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Evaluating the Impact of High-Dose Hydrocortisone on Serum CRP Concentrations in Healthy Dogs

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ABSTRACT

Serum C-reactive protein (CRP) is widely used to assess inflammatory disease progression and treatment response in dogs. High-dose glucocorticoids are a common therapy for such conditions, but their independent effect on CRP is not well understood. This study measured CRP using two immunoassays—sandwich ELISA and particle-enhanced turbidimetric immunoassay—in 12 healthy beagle dogs treated with either oral hydrocortisone (8 mg/kg every 12 hours) or a placebo over 28 days, with samples collected on days 0, 1, 5, and 28. Minor fluctuations in CRP occurred in both groups, and no significant effect of hydrocortisone was observed ($p = 0.761$). On day 28, CRP decreased by more than 2.7-fold in three dogs receiving hydrocortisone and two placebo dogs, while one placebo dog showed a similar increase. These results suggest that high-dose hydrocortisone does not confound CRP measurement in healthy dogs, supporting CRP as a reliable biomarker for monitoring inflammation. Future studies should explore potential cellular effects of corticosteroids, including hepatic protein and transcriptome changes.

Keywords: Canine, CRP, Biomarker, Corticosteroid, Inflammation, Immunosuppression, Treatment monitoring

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Introduction

C-reactive protein (CRP) is a liver-produced acute-phase protein that belongs to the pentraxin family and rises in the blood during inflammatory states, infections, and certain cancers [1]. Its synthesis is triggered by proinflammatory cytokines, particularly interleukin-6 (IL-6) and interleukin-1β (IL-1β), under the regulatory control of nuclear factor kappa-B (NF-κB) [2]. In canine medicine, CRP serves as an important biomarker for tracking disease progression and evaluating treatment response in autoinflammatory disorders [3,4]. It may also help differentiate dogs with chronic inflammatory enteropathy (CIE) who need anti-inflammatory or immunosuppressive therapy from those responding to dietary or antibiotic interventions [5].

Glucocorticoids are commonly administered at anti-inflammatory or immunosuppressive doses in dogs with conditions such as idiopathic inflammatory bowel disease [4], primary immune-mediated hemolytic anemia [6], steroid-responsive meningitis-arteritis [7], and primary immune-mediated polyarthritis [3]. In these cases, serum CRP is frequently used to monitor patient status during therapy.

In human studies, corticosteroid therapy has been shown to lower CRP levels independently of improvements in the underlying disease [8]. Differentiating between a true therapeutic response and a steroid-induced reduction in

CRP is challenging, especially in clinical patients. The potential impact of high-dose glucocorticoids alone on serum CRP levels in dogs remains largely unexplored.

Establishing whether corticosteroids can independently modify CRP concentrations is essential for confirming the reliability of repeated CRP measurements as a monitoring tool in veterinary practice [1,9,10]. Accordingly, this study aimed to investigate the effects of a four-week high-dose corticosteroid regimen on serum CRP in healthy dogs.

Several analytical approaches exist to measure canine CRP, including species-specific enzyme-linked immunosorbent assays (ELISAs), immunoturbidimetric methods, and point-of-care lateral flow tests [8,11–13]. A secondary goal of this research was to compare results obtained from two species-specific canine CRP assays to evaluate consistency between methods.

Materials and Methods

Serum samples were obtained from a prospective, randomized, placebo-controlled experimental study involving 12 clinically healthy 3-year-old Beagle dogs in which hypercortisolism was experimentally induced. Clinical findings and additional biomarker results for these animals have been published previously [14-16], confirming successful and consistent induction of hypercortisolism in the hydrocortisone-treated group [14,16]. The study protocol, including the serum sampling procedure, was approved by the Cantonal Committee for Animal Experimentation of the Canton of Zurich, Switzerland (permit no. 150/2004; approval date: 6 August 2004).

