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Hypothalamic-pituitary-adrenal axis recovery after intermediate-acting glucocorticoid treatment in client-owned dogs

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Abstract

Background: In dogs, duration of hypothalamic-pituitary-adrenal (HPA) axis suppression after systemic glucocorticoid treatment is reported to vary from a few days to up to 7 weeks after glucocorticoid discontinuation. These data are derived mainly from experimental studies in healthy dogs and not from animals with spontaneous disease.

Hypothesis and Objective: To determine the timeline for recovery of the HPA axis in a group of ill dogs treated with intermediate-acting glucocorticoids (IAGCs).

Animals: Twenty client-owned dogs that received IAGC for at least 1 week.

Methods: Single-center prospective observational study. An ACTH stimulation test, endogenous ACTH concentration, serum biochemistry profile, and urinalysis were performed at T0 (2-6 days after IAGC discontinuation) and then every 2 weeks (eg, T1, T2, T3) until HPA axis recovery was documented (post-ACTH cortisol concentration > 6 μg/dL).

Results: The median time of HPA axis recovery was 3 days (range, 2-133 days). Eleven of 20 dogs showed recovery of the HPA axis at T0, 6/20 at T1, and 1 dog each at T2, T5, and T9. Dose and duration of treatment were not correlated with timing of HPA axis recovery. Activities of ALT and ALP were significantly correlated with the post-ACTH cortisol concentration (rs = −0.34, $P = .03$; rs = −0.31, $P = .05$). Endogenous ACTH concentration was significantly correlated with pre ($r = 0.72$; $P < .0001$) and post-ACTH cortisol concentrations ($r = 0.35$; $P = .02$). The timing of HPA axis recovery of the dogs undergoing an alternate-day tapering dose was not different compared to dogs that did not (3.5 vs 3 days, $P = .89$).

Conclusion and Clinical Importance: Most dogs experienced HPA axis recovery within a few days after IAGC discontinuation. However, 2/20 dogs required >8 weeks.

Abbreviations: ACTHst, ACTH stimulation test; ALP, alkaline phosphatase; ALT, alanine aminotransferase; eACTH, endogenous ACTH; EEH, eunatremic, eukalemic HA; GCs, glucocorticoids; GGT, gamma-glutamyl transpeptidase; HA, hypoadrenocorticism; HPA, hypothalamic-pituitary-adrenal; IAGCs, intermediate-acting glucocorticoids; IMHA, immune mediated hemolytic anemia; Rs, Spearman's rank correlation coefficient; UPC, urinary protein to creatinine ratio; UPC, urine protein: creatinine ratio; USG, urine specific gravity.

KEYWORDS
ACTH stimulation test, endogenous ACTH, eunatremic eukalemic-hypoadrenocorticism, false positive results, hypoadrenocorticism

1 | INTRODUCTION

Hypoadrenocorticism (HA) is an uncommon disease in dogs.[1] Dogs with HA frequently are presented with chronic nonspecific clinical signs, including anorexia, vomiting, weight loss, and diarrhea.[2-5] Because of the vague clinical signs, dogs with HA and, in particular, those with eunatremic, eukalemic HA (EEH), often receive empirical treatment with glucocorticoids (GCs) before reaching a final diagnosis. The use of GCs results in the suppression of endogenous hypothalamic-pituitary-adrenal (HPA) axis function by exerting negative feedback effects at the level of the pituitary gland and hypothalamus.[6] The ACTH stimulation test (ACTHst) is the gold standard for HA diagnosis.[3] However, previous GC administration can cause false positive results on the ACTHst, resulting in a misdiagnosis of HA.[3] For this reason, dogs with HA, in particular EEH, represent a diagnostic challenge. Currently, no guidelines exist regarding the required time until the ACTHst can be carried out after a dog has been treated with different GC formulations. Generally, the extent and duration of suppression of the HPA axis depends on the dose, potency, half-life, and duration of GC treatment.[6] However, in humans, the duration and severity of HPA suppression cannot be reliably predicted by dose, duration or type of GC treatment.[7,8] Few published studies are available regarding the duration of HPA axis suppression in dogs receiving systemic GCs. In these studies, HPA axis recovery in dogs treated with systemic GCs is reported to range from a few days to up to 7 weeks after GC discontinuation.[9-15] However, the majority of these studies were carried out on healthy experimental dogs and, as such, possible interference on HPA axis from concurrent diseases has not been investigated. Moreover, in clinical practice, gradual tapering of the GC dose is recommended if the treatment lasts for ≥2 weeks, or if high doses are used.[6] The effect of alternate-day treatment on HPA axis recovery in a clinical context has not been investigated.

