dog is clinically unwell (clinical signs of hypoadrenocorticism) or post-ACTHst is <25 nmol/L, mitotane treatment should be stopped. The electrolytes should be checked and prednisone given as necessary. An ACTHst is done in 3-4 weeks to assess response (Figure 5).

Mitotane- Monitoring treatment

*Modified from "Mitotane (Lysodren®) Dosing and Monitoring" flowchart published by IDEXX

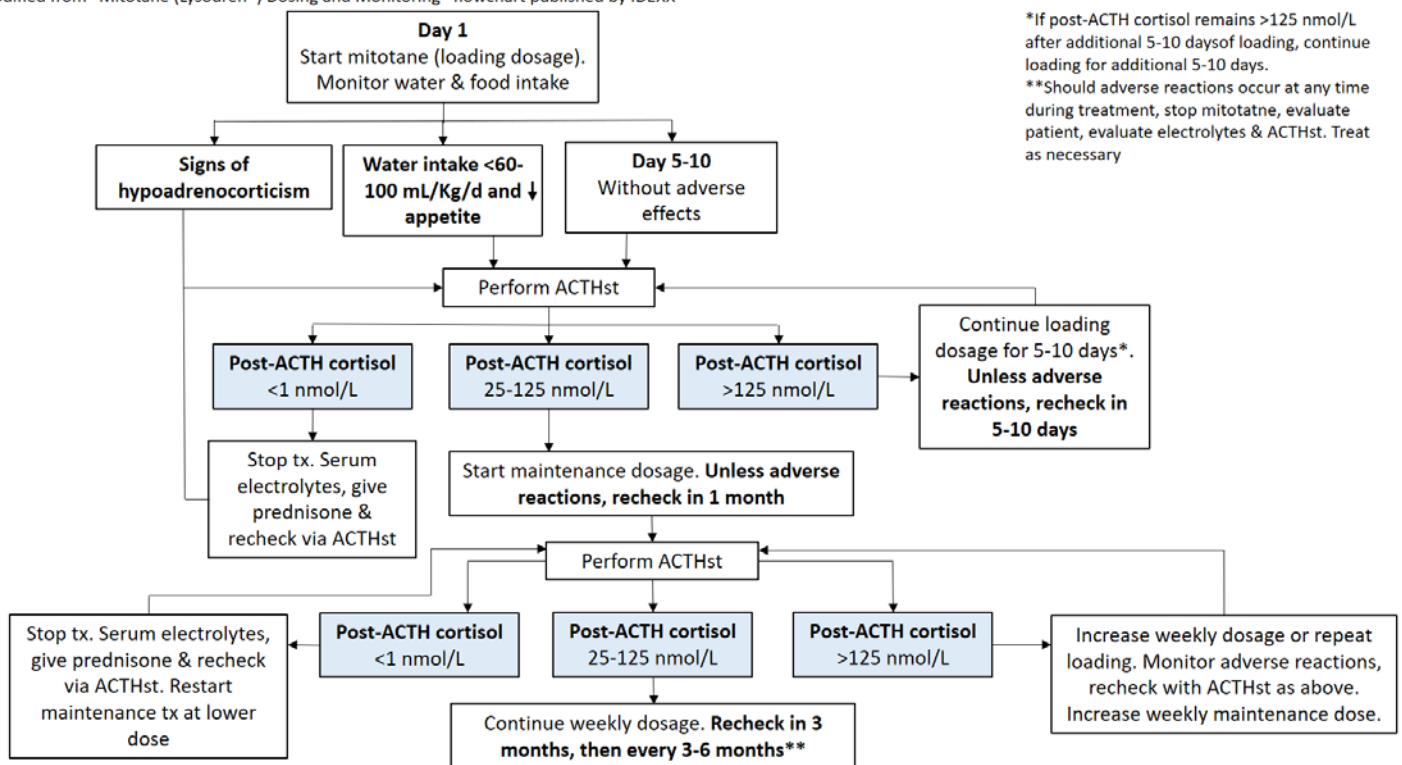


Figure 5. Flowchart of proposed monitoring protocol of dogs undergoing treatment for HAC with mitotane based on clinical response and post-ACTH serum cortisol concentration.

References

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