Blood samples were collected from all dogs prior to treatment initiation (baseline, day 0) and on days 1, 5, and 28 of treatment. Dogs were randomly allocated to receive either hydrocortisone (8 mg/kg PO q12h; Hotz Pharmacy, Kusnacht, Switzerland; n = 6) or placebo (empty gelatin capsule; n = 6) as previously described [14]. Serum was separated and stored at −80 °C. Samples remained frozen for 96 months until initial analysis of C-reactive protein (CRP) using the TriDelta Phase™ canine CRP sandwich ELISA (Tri-Delta Diagnostics, Boonton Township, NJ, USA; reference interval 0.1–7.6 mg/L [13]). The same aliquots were then kept frozen for an additional 96 months before re-analysis with the Gentian Canine CRP particle-enhanced turbidimetric immunoassay (Gentian Diagnostics, Moss, Norway; reference interval <10 mg/L). This extended storage period was deemed acceptable based on documented long-term stability (≥11 years) of CRP in human serum stored at −80 °C [17].

All serum CRP measurements with the TriDelta assay were performed in duplicate across two separate 96-well plate runs, whereas the Gentian assay was performed in a single run on a fully automated clinical chemistry analyzer. Identical reagent lots, calibrators, and quality-control materials were used throughout to minimize inter-assay variation.

Data distribution and homogeneity of variances were assessed using the Shapiro–Wilk and Brown–Forsythe tests, respectively. Agreement and correlation between the TriDelta and Gentian assays were evaluated by Spearman's rank correlation coefficient (ρ), Bland–Altman analysis (performed on common log-transformed data), and Cohen's kappa statistic (using the respective upper reference limits as diagnostic cut-offs).

For longitudinal comparison of CRP concentrations obtained with the TriDelta assay, data were Box–Box-Cox transformed ($\lambda = -0.93$) and analyzed by multivariate analysis of variance (MANOVA) for repeated measures, followed by pairwise t-tests to identify differences between treatment groups at individual time points. Statistical analyses were conducted using JMP® version 13.0 (SAS Institute, Cary, NC, USA) and GraphPad Prism versions 9.0 and 10.0 (GraphPad Software, San Diego, CA, USA). A p-value < 0.05 was considered statistically significant.

Results

Using the TriDelta assay (LOD = 0.1 mg/L), serum CRP concentrations exceeded the assay's lower detection limit in 17 of 48 samples (35%), whereas only 3 samples (6%) were above the detection limit of the Gentian assay (LOD = 9.9 mg/L). CRP values surpassed the reference interval in 3 of 48 samples (6%)—one sample in each group at baseline and one sample from a dog on day 1 of treatment—with levels exceeding 7.6 mg/L for TriDelta and ≥10 mg/L for Gentian.

Comparing the two assays across all 48 serum samples revealed a moderate correlation between their measurements **(Figure 1)**. Bland–Altman analysis of log-transformed CRP concentrations indicated that the TriDelta assay produced slightly lower values than the Gentian assay **(Figure 2)**, with a mean bias of −3.49 (95% CI: −3.97 to −3.01, log scale; equivalent to 0.03 mg/L, 95% CI: 0.02–0.05 mg/L). The limits of agreement ranged

from −6.71 (95% CI: −7.53 to −5.89, log scale; 0.01 mg/L, 95% CI: 0–0.01 mg/L) to −0.27 (95% CI: −1.09 to 0.55, log scale; 0.76 mg/L, 95% CI: 0.34–1.74 mg/L).

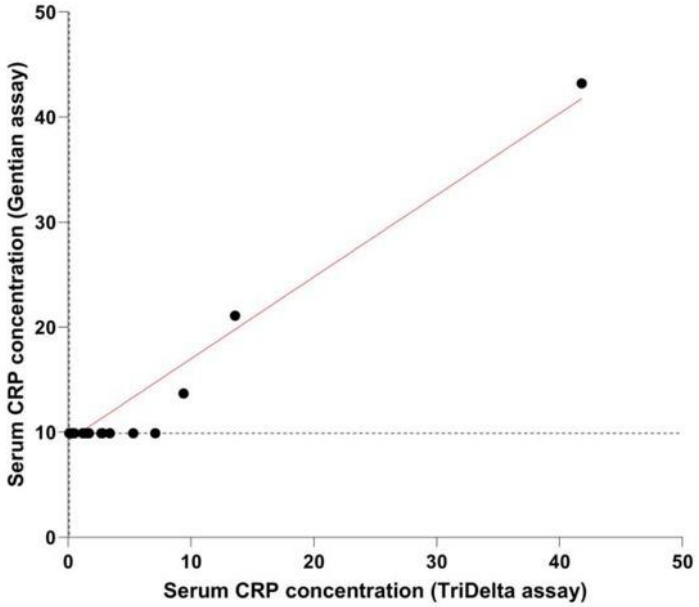

Figure 1. Comparison of serum CRP concentrations in dogs measured by two assay methods