Our aim was to determine the timeline for recovery of the HPA axis in a group of ill dogs treated with systemic intermediate-acting GCs (IAGCs). Our hypothesis was that the timing of HPA axis recovery is dependent on the individual and that dogs that underwent the alternate-day tapering process may have more rapid recovery than those that did not.

2 | MATERIALS AND METHODS

2.1 | Study design

A single-center prospective observational longitudinal study involving client-owned dogs receiving systemic treatment with IAGCs (prednisone, prednisolone or methylprednisolone) that were admitted to the Veterinary Teaching Hospital of the University of Bologna from September 2020 to December 2022 was carried out. Dogs with different medical conditions (immune-mediated, neoplastic, and inflammatory) treated using IAGCs for at least 1 week were eligible for inclusion in the study. Only dogs in which the therapeutic protocol, in terms of dose and timing, was well defined were included. Dogs on topical GC treatment (alone or in combination with the systemic treatment) and dogs on a different type of GC (eg, dexamethasone, betamethasone) were not eligible for inclusion.

All dogs were enrolled according to the study protocol which was approved by the Scientific Ethics Committee of the University of Bologna.

2.2 | Animals

The following data were collected at the time of the enrollment (T0; 2-6 days after IAGC discontinuation): signalment, body weight, physical examination abnormalities, date of clinical evaluation, date of the beginning of GC treatment, type of GC administered, therapeutic protocol used (including dose of GC, tapering process and date of GC discontinuation), and the disease for which the dog was receiving GCs. At T0, an ACTHst, endogenous ACTH concentration (eACTH), serum biochemistry profile, and urinalysis including urinary protein-to-creatinine ratio (UPC) were performed. Serum biochemistry profile, urinalysis, eACTH and ACTHst were performed every 2 weeks (eg, T1 = 14 days post-T0, T2 = 28 days post-T0, T3 = 42 days post-T0, T4 = 56 days post-T0) until HPA axis recovery, defined as post-ACTH serum cortisol concentration > 6 μg/dL (endpoint), was documented.

2.3 | Endocrine testing and analytical procedures

For the ACTHst, blood samples were taken before and 60 minutes after IV injection of 5 μg/kg synthetic ACTH (Synacthen, Alfasigma S.P.A., Bologna, Italy). Blood samples for the determination of eACTH concentrations were collected before the injection of synthetic ACTH.

All of the analytical procedures were carried out at the veterinary laboratory of the University of Bologna. Blood samples for the determination of the eACTH were collected into EDTA-coated plastic tubes placed on ice. The samples were immediately centrifuged at 4°C, 500 × *g* for 8 minutes, and the plasma was immediately transferred to plastic tubes, stored at 4°C and analyzed within 8 hours, or stored at −80°C and thawed immediately before analysis. Blood samples for cortisol determination were collected in serum separating tubes. The coagulated blood samples were centrifuged for 10 minutes at 3000 × *g*; the serum was immediately transferred to plastic tubes, stored at 4°C and analyzed the same day or stored at −80°C and thawed immediately before analysis. Serum cortisol and eACTH concentrations were measured using a chemiluminescent enzyme

TABLE 1 (A) and (B) Results of the ACTH stimulation test and endogenous ACTH concentration in the two dogs diagnosed with eunatremic eukalemic hypoadrenocorticism.

