Effects of Oral Prednisone Administration on Serum Cystatin C in Dogs

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Background: Oral administration of glucocorticoids alters serum cystatin C (sCysC) concentration in humans.
Objective: To determine if oral administration of prednisone alters sCysC in dogs without pre-existing renal disease.
Animals: Forty-six dogs were included: 10 dogs diagnosed with steroid responsive meningitis arteritis (SRMA; group A), 20 dogs diagnosed of pituitary-dependent hyperadrenocorticism (PDH; group B), and 16 healthy control dogs (group C).
Methods: Retrospective observational study. SRMA diagnosed dogs were administered prednisone 4 mg/kg/24 h PO 7 days, reducing the dose to 2 mg/kg/24 h 7 days before medication withdrawal. In group A, sampling was performed at days 0, 7, 14, and a final control at day 21. Blood and urine samples were collected in the 3 groups, and in group A, sampling was performed at all time points (days 1, 7, 14, and 21).
Results: In group A, sCysC was significantly higher at day 7 compared to the control group (0.4 ± 0.04 mg/L vs. 0.18 ± 0.03 mg/L mean ± SEM respectively P < 0.01); sCysC values decreased to basal at day 14 when the dose was decreased and after 1 week of withdrawal of prednisone (0.27 ± 0.03 mg/L for group A at day 14 and 0.15 ± 0.02 mg/L at day 21; P > 0.05). Dogs with PDH included in group B did not have significant differences in sCysC (0.22 ± 0.03 mg/L) compared to control (P > 0.05).
Conclusions and Clinical Importance: Oral administration of prednisone unlike altered endogenous glucocorticoid production, increases sCysC in dogs in a dose-dependent fashion.
Key words: Cystatin C; Dog; Glucocorticoid; Meningitis.

Serum urea and creatinine levels are commonly used in veterinary medicine as indirect markers of glomerular filtration rate (GFR) to estimate renal function in dogs. However, these are delayed markers of renal failure as substantial variations in these parameters are only observed when approximately 75% of the functional renal mass is lost1 and can be influenced by nonrenal factors. For example, urea can vary due to a high protein diet and both markers (urea and creatinine) are modified by age, hydration status, and muscle mass.2 Therefore, when using serum urea or creatinine levels to estimate renal function, the results need to be carefully evaluated in light of the previously mentioned factors.

Cystatin C (Cys-C) is a 120 amino acid polypeptide constantly produced by most nucleated cells in the body;3 this molecule exhibits no tubular reabsorption, secretion, or metabolism and is freely filtered through the glomerulus.3 Thus, serum cystatin C (sCysC) levels can be considered as another renal marker with superior reliability compared to creatinine.4 In addition, sCysC shows lower individual variability than creatinine and has been reported not to be influenced by sex, age, or muscle mass in human medicine.5,6 With these physiologic characteristics, sCysC has a great potential to be an excellent surrogate marker of GFR.7,8

Nevertheless, as its clinical application was initiated in human medicine, it has been reported that several conditions unrelated to renal failure such as thyroid dysfunction, chronic liver disease, malignancies, or asthma among others can alter sCysC.9,10,21 However, it has to be noted that the treatment of choice for all these conditions include exogenous glucocorticoid administration and that methylprednisolone or prednisone administration deeply influence sCysC in humans.10,15,22

In veterinary medicine, reference values for sCysC canvary with age and body weight (<15 kg),23 although

**Abbreviations:**
- ALP: alkaline phosphatase
- ALT: alanine aminotransferase
- CL: critical limit
- CSF: cerebrospinal fluid
- CV: coefficient of variation
- GFR: glomerular filtration rate
- LOD: limit of detection
- LOQ: limit of quantification
- PDH: pituitary-dependent hyperadrenocorticism
- sCysC: serum cystatin C
- SRMA: steroid responsive meningitis arteritis
- UP/C: urinary protein:creatinine ratio
- USG: urinary specific gravity
these results are controversial.24,25 Other conditions such as fasting23 or leishmaniasis26,27 influence sCysC in dogs.

Until now, no reports have been published regarding the effect of glucocorticoid supplementation on sCysC in dogs. We therefore hypothesized that, as reported in humans, oral administration of prednisone would increase sCysC in dogs in the absence of a pre-existing renal condition. To achieve this goal, a cohort of 10 dogs affected with steroid responsive meningitis arteritis (SRMA) was selected and the levels of sCysC before and after prednisone administration were evaluated.

