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Longitudinal assessment of thyroid function in dogs with hypoadrenocorticism: Clinical outcomes and prevalence of autoantibodies

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Abstract

Background: Knowledge about primary hypoadrenocorticism coexisting with immune-mediated thyroiditis (Schmidt's syndrome) in dogs is limited.

Objective: To evaluate thyroid function in dogs with naturally occurring hypoadrenocorticism before and during treatment.

Animals: Sixty-six client-owned dogs.

Methods: Measurement of canine thyroid stimulating hormone (cTSH), total thyroxine (T4), free thyroxine, and autoantibodies against thyroglobulin, T4, and total triiodothyronine.

Results: Thirty-eight dogs were assessed before and 28 during treatment. Follow-up data were available for 24/38 and 17/28 dogs, with median follow-up duration of 3.8 years (range, <1.0-8.8 years) and 4 years (range, 1.1 weeks to 10.5 years), respectively. Canine thyroid stimulating hormone was above the reference range at the time of diagnosis of hypoadrenocorticism in 10 of 38 dogs but decreased into the reference range in 7 for which follow-up data was available. Hypothyroidism was confirmed in 5 dogs at a median age of 11 years (range, 7-15 years). In 4 dogs, the condition was diagnosed after a median treatment duration of 5.75 years (range, 2.6-10 years), while in 1 dog, the diagnosis was made concurrently. One dog had detectable thyroid autoantibodies.

Conclusions and Clinical Relevance: Hypothyroidism occurs as a rare concurrent condition in dogs with hypoadrenocorticism, potentially at any phase of treatment. Close monitoring of cTSH levels in these dogs could be beneficial, as early changes might indicate the onset of hypothyroidism. The low prevalence of detectable thyroid

Abbreviations: AA, autoantibody; cTSH, canine thyroid stimulating hormone; DOCP, desoxycorticosterone pivalate; fT4, free thyroxine; T3, total triiodothyronine; T4, total thyroxine; TgAA, thyroglobin-AA.

autoantibodies suggests that nonimmune mechanisms might contribute to thyroid dysfunction.

KEYWORDS
ACTH, Addison's disease, cortisol, cTSH, hypothyroidism, polyglandular

1 | INTRODUCTION

The predominant form of hypoadrenocorticism in dogs is characterized as primary hypoadrenocorticism, involving the destruction of the adrenal cortex, leading to complete deficiency of glucocorticoids and mineralocorticoids. Histopathological examination typically shows lymphocytic adrenalitis in the early stages and atrophy in the later stages of the disease. Antibodies against the cytochrome P450 side-chain cleavage enzyme are identified in 24% of dogs with hypoadrenocorticism, indicating an autoimmune-mediated pathogenesis.[1] In human medicine, primary hypoadrenocorticism often co-occurs with immune-mediated thyroiditis, commonly known as Schmidt's syndrome. This syndrome denotes the combination of autoimmune primary adrenal insufficiency (Addison's disease) with autoimmune hypothyroidism and sometimes type 1 diabetes mellitus, representing a component of the broader autoimmune polyendocrine syndrome type 2 or polyglandular autoimmune syndrome type II. In humans, autoimmune polyendocrine syndromes frequently manifest with circulating autoantibodies and lymphocytic infiltration in affected organs, ultimately leading to organ failure.[2] Consequently, autoantibodies play a crucial role in diagnosing autoimmune endocrine diseases and are employed as a screening tool to identify individuals at risk during the early stages of the disease, as autoantibodies are often detectable years before disease onset. Among humans with hypoadrenocorticism, the prevalence of autoimmune thyroid disease ranges from 0.3% in the United Kingdom to 47% in Norway, with an intermediate prevalence of 24% in Poland.[3-5]

In dogs, there are 2 case series and several case reports describing polyendocrine syndromes.[6-15] Intriguingly, both case series highlight that the combination of hypoadrenocorticism and hypothyroidism ranks as either the most common or second most common occurrence.[6,12] Thyroid autoantibodies were assessed in 6 dogs, with positive finding in 4 cases.[6,8,12,14,15] Moreover, hypothyroidism has been documented in 4% of dogs with hypoadrenocorticism.[16] The study does not classify this occurrence as polyendocrine syndrome, and the authors do not specify whether these dogs exhibited autoantibodies.[16]

To our knowledge, there are no extensive studies investigating the prevalence of thyroid autoantibodies in dogs with hypoadrenocorticism during the chronic treatment of the disease. Additionally, the evaluation of thyroid function in dogs with primary hypoadrenocorticism has not been undertaken during chronic treatment of hypoadrenocorticism.