Time points	Pre-ACTH cortisol (µg/dL)	Post-ACTH cortisol (µg/dL)	eACTH (pg/mL)
(A)			
T0	0.30	0.30	95
T1	0.39	0.44	300
T2	0.71	0.84	246
T3	0.84	0.87	184
T4	1.31	1.29	267
T5	1.70	1.99	193
T6	1.34	2.15	122
T7	1.54	2.06	156
T8	1.51	2.72	146
T9	1.10	1.96	143
T10	0.86	1.13	238
T12	0.53	0.60	780
T13	0.43	0.37	843
(B)			
T0	0.30	0.30	29
T1	0.30	0.30	196
T2	0.30	0.30	456
T3	0.30	0.30	760
T4	0.30	0.30	755
T5	0.30	0.30	661
T6	0.30	0.30	967

immunoassay (Immulite 2000, Siemens Healthcare) that had been validated for dogs and is widely used in laboratories throughout the world.[16,17]

Serum biochemistry profiles (AU 480, Beckman Coulter/Olympus, Brea, California) and urinalyses were carried out using standard laboratory methods at the medical laboratory of the referral institution.

2.4 | Statistical analysis

Statistical analysis was carried out using commercial statistical software packages (GraphPad Prism 7, San Diego, California). Descriptive statistics were generated to characterize the study population. Continuous variables were presented as mean ± SD or median and range (minimum and maximum value), depending on whether the data were normally or not normally distributed, respectively. Categorical variables were described with frequencies, proportions or percentages. Cumulative, maximum and median/mean daily GC dose and overall duration of treatment were extrapolated from the therapeutic protocol of each dog. The time of HPA axis recovery was calculated as the interval between the last GC administration and a post-ACTH serum cortisol concentration > 6 µg/dL.

Correlations between the timing required for HPA axis recovery and cumulative dose, maximum dose, median daily dose, duration of treatment, and body weight were evaluated using Spearman's rank correlation coefficient (Rs). The same statistical analysis was used to investigate the correlation between post-ACTH cortisol concentration and clinicopathological abnormalities associated with GC treatment (alanine aminotransferase [ALT], alkaline phosphatase [ALP], gamma-glutamyl transpeptidase [GGT], haptoglobin, cholesterol, triglycerides, urine specific gravity [USG], and UPC) as well as between pre- and post-ACTH cortisol concentration and eACTH concentration.

Comparison between the timing required for HPA axis recovery in dogs that did and those that did not undergo the alternate-day tapering process was carried out using a Mann Whitney or *t* test. The level of significance was set at $P < .05$.

3 | RESULTS

3.1 | Animals

A total of 23 dogs were included in the study. Of them, 2 dogs were excluded because they were diagnosed with primary EEH. In particular, the diagnosis of EEH was based on the presence of compatible clinical signs (eg, lethargy, hyporexia, diarrhea) coupled with (1) a persistent post-ACTH serum cortisol concentration < 2 µg/dL