Material and Methods

Animals

This study has not been subjected to any animal ethics committee as all the animals enrolled in this study were dogs referred to the Veterinary Hospital of the University of Extremadura. The excess of blood and urine samples were used for this study with the owner consent.

This study includes 46 dogs seen at the Veterinary Hospital of the University of Extremadura. The dogs were divided into the following groups: 10 dogs diagnosed with SRMA (group A), 20 dogs diagnosed of pituitary-dependent hyperadrenocorticism (PDH; group B), and 16 healthy control dogs (group C).

Experimental Groups

The animals included in group A were sampled from January of 2015 to February of 2016) had a body weight ≥15 kg, different ages (range: 1–2 years), sex (6 males and 4 females), and breeds. They were selected based on the following inclusion criteria: having SRMA, absence of clinical and laboratorial signs of kidney disease, and proper state of hydration. All the dogs received a similar treatment with prednisone (Prednisona Alongaa ) and none of them had previously been treated with glucocorticoids.2 Corticosteroid therapy consisted of oral administration of prednisone alone at 4 mg/kg/24 h for 7 days, reducing the dose to 2 mg/kg/24 h for further 7 days; after 14 days, prednisone was removed. Blood samples were collected in prednisone-treated animals on days 1, 7, 14, and 21 after the onset of the treatment and were immediately processed; for all the rest of the groups (control and PDH), blood was obtained once in the absence of any treatment.

SRMA was diagnosed on the basis of: (1) characteristic clinical signs (reluctance to move, kyphosis, stiff gait, cervical and/or thoracic pain, muscle rigidity, or apparent pain on opening the mouth), (2) hematology (WBC higher than 14.00 cells ×10⁹/L due to neutrophilia) and normal biochemistry profile, (3) normal urinalysis, and (4) modifications in the cerebrospinal fluid or CSF (increased WBCs >10 cells/µL due to neutrophilia) and normal biochemistry profile, (3) normal urinalysis, and (4) modifications in the cerebrospinal fluid or CSF (increased WBCs >10 cells/µL due to neutrophilia) and normal biochemistry profile.

Clinical Pathology Testing

Blood samples were collected from the cephalic vein after a 12-hour fasting and placed in tubes containing EDTA for the hematology examination or with a clotting activator for the serum biochemistry. Sera were prepared by centrifuging blood samples at 200 × g for 10 min. The hematologic analyses were performed with an automated analyser (Mindray BC-5300; Vet Spinreact), and blood smears were stained with Diff-Quick. The biochemical variables determined included urea, creatinine, ALT, ALP, total protein, albumin, cholesterol, calcium, and phosphorus by a commercial kit (Spinreact, Barcelona, Spain) as previously validated by Almy et al.28 As an indication of assay precision, the intraday coefficient of variation (CV) was calculated from 10 samples assayed on the same day, and the interday CV was calculated from 10 samples assayed on separate days. The accuracy of the assay was investigated by linearity during dilution using the mean of three calibration curves of four standards with known cystatin C concentrations (Cystatin C Calibrator; Spinreact, Barcelona, Spain). The CL (critical limit), LOD (limit of detection), and LOQ (limit of quantification) were calculated as follows:21: CL = standard deviation (SD) × t (0.05;69); LOD = SD × 2 t (0.05;69); LOQ = 10 × SD, where the parameter t represents Student’s t-test. The repeatability and reproducibility of the cystatin C turbidimetric assay had satisfactory variability with a within-day CV = 5.4% and a between-day CV = 7.0%, both less than 10%. Regression analysis showed a linear relationship (R = 0.9997) between the real and theoretical values of the cystatin C concentration. All dogs were tested for the absence of canine heartworm disease, Anaplasma phagocytophylum, Borrelia burgdorferi, Ehrlichia canis antibodies (Canine SNAP 4Dx, IDEXX Laboratories, USA), and leishmaniasis (direct visualization of Leishmania infantum amastigotes in ganglia or bone marrow smears and/or a positive immunosassay commercial kit; kit Q lectin ELISA leishmania; Laboratorios Leti, Spain).