Therefore, the objective of this study was to analyze thyroid function both before and during chronic treatment of hypoadrenocorticism. For this purpose, we assessed concentrations of canine thyroid stimulating hormone (cTSH), total thyroxine (T4) and free thyroxine (fT4), and autoantibodies against thyroglobulin, T4 and triiodothyronine (T3) in dogs with naturally occurring primary hypoadrenocorticism.

2 | MATERIALS AND METHODS

2.1 | Dogs

Dogs with naturally occurring hypoadrenocorticism, either newly diagnosed or previously treated, were prospectively enrolled in the study between March 2014 and November 2022. Hypoadrenocorticism was confirmed by an insufficient ACTH-stimulated serum cortisol concentration (<2 μg/dL). Primary hypoadrenocorticism was diagnosed on the basis of abnormal serum sodium and potassium concentrations and if available high plasma cACTH concentrations. In dogs with electrolytes within the reference range, determination of cACTH was mandatory. Dogs with iatrogenic causes of hypoadrenocorticism (eg, previous steroid or trilostane treatment) were excluded from the study.

After the ACTH-stimulation test (performed with synthetic ACTH [Synacthen, Future Health Pharma GmbH, Wetzikon, Switzerland]), all newly diagnosed dogs were treated with prednisolone; the starting dose in the hospital ranged between 0.5 and 1 mg/kg IV, q6 to q12 hours, depending on the severity of the signs for a duration of 12 to 48 hours.[17] If serum electrolytes were within the reference range, dogs received prednisolone only. Otherwise, they were started with mineralocorticoid treatment (desoxycorticosterone pivalate [DOCP; Percorten-V; Novartis Animal Health US, Greensboro, North Carolina or Zycortal; Dechra Pharmaceuticals, Overland Park, Kansas] or fludrocortisone [Florinef; Bristol-Myers Squibb SA, Baar, Switzerland]) in addition to the prednisolone. In all dogs, the prednisolone dose was gradually reduced over several weeks after discharge to a final dose of 0.05 to 0.1 mg/kg per day.

2.2 | Analytical procedures

If there was insufficient serum to measure all thyroid variables (eg, T4, fT4, fT3, TSH, AA), the attending clinician prioritized the most critical variables and informed the laboratory to measure these first.

2.2.1 | Determination of serum cTSH and serum T4 concentration

Serum cTSH concentrations were measured by use of a solid-phase, 2-site chemiluminescent enzyme immunometric assay (DPC Immulite

1000 [samples received from March 2014 to September 2016], DPC Immulite 2000 [samples from September 2016 until November 2022], Siemens Schweiz AG, Zurich, Switzerland). The upper limit of the reference range was 0.5 ng/mL. Serum T4 concentrations were determined with a homologous solid-phase, chemiluminescent enzyme immunoassay (DPC Immulite 1000 [samples received from March 2014 to September 2016], DPC Immulite 2000 [samples from September 2016 until November 2022], Siemens Schweiz AG, Zurich, Switzerland). The reference interval was between 0.9 and 4.7 μg/dL.

2.2.2 | Determination of fT4

Determination of serum fT4 was performed at the Michigan State University, Veterinary Diagnostic Laboratory using equilibrium dialysis, as previously described.[18] The reference interval reported by the laboratory was 0.5 to 3.3 ng/dL.