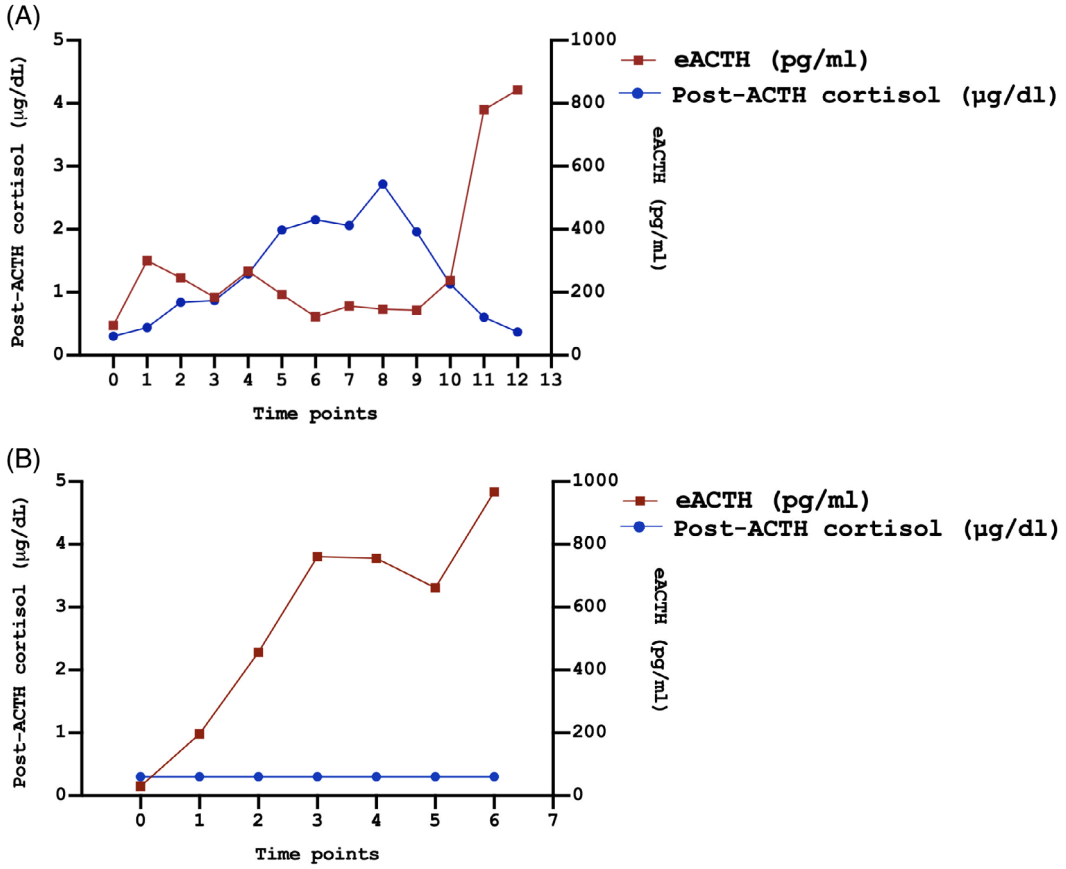


FIGURE 1 (A) and (B) serum post-ACTH cortisol concentration and endogenous ACTH concentration during the different time points in the two dogs diagnosed with eunatremic, eukalemic hypoadrenocorticism.

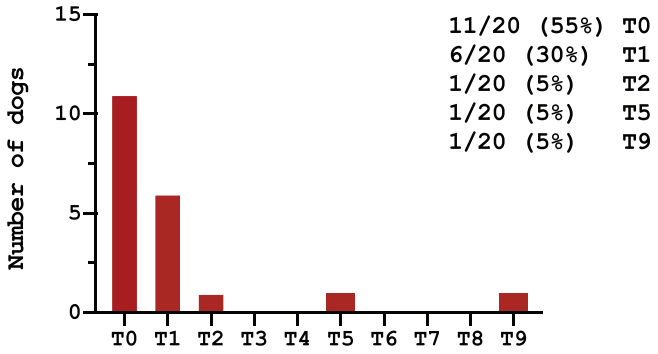


FIGURE 2 Barplot representing the percentage distribution of the population based on time of hypothalamic-pituitary-adrenal axis recovery.

(<55.0 nmol/L); (2) high (>58 pg/mL) plasma eACTH concentration, and (3) absence of electrolyte abnormalities (Table 1 and Figure 1). Moreover, 1 additional dog was excluded owing to immune-mediated hemolytic anemia (IMHA) relapse and the necessity of reintroducing GC treatment. The final study population included 13 females, of which 10 were spayed, and 7 males of which 3 were neutered. The median (range) age was 8.25 years (5 months-11.75 years) and the median body weight was 22 kg (4.5-44 kg).

The breeds included mixed-breeds (8), German Shepherds (2), Border Collies (2), American Staffordshire Terriers (2), Cocker Spaniels (1), Springer Spaniels (1), Maltese (1), Maremma Sheepdogs (1), Doberman Pinschers (1), and Spanish Greyhounds (1). The dogs had been treated with IAGCs for the following medical conditions: IMHA (7), immunosuppressant-responsive enteropathy (3), immune-mediated polyarthritis (3), mast cell neoplasia (2), immune-mediated thrombocytopenia (1), meningoencephalitis of unknown origin (1), sterile steroid-responsive lymphadenitis (1), suspicion of atypical hypoadrenocorticism (1), and protein-losing enteropathy (1). The most commonly used GC preparation was prednisolone in 16 of the 20 dogs, followed by methylprednisolone in 4/20 dogs. The therapeutic protocol used for each case is reported in Table S1. Table S2 shows the IAGC dose each dog was receiving during the last 14 days of treatment before T0. The median cumulative dose was 58.5 mg/kg (range, 14.7-370.5). The median maximum dose was 1.25 mg/kg/day (range, 0.2-4). The median of the mean daily dose was 0.7 mg/kg/day (range, 0.1-1.9). The median duration of the GC treatment was 65 days (range, 35-534).

