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Ultrasonographic Changes of Adrenal Glands Size under Trilostane Therapy, Their Correlation with Clinical Signs and Endocrine Tests and Possibility of Introducing Ultrasound as Additional Monitoring Modality of Cushing's Syndrome

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Abstract

A prospective study of 15 dogs with diagnosed Cushing's syndrome was made to find a correlation between adrenal size enlargement, clinical signs and results of an ACTH stimulation test. To our knowledge, no study was made trying to correlate changes in adrenal size, response to therapy and ACTH stimulation test results. Ultrasonographic evaluation and an endocrine test (ACTH stimulation test/"pre pill") were performed in each dog two weeks after initiating the therapy, two to four weeks after changing the dosage of trilostane and ideally 10 weeks after establishing the right dosage of trilostane (two owners showed up later on date due pandemic of COVID-19). The results indicate that there is correlation between adrenal size enlargement and response to trilostane therapy. In this study, all dogs that had increase in adrenal gland size of more than 7% showed as well clinical improvement of HAC (hyperadrenocorticism) signs under trilostane therapy. Further, possible multicentric, studies should be performed to verify assumed correlation between adrenal size enlargement and positive response to trilostane therapy.

Keywords

Adrenal Gland, Ultrasonography, Trilostane, Monitoring

1. Introduction

Hyperadrenocorticism (HAC) in dogs and cats is a well-described disease in li-

terature and one of the most common endocrinopathies in dogs. There is little information concerning adrenal gland size enlargement under trilostane therapy. Trilostane is competitive inhibitor of 3-beta-hydroxysteroid dehydrogenase [1] and is the most commonly used modality in treatment of canine HAC. The level of cortisol production inhibition is dosage dependent and reversible. Few studies have been published describing ultrasonography in dogs with pituitary dependent HAC treated with trilostane [1], sonographic changes of adrenal glands under trilostane therapy [2] and contrast enhanced ultrasound of adrenal glands in dogs with pituitary dependent Cushing's syndrome [3]. This prospective study was performed to evaluate whether there is a correlation between adrenal gland enlargement and positive reaction to trilostane therapy given twice daily. Presently, endocrine tests and clinical examination are used to evaluate and establish the correct dose of trilostane in dogs. The aim of this study is to evaluate an additional objective parameter that can help to decide whether to adjust or not the dosage of trilostane. Additionally, with this modality, suspicion of possible concurrent disease could be confirmed if the dog is not reacting to therapy as expected, but laboratory tests are within normal limits.

2. Materials and Methods

2.1. Study Design

In this prospective study 15 client owned dogs (six males and nine females with mean age of 11.6 years) were referred to the referral veterinary clinic Tierklinik am Kaiserberg in Duisburg, Germany, between December 2019 and March 2021. Criteria for including the dogs in this study were: diagnosed HAC, owner's compliance (with prescribed therapy and check up's), patient compliance during ultrasound examination (aggressive and extremely stressed patients were excluded) and irregular shaped adrenal glands (two dogs were excluded from the study because of the irregular adrenal shape and measurement at the same point on repeated scans was not possible). All patients presented had at least one of the signs of HAC (pu/pd, hair loss, lethargy, abdominal enlargement, weight gain). pu/pd was reported in 14/15 patients, 11/15 were lethargic, 7/15 had hair loss, weight gain was reported in 3 patients and polyphagia in 2. The diagnosis of Cushing's syndrome was established using endocrine tests (Dexa low dose suppression—LDDS test and ACTH stimulation test). Synacthen© and Cosacthen© were used (dosage 5 µg/kg), i/v for the ACTH stimulation test and Dexatat© (dosage 0.01 mg/kg), i/v for the LDDS test. All patients received trilostane (Vetoryl©) two times daily, except patient named 2 L who received trilostane once per day because of the owner's compliance. Starting dosage of trilostane of 0.5 mg/kg 2xd was used for pituitary depended HCA and 1 mg/kg 2xd for adrenal depended HCA. Given that CT scan of pituitary gland was done only in one patient, determination whether HAC is pituitary or adrenal dependent was based on ultrasound appearance of adrenal glands (patients with bilateral adrenal enlargement were assumed to have a pituitary dependent cushing's syndrome and