2.2.3 | Determination of serum thyroid autoantibodies

Determination of serum thyroid autoantibodies (AA) was performed at the Michigan State University, Veterinary Diagnostic Laboratory. Thyroglobulin autoantibodies (TgAA) were measured by an ELISA (Canine thyroglobulin autoantibody, Oxford Biomedical Research, Oxford, Michigan) as described in Nachreiner et al.[19]

T4- and T3-AA were measured using an in-house charcoal-separation binding assay as described in Nachreiner et al.[20]

In most instances, thyroid AA measurements were performed contemporaneously at the direction of the treating clinician, in accordance with the project description. However, in 10 dogs, these measurements were done retrospectively for the present study. All 10 dogs had been included in a previous study, with samples kept at −80°C. In total, 31 dogs had been previously included in either Reusch et al or Sieber-Ruckstuhl et al.[17,21] All dogs were treated according to established standards of veterinary care and ethical approval.

2.2.4 | Statistical analyses

Statistical analysis was performed by commercial software using non-parametric tests (GraphPad Prism 10, GraphPad Software, San Diego, California; SPSS, Statistical Package for the Social Science, Software Packets for Windows, Version 24). Data are expressed as median and range. To analyze several time points during hypoadrenocorticism treatment, the Friedman's repeated measures test and Dunn's multiple comparisons posttest were used. If only 2 time points were compared, the Wilcoxon matched-pairs signed rank test was used. To compare the sex distribution between dogs with hypoadrenocorticism only and those with hypoadrenocorticism and hypothyroidism the Fisher's exact test was used. For all statistical analyses, values of $P < .05$ were considered significant.

3 | RESULTS

Sixty-six dogs with ages at the time of diagnosis of hypoadrenocorticism ranging from 0.5 to 13 years (median, 5.0 years) and body weight (time of diagnosis of hypoadrenocorticism) from 3.2 to 55.0 kg (median, 14.6 kg) were included.

There were 25 males (19 castrated) and 41 females (32 spayed), 16 of them were mixed breed, 50 purebred dogs. Breeds including more than 1 dog were: Jack Russel Terrier [6], Labradoodle [4], Poodle [3], Labrador Retriever [3], Pinscher [2], St. Bernhard dog [2], and Chihuahua [2]. Most presenting clinical signs of the newly diagnosed dogs included vomiting, diarrhea, anorexia or hyporexia, weight loss, weakness, lethargy, polyuria, polydipsia, or some combination of these.

In 5 dogs, eunatremic and eukalemic hypocortisolism was diagnosed. All 5 dogs suffered from primary hypoadrenocorticism, which was based on high cACTH concentrations after receiving the result of the abnormal ACTH stimulation test.

3.1 | Dogs assessed at the time of diagnosis of hypoadrenocorticism (n = 38)

Canine thyroid stimulating hormone concentrations were above the reference range in 10 of the 38 dogs tested. Total T4 and free T4 concentrations were below the reference range in 8 out of 36 and 1 out of 31 dogs, respectively. The results (median, range) are presented in Table 1. Autoantibodies were measured in 31 dogs, none of which had a positive AA titer. Among the 10 dogs with cTSH concentrations above the reference range, total T4 was measured in 9 and fT4 in 5, with low levels found in 1 and 0 dogs, respectively.

In 1 dog (Dog 1), hypothyroidism (normal TSH, low T4, and low fT4) was diagnosed concurrently with hypoadrenocorticism (see Tables S1 and S2).

3.1.1 | Follow-up assessments

In 24 of the 38 dogs, 1 (n = 7) or more (n = 17) follow-up assessments were performed. The median follow-up duration was 3.8 years (range, <1.0-8.8 years), with a median of 3 follow-up assessments (range, 1-6) per dog, resulting in a total of 69 assessments. For dogs with more than 1 assessment, the median number was 3 (range, 2-6) per dog. The number of available samples, as well as T4 and fT4 concentrations, and the number of dogs with low T4 and fT4 concentrations at each time point, are presented in Table 2.

There was a significant decrease in cTSH after starting glucocorticoid treatment ($P < .05$). Thyroid stimulating hormone decreased into the reference range in all dogs where it was initially high, and follow-up samples were available (n = 7). Canine thyroid stimulating hormone concentrations remained within the reference range during chronic hypoadrenocorticism treatment in all but 2 dogs (Dogs 2 and 3), which developed hypothyroidism (see Tables S1 and S2). Results from dogs with follow-up cTSH concentrations are shown in Figure 1.

TABLE 1 Thyroid function variables before treatment of hypoadrenocorticism.