3.2 | Timing of the HPA axis recovery

The median time of HPA axis recovery was 3 days (range, 2-133 days). In particular, 11/20 dogs experienced recovery of the

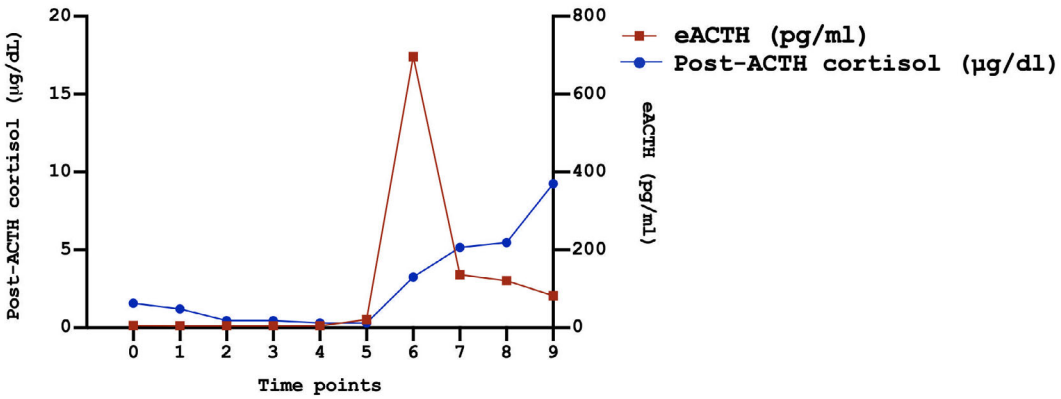


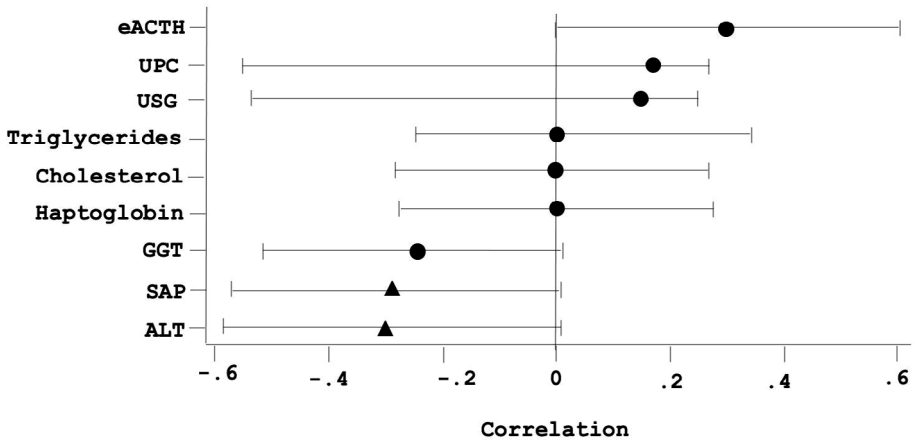
FIGURE 3 Post-ACTH cortisol concentration and endogenous ACTH concentration during the different time points in Case 11.

TABLE 2 The correlations between the timing of hypothalamic-pituitary-adrenal (HPA) axis recovery and cumulative dose, maximum dose, median daily dose, duration of treatment, and body weight.

Variables	Rs	(95% confidence interval)	*P* values
Cumulative dose	0.062	−0.501 to 0.402	.79
Maximum dose	0.042	−0.419 to 0.486	.85
Median daily dose	0.010	−0.445 to 0.461	.96
Duration of treatment	0.008	−0.460 to 0.447	.97
Body weight	−0.185	−0.589 to 0.293	.43

Note: None of the variables were correlated with the timing of HPA axis recovery.
Abbreviation: Rs, Spearman's correlation coefficient.