Urine was obtained by ultrasound-guided aseptic cystocentesis. Three microliter of urine was used for routine urinalysis (Multitest Reagent Strips, Bayer Corporation, Madrid, Spain) according to the manufacturer’s instructions using an Ursipin reader.
Statistical Analysis

Data were tested for normality by a Shapiro-Wilk test; results are reported as mean ± standard error of the mean (SEM). Groups were compared using ANOVA on ranks due to their non-Gaussian distribution. When statistically significant differences were found, a Dunn’s posthoc test was used. All statistical analyses were performed by Sigma Plot software version 11.0 for Windows (Systat Software, Chicago, IL, USA). Differences among means were considered as statistically significant when \( P < 0.05 \) or \( P < 0.01 \).

Results

Hematology

The hematologic, biochemical, and urinalysis values of control group (Group C) were within the normal reference range established by the Clinical Pathology Service of the Clinical Veterinary Hospital of the UEx. WBC (Table 1) was significantly enhanced in groups A (over 18.00 \( \times 10^9 \) /L cells) and B (11.78 ± 1.14 cells \( \times 10^9 \) /L) compared to group C (9.47 ± 0.78 cells \( \times 10^9 \) /L; \( P < 0.001 \)). Lymphopenia was observed in some dogs affected with PDH (11 out of 20) and leukocytosis (9 out of 20) due to neutrophilia; neutrophilia and monocytosis were detected in all SRMA cases throughout the study (data not shown). Platelet count (Table 1) differed statistically in Group A (day 14) and Group B compared to control (Table 1; \( P < 0.05 \)).

Serum Biochemistry and Urinalysis

Serum concentrations of total proteins, albumin, calcium, and phosphorus remained within the reference values in all dogs in Group A (Table 2). A significant raise in serum cholesterol (Table 2) was observed after prednisone administration at day 7 (280.04 ± 48.23 mg/dL; mean ± SEM) and 14 (324.43 ± 45.43 mg/dL) compared to the control group (186.19 ± 60.35 mg/dL group C; \( P < 0.01 \)); ALP values were significantly enhanced in all groups (A and B) compared to control (group C; Table 2, \( P < 0.01 \)). The dogs included in Group A (day 14) and B showed an increased ALT (Table 2; \( P < 0.01 \)). In dogs diagnosed with PDH, endogenous corticosteroid production was altered (mean pre-ACTH cortisol of 8.7 ± 1.2 µg/dL; \( n = 20 \)), while in the day 0 of the SRMA-affected group, cortisol values remained in the reference range (2.2 ± 0.6 µg/dL; \( n = 16 \)), as 8 µg/dL is the threshold value of the laboratory below which serum cortisol is considered as normal. The other biochemical determinations were within the normal intervals. No changes were observed in urinalysis in either group of dogs studied. The UP/C was lower than 0.4 in all groups (Table 2) although the higher value was observed in group B (0.35 ± 0.02; \( P < 0.01 \) vs. control), which is commonly found in dogs affected with PDH. 32

| Group | A       | B       | C       |
|-------|---------|---------|---------|
| Day   |         |         |         |
|       | 1       | 7       | 14      | 21      |
| PCV (%)| 45.64 ± 3.11 | 42.07 ± 1.88 | 43.20 ± 1.71 | 43.55 ± 1.55 | 46.72 ± 1.61 | 45.89 ± 1.09 |
| WBC \((\times 10^9/\text{L})\) | 20.21 ± 2.46** | 26.93 ± 2.56** | 24.57 ± 2.88** | 18.36 ± 1.87** | 11.78 ± 1.14** | 9.47 ± 0.79 |
| PLT \((\times 10^9/\text{L})\) | 334.60 ± 54.56 | 351.20 ± 45.44 | 367.00 ± 25.17* | 303.20 ± 27.16 | 434.25 ± 44.35* | 246.80 ± 26.55 |

PCV, white blood cell (WBC) and platelet (PLT) counts in dogs affected with steroid responsive meningitis arteritis (SRMA; group A, \( n = 10 \) at days 1, 7, 14, and 21 after treatment onset), dogs with hyperadrenocorticism (PDH; group B, \( n = 20 \)), and control dogs (group C, \( n = 16 \)). Values are presented as mean ± SEM. Values marked with * differ statistically from the control group; ** \( P < 0.05 \) and \( * * P < 0.01 \).