Thyroid function variables	Median	Range	Number of dogs included
cTSH (ng/mL)	0.2	<0.03-2.6	38
T4 (μg/dL)	1.4	<0.5-4	36
fT4 (ng/dL)	1.5	0.08-3.5	31

Note: Reference ranges: cTSH, < 0.5 ng/mL; T4, 0.9-4.7 μg/dL; free T4, 0.5-3.3 ng/dL.
Abbreviations: cTSH, canine thyroid stimulating hormone; fT4, free thyroxine; T4, total thyroxine.

Total thyroxine levels significantly increased after the initiation of hypoadrenocorticism treatment ($P < .05$). Of the 5 dogs with initially low T4 and follow-up information, 3 normalized and remained stable. In 1 dog with initially low T4 and high TSH, both T4 and TSH normalized within the first year of treatment. Between 1 and 2 years, T4 decreased while TSH remained normal. Subsequently, TSH increased while T4 remained low, leading to a diagnosis of hypothyroidism after 2.6 years (Dog 2). Another dog with initially low T4 exhibited fluctuating T4 levels over 6 years (low within the first year, normal after 1-2 years, low after 2-3 years, and normal after 4-6 years), while TSH remained within the reference range. Due to signs of recurrent

TABLE 2 Median (range) of T4 and free T4 concentrations and the number of dogs with T4 or free T4 below the reference range before and after chronic treatment of hypoadrenocorticism in the dogs with follow-up assessments.

Duration of HA treatment	T4 (μg/dL)	Number of dogs with T4 below RR	Number of dogs included	fT4 (ng/dL)	Number of dogs with fT4 below RR	Number of dogs included
Before	1.3 (0.3-4)	5	24	1.5 (0.8-3.5)	0	15
<1 year	1.6 (0.8-4.7)	1	12	1.8 (0.8-2.9)	0	10
1-2 years	2.0 (0.4-3.5)	2	12	1.7 (1.2-3.0)	0	9
2-3 years	2.4 (0.2-3.4)	3	13	1.7 (0.7-2.6)	0	8
3-4 years	3.0 (1.1-4.1)	0	7	1.8 (1.6-3.3)	0	6
4-6 years	1.9 (0.9-4.0)	0	10	2.2 (1.1-3.5)	0	4
6-8 years	1.3 (1.1-3.3)	0	3	1.5 (1.0-1.7)	0	3
>8 years	0.6/0.8	2	2	2.0	0	1

Note: Reference ranges: T4, 0.9-4.7 μg/dL; free T4, 0.5-3.3 ng/dL.
Abbreviations: fT4, free thyroxine; NA, not applicable; RR, reference range; T4, total thyroxine.