patients with enlargement of a single adrenal gland with atrophic contralateral adrenal were suspected with adrenal dependent cushing's syndrome). In all patients that needed dosage adjustment, dosage of trilostane needed to be increased (decreasing dosage was not necessary—none of the patients showed signs of hypoadrenocorticism such as lethargy, vomiting, diarrhea, bradycardia). The criteria for increasing the dosage of trilostane were absence or minimal improvement of the clinical signs in combination with elevated cortisol values. In patients that needed adjustment of the treatment the dosage was increased with 25% - 50% more for the whole daily dosage. In one dog diabetes mellitus was diagnosed and insulin therapy with Caninsuline© (0.25 IE/kg) was started after second control. In 13 dogs the ACTH stimulation test was used during the control (2 weeks after the first visit), and in 2 dogs "pre pill" endocrine test was performed. The ACTH stimulation test was performed 3 - 4 hours after administration of trilostane (time of expected cortisol nadir after administration of trilostane).

2.2. Diagnostic Imaging

At the moment of the diagnosis of HCA and at every check-up (after clinical examination and performing of the endocrine test) an ultrasound examination of the adrenal glands was performed (in this manner additional stress during ultrasound examination would not interfere with measured cortisol levels). The examinations were done with a Philips Epiq 7 (c8 - 5 MHz transducer), Esaote my Lab Class 9 (mC 3 - 11 MHz transducer) or Esaote my Lab Class C (CA 123 transducer, 3 to 9 MHz) ultrasound devices. Ultrasound was performed in dorsal recumbency or in standing position, depending on patient and owner compliance, and the hair on ventral abdomen was clipped. Ultrasonography was performed by a veterinarian in training for "Fachtierarzt für innere Medizin" under supervision of an internal medicine specialist certified by the German national board. The maximal dorsoventral thickness in sagittal and transversal planes was measured (**Figures 1-3**). The clinical examinations, endocrine test and ultrasound examinations were performed at the day of first visit, 10 - 14 days after initiating trilostane therapy, ideally 10 weeks after establishing the right dosage of Vetoryl© (trilostane) and 14 - 28 days after every change of the dosage of trilostane. Measurements of the adrenal glands during each ultrasound examination were compared with the size estimated during the time of first examination. Some dogs showed up later for their second control due to COVID 19 pandemic (patients named 7S and 5P showed up 6 and 18 weeks later than planned, respectively).

2.3. Dividing Criteria

Response of the patients to the introduced therapy was evaluated based on history, clinical examination and the endocrine results (ACTH stimulation test and "pre pill"). The patients were divided in two groups, the one with satisfactory endocrine tests (ACTH: preserved adrenal reserve, at least two times higher

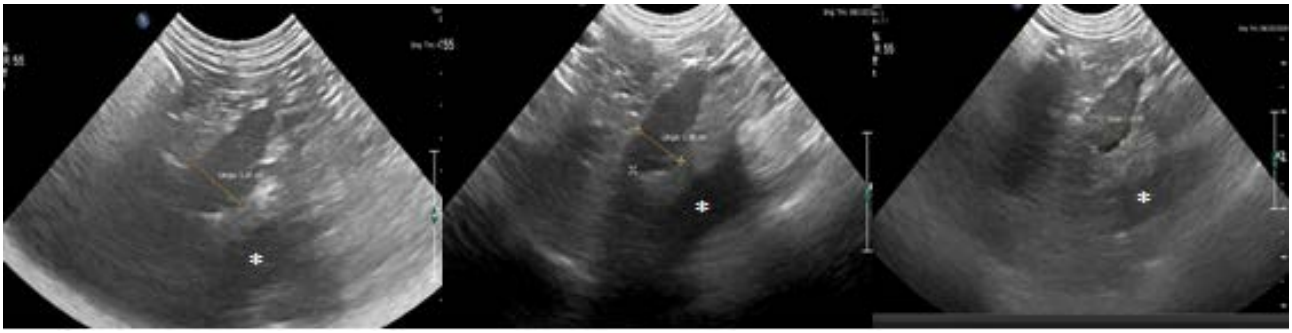


Figure 1. Right adrenal gland on three consecutive scans. From left to right: time of establishing diagnosis; first check-up, second check-up. Aorta is visible in far field (asterisk). Uniform enlargement of right adrenal gland is to be observed on all three scans.