FIGURE 4 Correlations of clinical-pathological abnormalities because of glucocorticoids treatment with post-ACTH cortisol concentrations. The triangles represent a statistically significant correlation and the circles represent no significant correlation. ALP, alkaline phosphatase; ALT, alanine aminotransferase; eACTH, endogenous ACTH; GGT, gamma-glutamyl transpeptidase; UPC, urine protein: creatinine ratio; USG, urine specific gravity.



HPA axis at T0, 6/20 at T1, and 1 dog each at T2, T5, and T9 (Figure 2). The pre- and post-ACTH cortisol concentrations and the respective eACTH concentrations in all dogs at each time-point are reported in Table S3. One dog (Case 11) had undetectable (<0.3 µg/dL) pre- and post-ACTH cortisol concentrations up to T5. At T6, the eACTH became increased (696 pg/mL) and, concurrently, pre- and post-ACTH cortisol concentrations were detectable (2.89 and 3.26 µg/dL) for the first time. This dog reached the endpoint of the study after 4 months of GC discontinuation (T9; Figure 3).

Thirteen dogs underwent an alternate-day tapering process and 7 dogs did not. The timing of the HPA axis recovery in the dogs that underwent the alternate-day tapering process (3.5 days) was not different as compared to the dogs that did not (3 days; $P = .89$).

3.3 | Correlation analysis

Cumulative dose, maximum dose, median daily dose, duration of treatment, and body weight were not correlated with the timing of HPA axis recovery (Table 2).

Of the clinico-pathological abnormalities associated with GC treatment, ALT and ALP activities were significantly negatively correlated with the post-ACTH cortisol concentration ($rs = -0.34$, $P = .03$; $rs = -0.31$, $P = .05$; Figure 4). Haptoglobin, GGT, cholesterol, triglycerides, UPC, and USG were not correlated with the post-ACTH cortisol concentration (Figure 4). Endogenous ACTH was significantly positively correlated with pre-ACTH ($r = 0.72$; $P < .0001$) and post-ACTH ($r = 0.35$; $P = .02$) cortisol concentrations (Figure 5A,B).

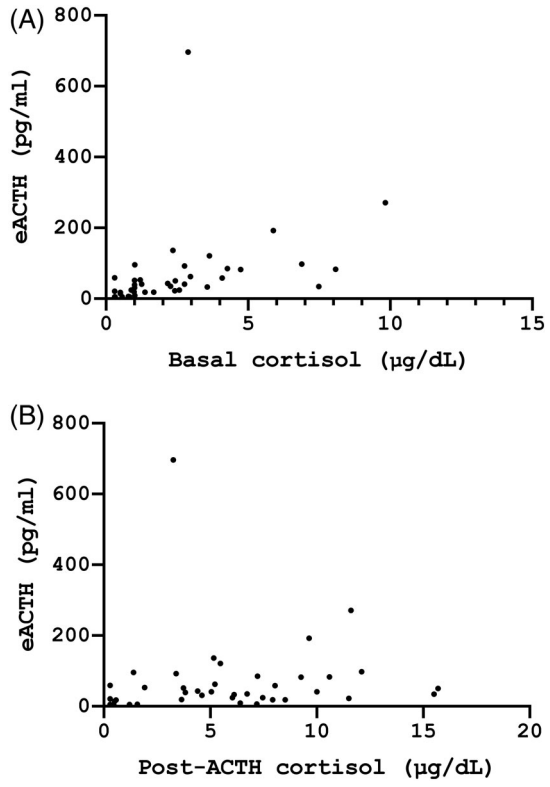


FIGURE 5 (A) The correlation between basal cortisol and endogenous ACTH (eACTH) concentration. Basal cortisol was positively correlated with eACTH (rs = 0.72; $P < .0001$). (B) The correlation between post-ACTH cortisol and eACTH concentration. Post-ACTH cortisol was positively correlated with eACTH (rs = 0.36; $P = .02$).