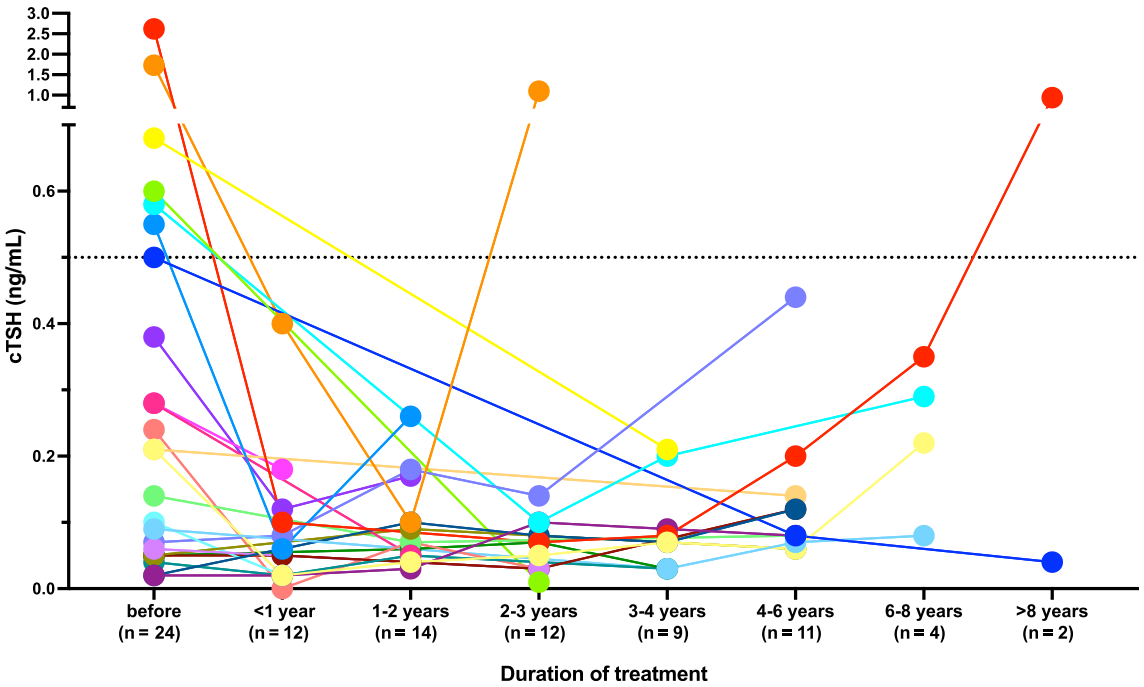

FIGURE 1 Scatter plot of cTSH concentrations in 24 dogs with hypoadrenocorticism before and during chronic hypoadrenocorticism treatment. The dotted line represents the upper limit of reference range. Reference range for TSH <0.5 μg/dL.

gastrointestinal disease, food-responsive enteropathy and nonthyroidal illness were suspected.

During chronic treatment of hypoadrenocorticism, 2 dogs developed low T4 concentrations at multiple rechecks (1 dog at 1-2 years and 2-3 years; the other dog at over 8 years). However, as no clinical signs of hypothyroidism were apparent and TSH remained normal, nonthyroidal illness was suspected.

One dog (Tables S1 and S2, Dog 3) with initially normal T4 but high TSH developed hypothyroidism after 8.5 years, indicated by low T4 and high TSH.

Free thyroxine did not significantly change during chronic treatment of hypoadrenocorticism and remained within the reference range in all dogs throughout the entire follow-up period.

Autoantibodies were measured in conjunction with fT4 determination, so the number of dogs included at each follow-up time point during treatment is documented in Table 2. None of the dogs had a positive AA titer.

3.2 | Dogs assessed during hypoadrenocorticism treatment (n = 28)

Twenty-eight additional dogs underwent 1 (n = 11) or more (n = 17) thyroid assessments during chronic treatment of hypoadrenocorticism. These assessments were conducted over a median period of 2.1 years (range, <4 weeks to 10.5 years) from the time of diagnosis, with a total of 61 assessments performed. For dogs with more than 1 assessment, the median number of assessments was 3 (range, 2-5) per dog and the median follow-up time 4 years (range, 1.1-10.5 years). The number of dogs assessed at each time point, along with T4 and fT4 concentrations and the number of dogs with low T4 and fT4 concentrations, are summarized in Table 3.

Two dogs (Dogs 4 and 5) developed high cTSH concentrations 3 and 10 years after their hypoadrenocorticism diagnosis. Both dogs had been assessed 2 and 1.5 years earlier (1 and 8.5 years after diagnosis of hypoadrenocorticism, respectively), at which time all thyroid function variables were within the reference range. When TSH concentrations were high, T4 levels (and in Dog 4 also fT4) fell below the reference range, and both dogs exhibited typical clinical signs of hypothyroidism. As 1 owner declined L-thyroxine therapy, 1 dog continued to have high TSH and low T4 and fT4 concentrations 2 years later (after 4-6 years of chronic treatment of hypoadrenocorticism).

No other abnormal thyroid function variables were detected in any of the other dogs during the study period. Autoantibodies were measured in conjunction with fT4, so the number of dogs included at each follow-up time point during treatment is documented in Table 3. Autoantibody titers were positive in only 1 dog, 3 years after the diagnosis and treatment of hypoadrenocorticism, at which point the dog had developed hypothyroidism (Dog 4).