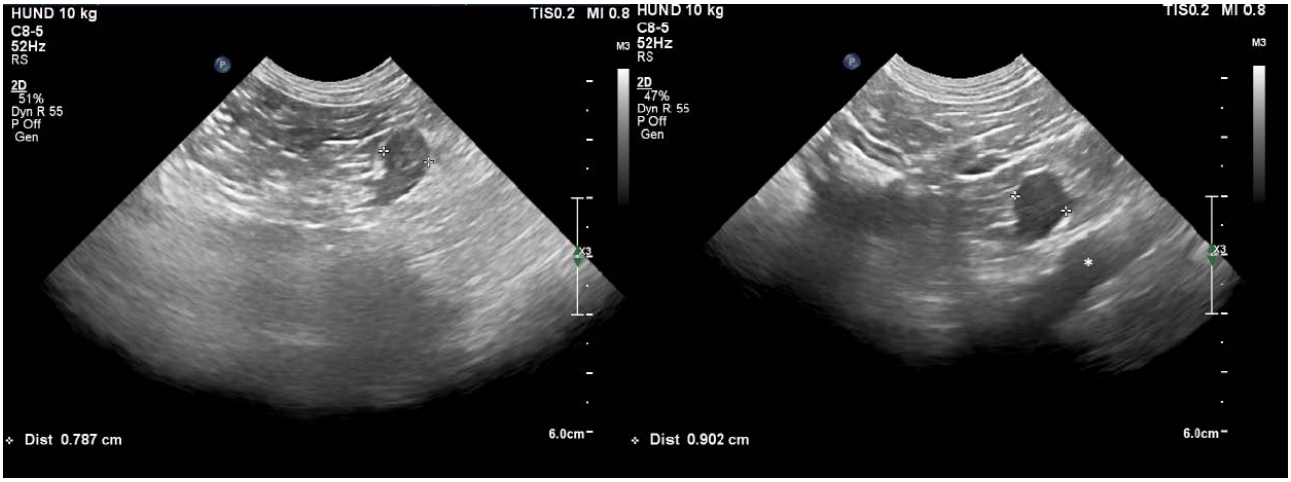


Figure 2. First and final left adrenal size (between cursors) evaluation. Effort is given to preform measurements at the same position. Aorta is visible in far field (asterisk). Increase in size and change of echogenicity is to be appreciated on these two consecutive scans.

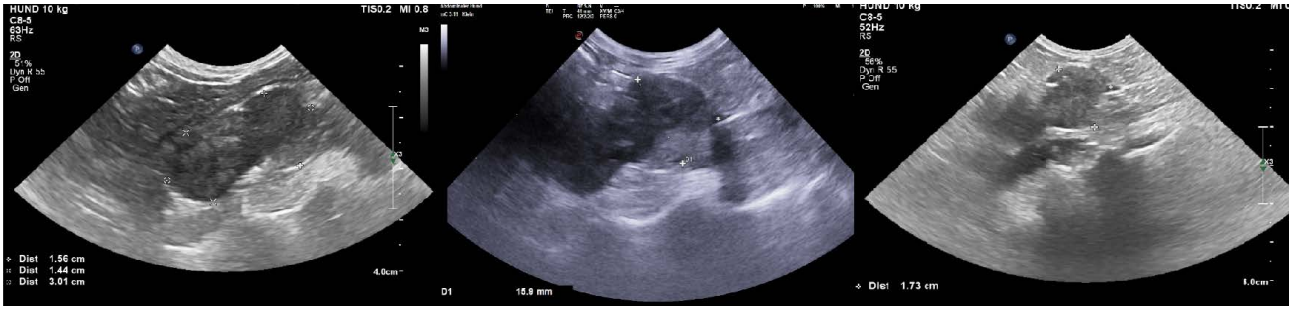


Figure 3. Neoplasia of left adrenal on three consecutive scans. The mass of the left adrenal gland has irregular and heterogeneous appearance. From left to right: time of establishing diagnosis; first check-up, second check-up. Caudal to adrenal gland tumor (between cursors), small amount of free fluid (asterisk) is to be observed.

stimulated cortisol value than the basal one and values in lower middle part of the reference interval(for basal and stimulated values 2 - 6 μg/dL and 6 - 22 μg/dL respectively) and "pre pill"—just around lower reference interval (1.45 - 4.5 μg/dL), history (improvement of lethargy, absence of pu/pd, improvement in hair coat) and clinical examination (normothermia, unremarkable auscultation of the heart and the lungs, pink mucous membranes, no pain during manipula-

tion), named good control group. The second group contains patients that still had signs of HAC such as pu/pd and lethargy and elevated cortisol values on their endocrine test named poor control group (see Table 1 and Table 2). In all patients of the good control group improvement in their lethargy and pu/pd was reported at the first check up. Patients of the bad control group showed improvement in their lethargy and pu/pd 2 weeks after establishing the right dosage of trilostane, or after initiating insulin therapy (Caninsuline© 0.25 IE/kg) for concurrent diabetes mellitus. Hair coat improvement was not reported after 10 weeks in 4/7 patients, two patients improved after 10 weeks and one after 20 weeks. No significant weight loss was reported in patients that were gaining weight before initiating trilostane therapy.