Serum CRP levels in 48 samples from 12 dogs (collected at four time points during 28 days of treatment with either placebo or hydrocortisone at 8 mg/kg every 12 hours) were measured using the TriDelta assay (x-axis; lower detection limit 0.1 mg/L, vertical dashed line) and the Gentian assay (y-axis; lower detection limit 9.9 mg/L, horizontal dashed line). The two assays showed a moderate correlation ($p = 0.49$, 95% CI: 0.23–0.69; $p < 0.001$; red line represents linear regression). Despite this, the paired measurements demonstrated perfect diagnostic agreement, with a kappa coefficient of 1.00 (95% CI: 1.00).

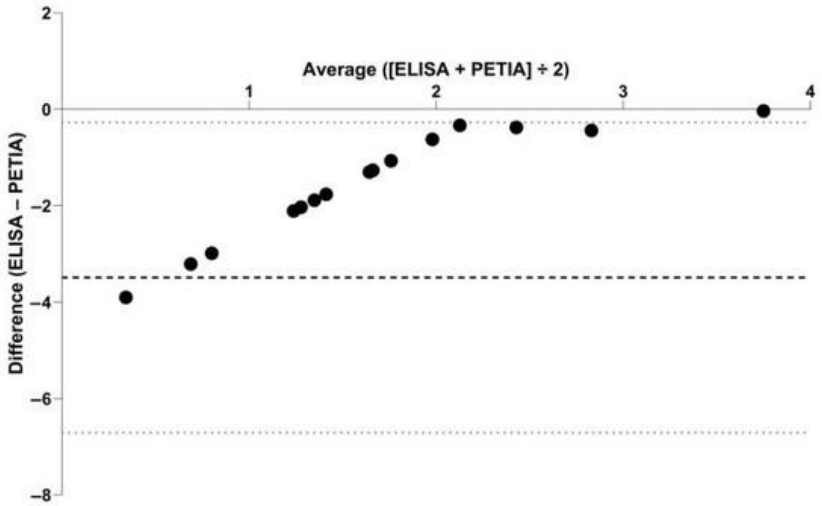

Figure 2. Bland–Altman comparison of serum CRP measured by ELISA and PETIA

This plot visualizes the agreement between serum CRP concentrations in 48 samples analyzed using a sandwich ELISA and a particle-enhanced turbidimetric immunoassay (PETIA). Each point (black dot) represents the difference between the two assays for an individual sample (ELISA minus PETIA) plotted against the average of the two measurements. The calculated mean difference (bias; dashed black line) was −3.49 on a log scale, with limits of agreement (dotted gray lines) of −6.71 and −0.27, corresponding to 0.03 mg/L, 0.01 mg/L, and 0.76 mg/L, respectively.

Throughout the 28-day study period, CRP concentrations showed only minor fluctuations in both the hydrocortisone and placebo groups, and statistical analysis did not reveal a significant effect of hydrocortisone on CRP levels (MANOVA, $p = 0.761$; **Figure 3**). Healthy dogs are known to exhibit substantial individual variability

in serum CRP, and changes exceeding 2.7-fold are considered clinically meaningful (minimum critical difference, MCD) [18]. In the first day of treatment, two hydrocortisone-treated dogs and one placebo dog experienced decreases greater than the MCD, while increases above this threshold were observed in two dogs from each group. Between days 1 and 5, four dogs receiving hydrocortisone and two placebo dogs showed reductions exceeding the MCD. From day 5 to day 28, one hydrocortisone-treated dog and two placebo dogs continued to display decreases beyond the MCD, whereas one dog in the placebo group showed an increase surpassing this threshold.