3.3 | Occurrence of hypothyroidism (n = 5)

Hypothyroidism was diagnosed concurrently with hypoadrenocorticism in 1 dog (Dog 1) and developed during the chronic treatment of hypoadrenocorticism in 4 dogs (Dogs 2-5). The signalment and presenting clinical signs of these 5 dogs at the time of hypothyroidism diagnosis, along with thyroid function variables at various time points, are detailed in Tables S1 and S2, respectively. Only 1 of the 5 dogs (Dog 2) had eunatremic and eukalemic hypoadrenocorticism.

The median age of the 5 dogs at the time of hypoadrenocorticism diagnosis was 5 years (range, 4-10 years). The median age of the other 61 dogs at the time of hypoadrenocorticism diagnosis was also 5 years (range, 0.5-13 years). There was no significant age difference between the dogs with hypothyroidism and those with hypoadrenocorticism only ($P = .33$). The median age of the 5 dogs at the time of hypothyroidism diagnosis was 11 years (range, 7-15 years).

Although all 5 dogs with both hypoadrenocorticism and hypothyroidism were spayed females, there was no significant difference in sex distribution between these 5 dogs and those with hypoadrenocorticism only ($P = .15$).

In 2 of the 4 dogs where hypothyroidism developed during chronic hypoadrenocorticism treatment, thyroid function variables were evaluated at the time of hypoadrenocorticism diagnosis. Both

TABLE 3 Median (range) of T4 and free T4 concentrations and the number of dogs with T4 or free T4 below the reference range during chronic treatment of hypoadrenocorticism in the dogs with follow-up assessments.

Duration of HA treatment	T4 (μg/dL)	Number of dogs with T4 below RR	Number of dogs included	fT4 (ng/dL)	Number of dogs with fT4 below RR	Number of dogs included
<4 weeks	2.0 (0.9-3.0)	0	6	2.0 (0.9-2.3)	0	8
<1 year	1.9 (1.1-2.6)	0	6	1.8 (1.1-2.2)	0	8
1-2 years	2.6 (1.3-4.0)	0	13	1.9 (0.8-3.6)	0	16
2-3 years	2.2 (1.3-2.8)	0	8	1.9 (1.0-2.4)	0	13
3-4 years	1.9 (0.3-3.2)	1	6	1.6 (0.38-2.1)	1	8
4-6 years	1.7 (0.6-2.7)	1	8	1.5 (0.46-2.1)	1	12
6-8 years	2.0 (1.1-3.4)	0	4	1.8 (1.0-2.1)	0	6
>8 years	0.2/1.3	1	2	0.95 (0.85-1.5)	0	4

Note: Duration of treatment is related to the time point of diagnosis. Reference ranges: T4, 0.9-4.7 μg/dL; free T4, 0.5-3.3 ng/dL.
Abbreviations: fT4, free thyroxine; NA, not applicable; RR, reference range; T4, total thyroxine.

dogs had high TSH concentrations, which normalized during hypoadrenocorticism treatment. In the other 2 dogs, thyroid function variables were evaluated 1 and 8.5 years after the hypoadrenocorticism diagnosis and were within the reference range. After a median of 5.75 years (range, 2.6-10 years), all 4 dogs developed typical clinical signs of hypothyroidism, with high TSH and low T4 concentrations (Tables S1 and S2). A decreased fT4 concentration was observed in 1 dog (Dog 4). In only 1 of the 4 dogs (Dog 4), TgAA (187%; reference range, <35) and T3AA (45%; reference range, <10) were detected. Three of the 4 dogs were treated with L-thyroxine (1 owner declined treatment), and all exhibited significant clinical improvement.

4 | DISCUSSION

In this study, we assessed thyroid function in dogs with primary hypoadrenocorticism, over an extended follow-up period of several years. Our findings indicate that most dogs maintained normal thyroid function throughout this period. The prevalence of hypothyroidism in our study cohort was 7.5% (5 out of 66 dogs). Comparing our study's prevalence with those described by others is challenging, given the limited data available in veterinary literature. Peterson and colleagues reported a 4% prevalence of hypothyroidism among 225 dogs with hypoadrenocorticism. Given the retrospective nature of the study, it was challenging to ascertain which endocrine disorder appeared first or determine the time gap between the 2 diagnoses.[16] In 2 additional case series involving dogs with multiple endocrine diseases, the pairing of hypoadrenocorticism and hypothyroidism was either the most prevalent or the second most prevalent combination.[6,12] Among the 10 cases, the diagnoses for both diseases were simultaneous in 5 instances, while hypothyroidism was identified first in 3 cases and hypoadrenocorticism in 2 cases.[6,12] In 1 of our cases, hypothyroidism was diagnosed simultaneously with hypoadrenocorticism, whereas in the remaining 4, it emerged during the chronic treatment of hypoadrenocorticism. The time lapse from diagnosis of hypoadrenocorticism to the onset of hypothyroidism ranged between 2.6 and 10 years, a duration comparable to findings reported in previous study.[12]