2.4. Data Analysis and Statistics

Measurement data were divided in two datasets, good control and poor control (explained previously). Each of the datasets had three timepoints: 1) starting (0

Table 1. Showing patients in "good control group" and their adrenal size, endocrine tests and clinical signs (ACTH—ACTH stimulation test, cr—cranial, ca—caudal, ce—central, W—week, p.P—"pre pill" test "+"—satisfactory).

Patient	Left Adrenal	Right Adrenal	L. Ad. %	Ri. Ad. %	ACTH/p.P	Clinical Signs
1C	0W.—ca 0.72 cm	0W.—ca 0.64 cm				
	2W.—ca 0.82 cm	2W.—ca 0.76 cm			+	+
2L	0W.—cr 0.75 cm; ca 0.99 cm	0W.—ce 0.93 cm				
	2W.—cr 0.81 cm; ca 1.05 cm	2W.—1.02 cm			+	+
3K	0W.—cr 0.56 cm; ce 0.7 cm	cr 1.91 cm				
	2W.—cr 0.58 cm; ce 0.74 cm	cr 1.87 cm			+	+
	10W.—cr 0.66 cm; ce 0.77 cm	cr 2.14 cm			+	+
4P	0W.—cr 0.61 cm; ca 0.6 cm	not the same angle and position as other measures				
	2W.—cr 0.62 cm; ca 0.65 cm	cr 0.77 cm; ca 0.72 cm			+	+
	10W.—cr 0.65 cm; ca 0.66 cm	cr 1 cm; ca 0.78 cm			+	+
5P	0W.—cr 1 cm; ca 1.01 cm	1.28 cm				
	22W.—cr 1 cm; ca 1.16 cm	1.34 cm			+	+
6T	0W.—cr 0.58 cm; ca 0.54 cm	ce 0.53 cm				
	2W.—cr 0.49 cm; ca 0.49 cm	ce 0.64 cm			+	+
	10W.—cr 0.67 cm	ce 0.73 cm			+	+
7A	0W.—cr 0.4 cm; ca 0.56 cm	cr 0.8 cm; ca 0.52 cm				
	2W.—cr 0.44 cm; ca 0.61 cm	cr 0.91 cm; ca 0.58 cm			+	+
8Sch	0W.—cr 0.76 cm;	ca 0.78 cm				
	2W.—cr 0.78 cm	ca 0.91 cm			+	+
	48W.—cr0.89 cm	ca 1.02 cm			+	+

Table 2. Showing patients in "good control group" and their adrenal size, endocrine tests and clinical signs (ACTH—ACTH stimulation test, cr—cranial, ca—caudal, ce—central, W—week, p.P—"pre pill" test, "+"—satisfactory, "+/-" small improvement in clinical signs, but still not satisfactory).