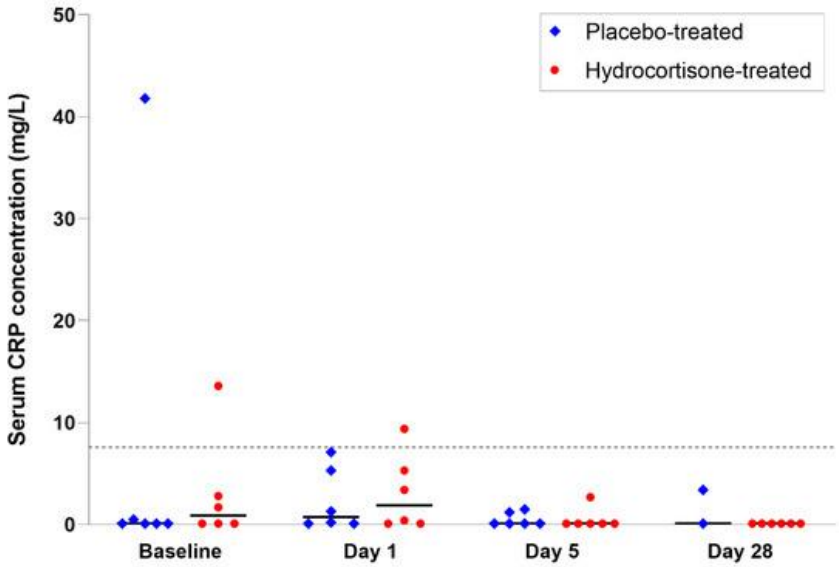

Figure 3. Serum CRP levels in healthy dogs treated with hydrocortisone versus placebo

CRP concentrations were measured in 12 dogs (hydrocortisone, n = 6; placebo, n = 6) over 28 days. Two baseline samples (one from each group) and one sample collected on day 1 from a hydrocortisone-treated dog exceeded the upper reference limit of the TriDelta CRP assay (>7.6 mg/L; gray dashed line), while all other values remained within the reference interval. Across the study period, CRP levels exhibited modest decreases in both groups, with no statistically significant effect of hydrocortisone administration observed ($p = 0.761$). Black horizontal lines indicate median values at each time point for each group.

Discussion

Our findings indicate that high-dose hydrocortisone does not substantially alter serum CRP concentrations in healthy dogs. This suggests that CRP can remain a reliable biomarker for monitoring inflammatory activity and treatment response in dogs receiving high-dose corticosteroids, and potentially in dogs with primary inflammatory diseases treated with other commonly used corticosteroids. Demonstrating that CRP levels are largely unaffected by potent corticosteroid doses—representing the standard first-line immunosuppressive therapy in dogs—supports the continued clinical utility of CRP for disease monitoring. Confirming these observations in dogs with active inflammatory conditions would be valuable to differentiate the direct impact of corticosteroid therapy from changes in CRP driven by treatment-induced disease modulation [19, 20]. Such studies, however, pose ethical challenges because withholding immunosuppressive therapy during the induction phase could jeopardize patient outcomes.

Although the TriDelta and Gentian assays showed only moderate correlation, their results were largely consistent, with the TriDelta assay providing the advantage of measurements within the reference interval. More sensitive canine CRP assays might have detected CRP in all samples, but such assays are not yet widely available. A limitation of this study is that serum samples had been stored at −80 °C for up to 16 years; nevertheless, CRP is known to remain highly stable under these conditions [17]. Ideally, repeated TriDelta measurements would have been performed concurrently with the Gentian assay to enhance comparability, but this was not feasible due to assay availability. Observed CRP levels were consistent with previously reported values in healthy beagle dogs using other immunoassays [21].

Finally, hydrocortisone was used to induce iatrogenic hypercortisolism in this study rather than prednisone or prednisolone, which are more commonly employed in clinical practice. Nevertheless, similar effects on CRP are expected because the administered hydrocortisone dose corresponded to an immunosuppressive level of prednisolone, and these dogs exhibited confirmed cortisol excess.

Conclusions

The findings of this study indicate that administering high-dose hydrocortisone does not significantly influence serum CRP levels in healthy dogs. This observation may extend to dogs with autoinflammatory disorders and to those receiving other high-dose corticosteroids, supporting the reliability of CRP as a biomarker for monitoring inflammatory processes. Further studies are needed to investigate the effects of corticosteroids on cellular transcriptome and proteome profiles in dogs, both with and without underlying inflammatory conditions.

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Conflict of Interest: Jan S. Suchodolski and Joerg M. Steiner are directors of the Gastrointestinal Laboratory at Texas A&M University School of Veterinary Medicine and Biomedical Sciences, where serum CRP analysis in dogs is offered on a fee-for-service basis. None of the other authors of this paper has any financial or personal relationship with other people or organizations that could inappropriately bias the content of the paper.

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Ethics Statement: None

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