Autoantibodies targeting thyroglobulin, T4 and T3 have been identified in numerous dogs, and TgAA particularly showing a significant correlation with the presence of lymphocytic thyroiditis.[19,22,23] Given the potential for hypothyroidism in dogs with hypoadrenocorticism as part of an immune-mediated polyendocrinopathy, one would expect to find thyroid autoantibodies in these dogs. However, these autoantibodies were detectable in only 1 of the 66 dogs with hypoadrenocorticism in our study. The prevalence of TgAA varies among different breeds. Among the breeds noted for a high prevalence (>10%) of TgAA, only a Golden Retriever, a Boxer, and a Maltese were among the 66 dogs included in our study. The Boxer and the Maltese developed hypothyroidism after 2.6 and 10 years of treatment of hypoadrenocorticism. However, even in these dogs, no antibodies were detectable.

The failure of the thyroid gland in hypothyroidism is assumed to be a slowly progressive process in which clinical signs and hormonal changes develop over several years.[24] If initially present, autoantibodies are expected to diminish in the late phase of the disease.[24] Therefore, it cannot be ruled out that the dogs, which had developed hypothyroidism but tested negative for TgAA, were already in the late phase of the disease. Another factor contributing to the lack of TgAA-positive dogs in our study could be their advanced age. Interestingly, the median age of 11 years at which 4 of our dogs with hypoadrenocorticism developed hypothyroidism was notably older than the range typically reported in the literature for hypothyroidism development, which is approximately 6.5 to 7 years.[25-27] Research indicates an inverse correlation between TgAA prevalence and the age of dogs, suggesting that older dogs were less frequently positive for TgAA.[24] Unfortunately, TgAA analysis was not available for all dogs at the time of hypoadrenocorticism diagnosis.

The initiation of glucocorticoid treatment after diagnoisis of hypoadrenocorticism might have reduced the presence of TgAA antibodies and led to a delay in the onset of the disease. In human cases, it has been demonstrated that relatively low doses of glucocorticoids can suppress the autoimmune response in autoimmune thyroiditis.[28] Finally, another explanation for the lack or extremely low prevalence of thyroid autoantibodies in our study could be that the origin of the endocrinopathy was nonimmune-mediated. The trigger for adrenal dysfunction in our dogs is unknown. It is generally assumed that hypoadrenocorticism arises from progressive immune-mediated destruction of the adrenal cortices. An autoimmune-mediated origin is suspected, as antibodies against the cytochrome P450 side-chain cleavage enzyme are detectable in some affected dogs.[1] We did not measure adrenal autoantibodies in this study, and therefore cannot comment on a potential autoimmune etiology. Autoantibodies play a crucial role in diagnosing autoimmune endocrine diseases in humans. They are used as a screening tool to identify individuals at risk during the early stages of the disease, as autoantibodies are often detectable years before the onset of symptoms.[29,30] Data from our study do not appear to support the findings seen in humans. However, further research is needed to understand the role of autoimmunity in the etiology of hypothyroidism in dogs with hypoadrenocorticism.