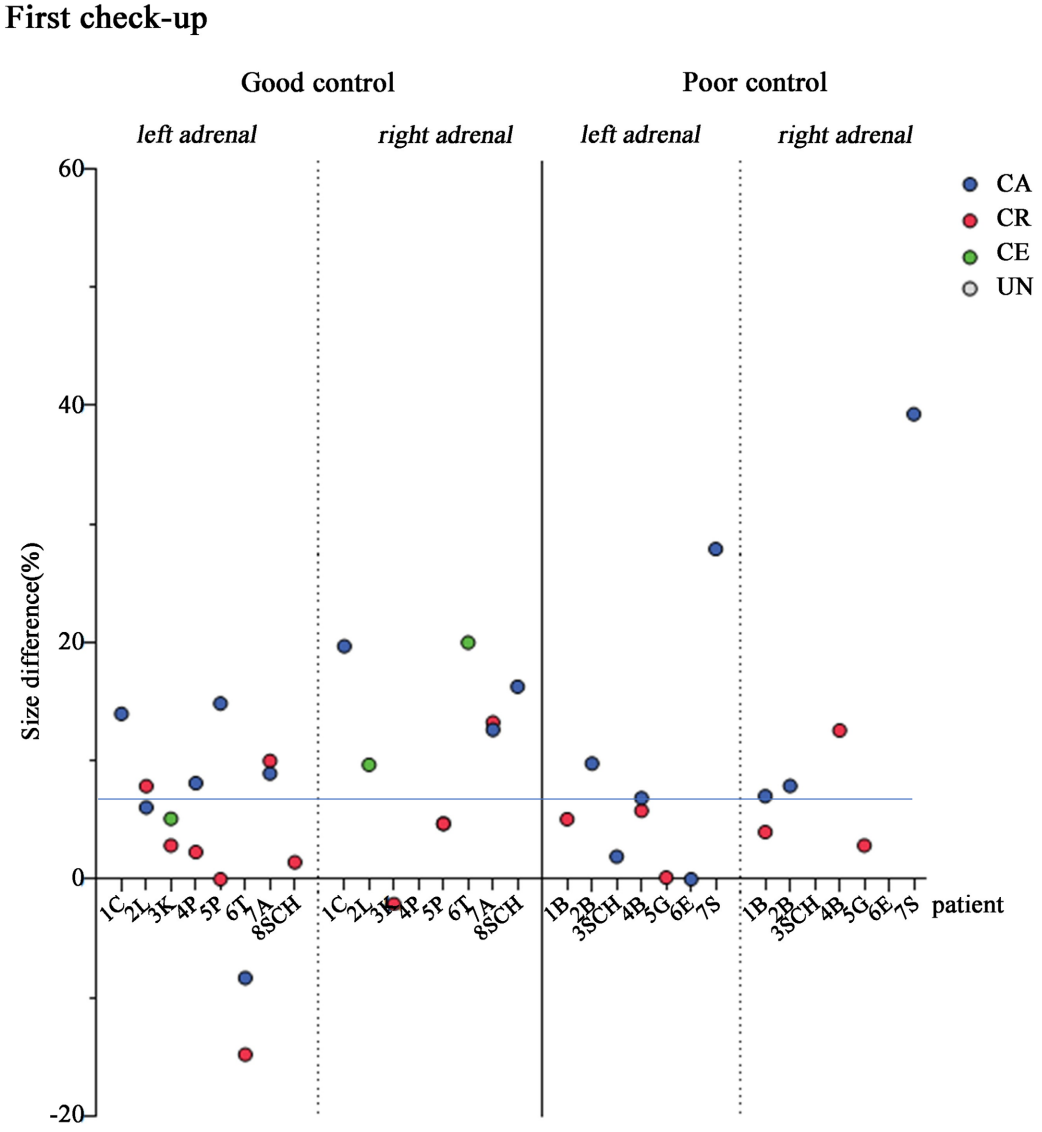
Patient	Left Adrenal	Right Adrenal	L. Ad. %	R. Ad. %	ACTH/p.P	Clinical signs	special notice
1B	0W.—cr 0.61 cm	cr 0.73 cm;	ca 0.57 cm				
	2W.—cr 0.64 cm	cr 0.76 cm;	ca 0.61 cm		-	-	
	10W.—cr 0.75 cm	cr 0.85 cm;	ca 0.68 cm		+	+	
2B (DM)	0W.—ca 0.79 cm	ca 0.74 cm					
	2W.—ca 0.86 cm	ca 0.79 cm			+	-	←dosage incease
	7W.—ca 0.88 cm	ca 0.84 cm			+	+	←begin of insuline th.
	13W.—ca 0.9 cm	ca 0.84 cm			+	+	
3Sch	0W.—ca 1.56 cm	0.31 cm					
	2W.—ca 1.59 cm	not to find			-	-	←dosage incease
	5W.—ca 1.7 cm	0.5 cm			-	+	
4B	0W.—cr 0.61 cm; ca 1.02 cm	cr 0.63 cm					
	2W.—cr 0.64 cm; ca 1.09 cm	cr 0.71 cm			+	-	←dosage incease
	5W.—cr 0.66 cm; ca 1.16 cm	cr 0.80 cm			+	+	
5G	0W.—cr 0.62 cm;	cr 1.05 cm					
	2W.—cr 0.63 cm; ce 0.7 cm	cr 1.08 cm			+	-	←dosage incease
	5W.—cr 0.75 cm; ce 0.75 cm	cr 1.15			+	+	
6E (DM)	0W.—ca 0.7 cm	cr 1.17 cm					
	2W.—ca 0.7 cm; cr 1.13 cm	cr 1.42 cm (Not the same angle and position)			+	-	←dosage incease
	8W.—ca 0.99 cm; cr 1.07 cm	cr 1.17 cm			+	-	←dosage incease
7S	0W.—ca 0.72 cm	0.57 cm					
	10W.—ca 0.92 cm	0.79 cm			-	-	←dosage incease
	34W.—ca 1.01 cm	0.871 cm			+	+	