4 | DISCUSSION

Our results showed that, in dogs treated with systemic IAGC for at least 7 days, the median time of HPA axis recovery was 3 days. Approximately half of the dogs (11/20) experienced complete recovery of the HPA axis within a few days after IAGC discontinuation. However, 2 of 20 dogs required >8 weeks to achieve complete HPA axis recovery. These data add important information to the current literature, which was based on limited studies carried out mainly in healthy research dogs receiving different types of GC preparations. In dogs, a single dose of methylprednisolone acetate (2.5 mg/kg IM) has been shown to suppress the HPA axis for up to 7 weeks.[10,12] A single dose of triamcinolone acetonide suppressed the HPA axis for 2 to 4 weeks[11] whereas a single dose of dexamethasone resulted in a decreased cortisol response after an ACTHst for up to 32 hours.[13] In contrast, prednisone, given at a single dose of 2.2 mg/kg IM, did not result in adrenocortical suppression.[11] However, even physiological doses of prednisone or prednisolone can suppress the HPA axis when given for a prolonged time.[6] In 1 study, PO administration of prednisone at 0.55 mg/kg q12h for 35 days resulted in HPA axis suppression for up to 2 weeks after prednisone discontinuation.[14] In general, all synthetic GCs suppress corticotropin-releasing hormone and ACTH secretion, but their effects are not equivalent. In our study, only dogs receiving IAGCs were included because these drugs are the PO GC medication most commonly used to treat chronic diseases in dogs. Considering only the previous studies in which IAGC (prednisolone or methylprednisolone) were used, the maximum time of HPA axis recovery was 2 weeks.[14] Our results are, for the majority of the dogs, comparable with those obtained in healthy experimental dogs in which IAGCs were administered. Indeed, 17/20 dogs showed complete HPA axis recovery within approximately 2 weeks after IAGC discontinuation. However, 2/20 dogs required >8 weeks to show complete HPA axis recovery. In particular, 1 of them (dog 11) showed complete HPA axis recovery 18 weeks after IAGC discontinuation. This interval was much longer compared to the maximum time previously reported in the veterinary literature using IAGCs or any type of GC. Recovery of the HPA axis after a single administration of methylprednisolone acetate required up to 7 weeks,[12] but the latter is a long-acting depot preparation of GCs and a longer duration of HPA axis suppression is expected as compared to IAGCs.

Especially interesting were the trends in pre- and post-ACTH cortisol and eACTH concentrations at the different time points in patient 11 (Figure 3) that required 18 weeks before reaching the endpoint of the study. This dog showed undetectable pre- and post-ACTH cortisol concentrations up to T5. At the same time point, eACTH was detectable (20 pg/mL) for the first time. At the next time point (T6), the eACTH concentration was very high (696 pg/mL), and the results of the ACTHst showed a subnormal response to ACTH stimulation (pre-ACTH cortisol = 2.9 µg/dL and post-ACTH cortisol = 3.3 µg/dL). Considering these results, a misdiagnosis of HA in a dog with potentially compatible clinical signs would have been possible. Therefore, it is necessary to consider that after GC treatment discontinuation, some dogs may require a long HPA axis recovery time. To confirm the presence of HA in such cases, sequential ACTHsts and eACTH measurements might be needed.

In our study, all the dogs underwent a progressive tapering of the IAGC dose, and 13 of the 20 dogs underwent an alternate-day tapering process. The latter should allow the HPA axis to recover on the off days and is assumed to allow more rapid HPA axis recovery. However, according to our results, the timing of HPA axis recovery in dogs that underwent an alternate-day tapering process (3.5 days) was comparable to the dogs that did not (3 days). These results suggest that a gradual decrease in the GC dose, even without an alternate-day tapering process, might allow rapid recovery of the HPA axis. Additional studies are needed to assess whether the alternate-day tapering process affects HPA axis recovery time.

The duration of time required for full HPA axis recovery is said to depend on the duration, dose, preparation, and frequency of administration of the GCs.[6] In our study, no correlations between the timing of HPA axis recovery and cumulative dose, maximum dose, median daily dose, and duration of treatment were found. Other studies in humans had similar findings.[18-21] Therefore, according to these results, the dose and the duration of treatment do not seem to affect the timing of HPA axis recovery. However, the small sample size could have caused a type II statistical error. In support of this, the 2 dogs

(Cases 3 and 11) that requires the longest time to show complete recovery of the HPA axis had received the longest duration of treatment and the highest median daily dose.