All 5 dogs with hypothyroidism in our study were female, reflecting a similar female predisposition seen in humans for polyendocrine syndromes.[29] However, in a previous case series, 2 of 3 dogs with concurrent hypothyroidism and hypoadrenocorticism were male.[6] Our study included more female dogs with hypoadrenocorticism, resulting in no significant statistical difference between sexes in those developing hypothyroidism. Some authors suggest that female dogs are more prone to hypoadrenocorticism, which might have influenced our results.[31] Nonetheless, there is debate, as another study found an even sex distribution, though it included only eunatremic, eukalemic dogs.[32]

An earlier study demonstrated that 37% of dogs with untreated hypoadrenocorticism exhibit high TSH concentrations alongside normal T4 concentrations.[21] This finding was confirmed in our study, as 26% of the dogs had high cTSH and usually normal T4 concentrations. An inverse relationship between the hypothalamic-pituitary-adrenal axis and the hypothalamic-pituitary-thyroid axis in dogs has been demonstrated in several studies.[33-35] High TSH concentrations are also observed in humans with hypoadrenocorticism. In addition to the

absence of cortisol's inhibitory effect on TSH secretion, it is hypothesized that some human patients with hypoadrenocorticism might also have concurrent chronic autoimmune thyroiditis, which could partly explain the increase in TSH levels.[36,37]

Notably, 2 dogs diagnosed with hypothyroidism initially showed high cTSH concentrations at the onset of hypoadrenocorticism. In both cases, cTSH levels normalized during treatment but increased again when hypothyroidism signs emerged after 2.6 and 8.5 years. Unfortunately, cTSH concentrations and antibody titers at diagnosis were not available for the other 2 dogs that developed hypothyroidism during chronic treatment. Five additional dogs exhibited high TSH concentrations at diagnosis. However, the follow-up period for most dogs was too short to determine if high initial TSH levels predict reduced thyroid function with later development of hypothyroidism. It might more likely reflect disruptions in the hypothalamic-pituitary-thyroid and hypothalamic-pituitary-adrenal axes rather than indicate hypothyroidism. This might better explain why TSH can rise even after more than 8 years, which is unlikely in long-standing classical hypothyroidism, where pituitary gland exhaustion has been hypothesized as a reason for low TSH concentrations in many hypothyroid dogs.[38] Closely monitoring thyroid variables in dogs with hypoadrenocorticism and high TSH at diagnosis might be warranted.

Our study longitudinally assesses thyroid function in dogs with hypoadrenocorticism, contributing to the limited literature on autoimmune polyendocrine syndromes in canines. We acknowledge that the lack of follow-up on dogs without hypoadrenocorticism to monitor for the development of hypothyroidism is a limitation of our study design.

Other limitations of our study include the low number of dogs with a complete thyroid panel, including thyroid autoantibodies, measured at the time of hypoadrenocorticism diagnosis. Additionally, we did not evaluate the occurrence of antibodies against other major thyroid proteins, such as thyroid peroxidase. Thyroid peroxidase antibodies are important in human autoimmune thyroiditis.[39] These antibodies have also been identified in dogs; however, their prevalence was low in 1 study with only 17% of the dogs with lymphocytic thyroiditis.[40] Moreover, they could not be detected in dogs lacking TgAAs. Therefore, it is unlikely that our dogs with hypoadrenocorticism would have had antibodies against thyroid peroxidase but not against thyroglobulin. We did not examine adrenal autoantibodies, such as antibodies against cytochrome P450 side-chain cleavage or 21-hydroxylase, to investigate an autoimmune origin of adrenal disease, as these tests are not commercially accessible.

In summary, autoantibodies against thyroid hormones in dogs with hypoadrenocorticism appear to be rare. It seems unlikely that screening for autoantibodies, as recommended for early detection of hypothyroidism in humans, would be similarly beneficial in dogs. Nonetheless, hypothyroidism can occur concurrently and was the only other endocrinopathy observed during hypoadrenocorticism treatment in our study (data not shown). Hypothyroidism can develop at any time after diagnosing hypoadrenocorticism and should be suspected if a clinically well-controlled dog under hypoadrenocorticism treatment exhibits signs such as an unexpected increase in body weight, reluctance to move, lethargy, or dermatological alterations. Untreated dogs with hypoadrenocorticism can have high TSH concentrations with normal total T4 or fT4 levels. Typically, TSH concentrations normalize during the first few weeks of hypoadrenocorticism treatment. However, since 2 of 4 dogs in our study that developed hypothyroidism had high cTSH concentrations at the time of hypoadrenocorticism diagnosis, it seems reasonable to monitor these dogs closely for signs of hypothyroidism during hypoadrenocorticism treatment.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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