weeks), 2) after the first checkup (mostly 2 weeks) and 3) after the second check-up (varied). Patients' left and right adrenal glands were measured. Size change at each check-up was estimated as a percentual increase/decrease compared to week 0. Data were plotted with GraphPad—Prism 8 using the "xy" dataset type showing size change on the y-axis and the patient ID on the x-axis. Further statistical tests to obtain p-values were performed using the unpaired t-test with the Welch's correction. The threshold of the statistical significance was set to be *p = 0.05.

3. Results

3.1. Percentage of Increase from Establishing the Diagnosis to First Check-Up

Greater increase in adrenal size was noticed in a group that reacted better at trilostane therapy, as visible from the Figure 4 (group that showed clinical improvement and had satisfactory ACTH/pre pill test). The right adrenal gland showed more enlargement in size and volume than the left adrenal gland. Some patients from the group that we named "poor control" showed increase of adrenal size. For example, patient named 2B showed enlargement in adrenal gland size, satisfactory ACTH stimulation test but absence of improvement in clinical symptoms. In this dog with further investigation diabetes mellitus was diagnosed.

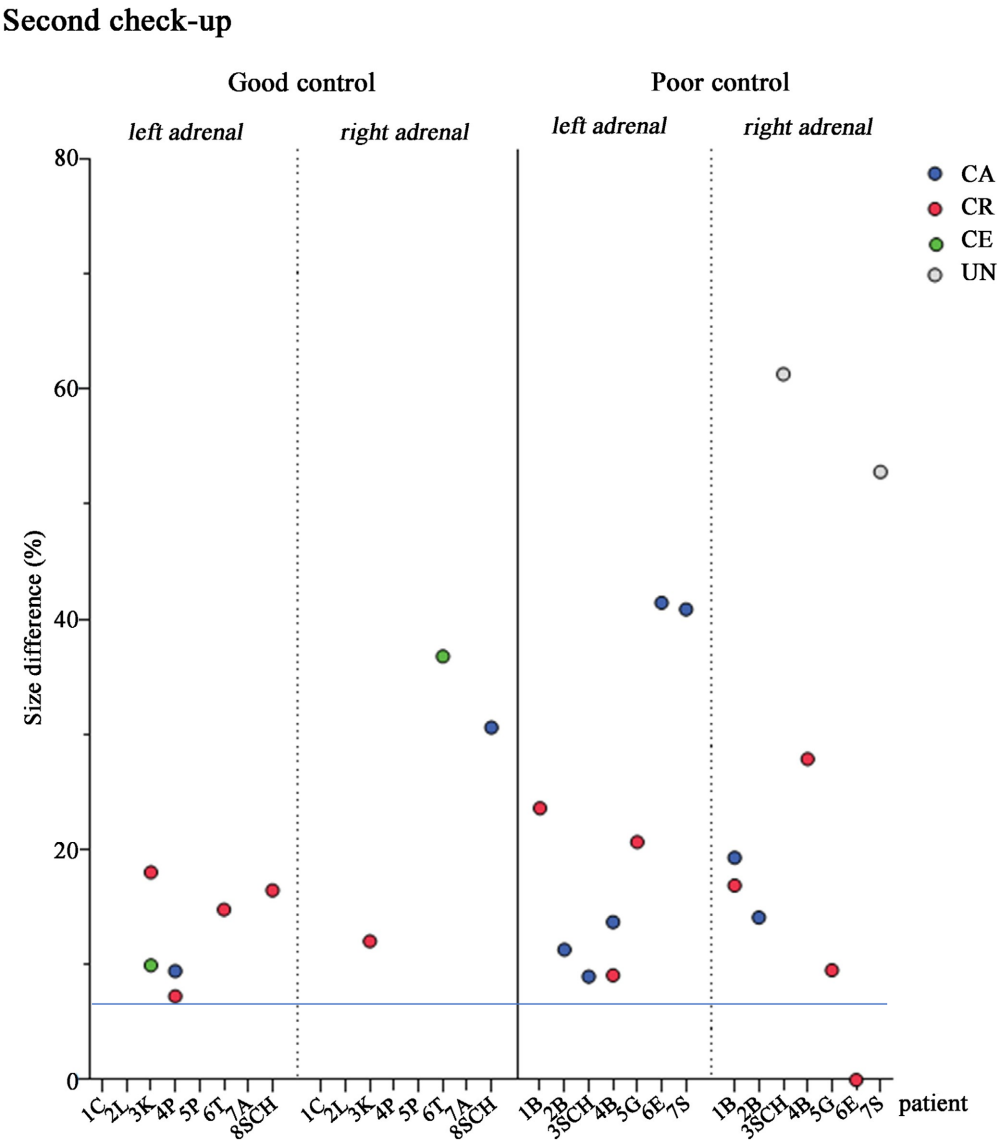


Ca—caudaly; Cr—cranialy; Ce—central; Un—unspecified.

Figure 4. First Check-up two weeks after initiating trilostane therapy.

3.2. Percentage of Increase from Establishing the Diagnosis to Second Check-Up

Figure 5 is representing findings on second check-up; we can see that almost all the patients but one, showed increase in adrenal size (bilateral). All the patients in „poor control" received increased dosage of trilostane and clinical symptoms improved, by every patient but one, in correlation with increase in adrenal size. The second check-up for the poor control group was performed 2 - 4 weeks after changing the dose of trilostane, one patient showed up later (six weeks after correcting the dosage). The patient named 6E had concurrent diabetes mellitus and was euthanized, because of absence of clinical improvement, 6 months after establishing diagnosis of Cushing's syndrome. The patient named 7S failed to show



Ca—caudaly; Cr—cranialy; Ce—central; Un—unspecified

Figure 5. Second check-up 2 - 4 weeks after changing trilostane dosage (for the poor control group, and approximately 10 weeks after initiating therapy for the good control group.

up 2 weeks after initializing the therapy, so the first check-up was done 10 weeks after starting with trilostane.