Anecdotally, and based on a recent study,[16] dogs with higher body weight experience a higher frequency of adverse effects as compared to dogs with lower body weights when receiving GC treatment. Considering this aspect, we wanted to investigate the correlation between the timing of HPA axis recovery and body weight. According to our results, the timing of HPA axis recovery was not significantly correlated with body weight. Once again, the lack of significance might be a consequence of the small sample size.

Increased liver enzyme activities are among the most common biochemical abnormalities in dogs receiving GC treatment.[6] In our study, ALT and ALP activities were significantly negatively correlated with the post-ACTH cortisol concentration whereas GGT activity was not. This finding can be explained by the fact that after GC treatment is discontinued, liver enzyme activities progressively decrease and return to baseline. At the same time, the post-ACTH cortisol concentration increases because of the progressive recovery of the HPA axis. The lack of significance for GGT activity might be the result of the less consistent effect of GC treatment on GGT as compared to ALP and ALT.[17,22-27]

Endogenous ACTH concentration was significantly positively correlated with both pre-and post-ACTH cortisol concentrations. The use of GCs results in the suppression of the endogenous HPA axis function by exerting negative feedback effects on the pituitary gland and hypothalamus. After discontinuing GC treatment, the negative feedback induced by the exogenous GC administration decreases, resulting in a progressive increase in eACTH and, at the same time, a progressive increase in basal and post-ACTH cortisol concentrations.

An interesting and unexpected finding of our study was that during case recruitment, 2 dogs were excluded from the final analysis because they had been diagnosed with EEH. The diagnosis of EEH was based on the presence of compatible clinical signs (eg, lethargy, hyporexia, diarrhea) coupled with (1) persistent post-ACTH serum cortisol concentration < 2 μg/dL (<55.0 nmol/L); (2) high (>58 pg/mL) plasma eACTH concentrations and (3) the absence of electrolyte abnormalities. In these dogs, GC treatment was discontinued 12 and 16 weeks before the EEH diagnosis. Both dogs had received prednisolone for the treatment of IMHA. The high occurrence of EEH in this population of dogs might reflect a common etiopathogenesis for both HA and IMHA. Indeed, IMHA involves autoimmunity to self-antigens on the erythrocyte cell membrane.[28] Several studies provide strong evidence for HA also being an immune-mediated condition.[29-31] Also, polyglandular endocrine disease has been reported in veterinary medicine.[32-37] Up to 2.3% of dogs diagnosed with endocrine disease are diagnosed with multiple endocrinopathies.[38] However, concurrent non-endocrine autoimmune disorders have only rarely been reported.[39-41] The occurrence of multiple immune-mediated diseases might be coincidental or reflect a common etiopathogenesis, but the latter often is considered to be likely owing to an underlying predisposition (genetic, environmental) or an immune trigger (infective, neoplastic, drug, toxin). Thus, the presence of 1 endocrine automimmune disorder should alert clinicians to the possibility of the patient developing concurrent immune-mediated diseases.

Our study had some limitations. First, the small sample size might have decreased statistical power, leading to type II errors. Second, the dogs included in the study underwent different therapeutic protocols in terms of dosage and duration of treatment. Third, the majority of dogs received IAGCs for immune-mediated diseases, which are associated with higher doses of GCs as compared to the doses usually received by dogs with suspected HA. This factor may have influenced our results, but it might reflect the real conditions of the clinical setting.

In conclusion, the optimal time to test for HPA axis recovery after prolonged GC use remains controversial because the variability of data regarding the recovery timelines. Clinicians should be aware that, after IAGC treatment for a prolonged period, the earliest that HPA axis recovery may be seen is approximately 2 to 6 days after GC discontinuation. However, some dogs can require >8 weeks. This extended time period could cause false positive results on the ACTHst, resulting in a misdiagnosis of HA.

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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

Approved by the Scientific Ethics Committee of the University of Bologna, Italy (protocol number 42842).

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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