On both charts we can see the blue line representing mark of 7% increase in adrenal gland size. At the time of first check up 5/8 (63%) patients in "good control" group were above 7% increase mark and at the same time 5/7 (71%) patients in "poor control" group were under above mentioned 7% mark. Meaning that some of dogs, 2/7 (29% of them), that did not show improvement in clinical signs, had increase in adrenal gland size (possibly because of later time of ultrasonographic control). On the second chart we can observe that every patient is above the 7% mark and at that point all the patients but one showed improvement in clinical signs and satisfactory endocrine tests. It appears that it might be that patients that have enlargement in adrenal size of 7% or higher might have as well an improvement in clinical sings and laboratory tests.

4. Discussion

All the patients that were included in our study matched criteria of adrenal gland enlargement established in the recent studies [4]. Increase in adrenal gland size was already described in previous reports [1] [2] [3] and the focus in our study was at correlation between increase in adrenal gland size and improvement of clinical symptoms of HAC. To the author's knowledge this is the first time that this correlation was observed. Establishing diagnosis of HAC can be challenging if the clinical symptoms are mild, or in patients with concurrent diseases [5]. Measurement of basal cortisol plasma level is not a useful screening tool for diagnostic of HAC [6] [7], because of elevation of endogenous cortisol levels in patients with non-adrenal diseases, chronical inflammation and stress [8] [9]. Endocrine tests should be performed if there is an indication according to clinical signs, CBC (complete blood count), biochemistry and urine analyses [6] [10]. In our case all patients showed strong indication for performing endocrine tests according to their clinical signs and blood work. The LDDS (low dose dexamethasone suppression) test is considered as the screening test of choice if iatrogenic HAC is not suspected [6] [11] [12] and in our study it was performed as a first choice test. ACTH stimulation tests were done if high clinical indication of HAC was present, but LDDS test showed negative results. One dog was suspected to have an atypical onset of HAC. Atypical Cushing's syndrome is suspected in a patient with enlarged adrenal glands who is showing historical, routine laboratory and clinical signs of Cushing's but the endocrine test remains normal [13] [14]. An ACTH stimulation test was done and post ACTH plasma concentration of 17 OH progesterone was increased. Good response to trilostane therapy, adrenal enlargement and excessive stimulation confirmed our suspicion of atypical onset of HAC. Trilostane is considered as a medical treatment of choice for dogs with HAC [15] [16] and it was used in this study as well. The dosage of 0.5 mg/kg twice daily for PD and 1 mg/kg twice daily for AD was the initial dose for all patients, which matches the current recommendation that can

be found in literature [17]. All dogs but one received trilostane twice daily, but once daily usage represents a good alternative and initial dose should be 1 - 2 mg/kg [17] [18]. The ACTH stimulation test represents until today the most used test for monitoring of HAC in dogs [19]. There are several protocols and recommendations when to perform a test based on time after drug administration [15] [20] [21] [22] in our case we have performed ACTH stimulation tests two to three hours after administration of trilostane, since that is the time when nadir of cortisol serum concentration occurs [21] [23] [24]. Two dogs were considered as suitable candidates for the "pre pill" test instead of ACTH stimulation test. Recent studies showed better correlation of the "pre pill" test with clinical signs and owners reports about patient's condition than the ACTH stimulation test [25] [26]. If difficulties during blood sampling occurred (for example excessive movement, vocalizing, etc.) as suggested in recent studies [27] the stress factor was included in interpretation of test results. After every endocrine test ultrasound examination of both adrenal glands was done. After two weeks of trilostane therapy all patients but one showed increase in adrenal size. On second ultrasound examination that was performed adrenal glands increased in size by every patient. Adrenal enlargement during second ultrasound examination was similar to already described in literature [3]. We believe that the level of adrenal enlargement is in direct correlation with clinical improvement receiving trilostane therapy. It is already suggested that adrenal enlargement during trilostane therapy occurs because of suppression of the negative feedback mechanism affecting production of cortisol in adrenal glands [2] and that ACTH not trilostane causes changes in adrenal gland tissue [28]. A possible theory that connects adrenal gland enlargement and clinical improvement could be that better suppression cortisol production causes higher production of endogenous ACTH [29] and therefore increase in adrenal gland size [30], but additional studies to support this theory are needed. It is important to be mentioned that adrenal gland enlargement is not only observed in patients with cushing's syndrome, but as well in patients with primary hyperaldosteronism, pheochromocytoma, incidentaloma, acromegaly and sometimes in patients with severe chronic or acute diseases (pyometra, severe gastro-enteritis, glomerulonephritis, etc.). Because in this study low dose of trilostane was used as initial dosage, no dog showed any sign of hypoadrenocorticism and therefore no data is available on adrenal gland enlargement in those cases. It might be interesting to see if in those cases adrenal glands increase in their size even more than in dogs that are showing improvement in clinical signs. Evaluation of adrenal gland enlargement might be a useful tool in monitoring of HAC patient, especially in the ones with concurrent diseases that are showing poor to no improvement of clinical sings of Cushing's syndrome. With this additional tool (to clinical examination and endocrine tests) we could determine better whether or not poor clinical improvement is because of insufficient dosage of trilostane or caused by some concurrent disease and gain more confidence in meeting the decision of adjusting the dosage of therapy. Possible combination of

novel methods of monitoring Cushing disease (measuring haptoglobin-Hp, serum alanine aminotransferase-ALT, gamma-glutamyl transferase-γGT) [16] with assessment of adrenal gland enlargement (together with clinical examination and endocrine tests) could as well be considered. Limitation of using abdominal ultrasound as a monitoring tool could be: demand for skilled sonographer and difficulties in obtaining measurements at the same position every time. The biggest advantage in using sonography for monitoring of HAC, especially in patients that do not improve under trilostane therapy, is the possibility of diagnosing concurrent diseases (pancreatic changes, increased thickness of bladder wall, etc.) that could be responsible for lack of clinical improvement.

Our study has several limitations. The number of dogs included in our study is small and the statistical correlation is lacking. A study on larger population of dogs would overcome this limitation. Secondly, some of the patients showed up later on date for scheduled appointments which could interfere with the level of change in adrenal glands size. The third limitation of our study might be that we didn't include control group of the dogs with diagnosed Cushing's syndrome that didn't received treatment with trilostane to observe level of change of adrenal glands in that population.

5. Conclusion

Evaluation of adrenal gland size increase might be a useful additional tool for monitoring dogs with HAC under trilostane therapy because adrenal gland enlargement could have correlation with improvement in clinical signs under trilostane therapy. In this study, all dogs that had an increase in adrenal gland size by more than 7% showed as well clinical improvement in HAC signs. Further and additional studies on this topic are needed. Adrenal gland enlargement evaluation alone should not be used as an only monitoring tool, because increase in adrenal gland size is seen as well in patients with poor control group.

Informed Consent Statement

Not applicable for studies involving humans.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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