Discussion

The aim of the present work was to elucidate if exogenous administration of corticosteroids in dogs without renal failure influence sCys-C. These data demonstrate that PO administered prednisone at 4 mg/kg enhances sCys-C and that dogs affected with PDH did not exhibit altered sCys-C values. These results are clinically relevant if sCys-C needs to be evaluated in any setting in which dogs are administered PO with prednisone administration at day 7 (280.04 ± 48.23 mg/dL; mean ± SEM) and 14 (324.43 ± 45.43 mg/dL) compared to the control group (186.19 ± 60.35 mg/dL group C; \( P < 0.01 \)); ALP values were significantly enhanced in all groups (A and B) compared to control (group C; Table 2, \( P < 0.01 \)). The dogs included in Group A (day 14) and B showed an increased ALT (Table 2; \( P < 0.01 \)). In dogs diagnosed with PDH, endogenous corticosteroid production was altered (mean pre-ACTH cortisol of 8.7 ± 1.2 µg/dL; \( n = 20 \)), while in the day 0 of the SRMA-affected group, cortisol values remained in the reference range (2.2 ± 0.6 µg/dL; \( n = 16 \)), as 8 µg/dL is the threshold value of the laboratory below which serum cortisol is considered as normal. The other biochemical determinations were within the normal intervals. No changes were observed in urinalysis in either group of dogs studied. The UP/C was lower than 0.4 in all groups (Table 2) although the higher value was observed in group B (0.35 ± 0.02; \( P < 0.01 \) vs. control), which is commonly found in dogs affected with PDH. 32
Table 2. Biochemical and urinary findings in the three groups of dogs included in the study.

| Group | Day | A | B | C |
|-------|-----|---|---|---|
|       | 1   | 7 | 14| 21|
| Urea (mg/dL) | 22.69 ± 7.67 | 28.26 ± 4.96 | 26.75 ± 8.49 | 24.55 ± 4.68* | 45.66 ± 34.93 | 34.31 ± 8.35 |
| ALT (IU/L) | 31.8 ± 28.8 | 43.7 ± 4.03 | 104.4 ± 27.77** | 59.3 ± 9.85 | 95.95 ± 16.19** | 33.68 ± 2.86 |
| Creatinine (mg/dL) | 0.82 ± 0.07 | 0.69 ± 0.07* | 0.83 ± 0.08 | 0.86 ± 0.14 | 0.97 ± 0.71 | 0.96 ± 0.12 |
| TP (g/dL) | 6.2 ± 0.1 | 6.4 ± 0.2 | 6.2 ± 0.1 | 6.4 ± 0.1 | 6.9 ± 0.1** | 6.4 ± 0.1 |
| Albumin (g/L) | 0.37 ± 0.01 | 0.38 ± 0.02 | 0.38 ± 0.02 | 0.37 ± 0.02 | 0.39 ± 0.01 | 0.36 ± 0.01 |
| Cholesterol (mg/dL) | 210.65 ± 27.08 | 280.04 ± 48.23** | 324.43 ± 45.43 | 237.50 ± 39.48 | 315.53 ± 131.52** | 186.19 ± 60.35 |
| ALP (UI/L) | 227.3 ± 27.97*** | 360 ± 46.14** | 456 ± 61.8** | 306.7 ± 36.6** | 494.35 ± 79.15** | 63.68 ± 5.40 |
| Calcium (mg/dL) | 11.07 ± 0.78* | 11.18 ± 0.66* | 11.09 ± 0.71* | 11.14 ± 0.60* | 11.09 ± 0.68* | 9.49 ± 0.44 |
| Phosphorus (mmol/L) | 3.96 ± 0.19 | 4.00 ± 0.19 | 4.00 ± 0.25 | 3.93 ± 0.22 | 4.31 ± 0.12 | 3.78 ± 0.15 |
| UP/C | 0.24 ± 0.02 | 0.26 ± 0.02 | 0.22 ± 0.02 | 0.25 ± 0.02 | 0.35 ± 0.02** | 0.2 ± 0.02 |

Serum urea, ALT, total protein (TP), albumin, cholesterol, ALP, calcium, phosphorus, and UP/C (urinary protein/creatinine ratio) values in dogs affected with steroid responsive meningitis arteritis (SRMA; group A, at days 1, 7, 14, and 21 after prednisone administration for 7 days at 4 mg/kg/24 hours; this finding is not related to impaired glomerular filtration as serum creatinine, urea and UP/C remain within reference ranges in all groups (Table 2). As previously mentioned, sCysC has been demonstrated to be an earlier indicator of decreased glomerular function compared to creatinine. However, exogenous corticosteroid administration does not seem to induce a raise in sCysC by decreasing the GFR. Instead, other mechanisms have been proposed, for example Bjarnadottir et al., in 1995 demonstrated that in vitro addition of dexamethasone to HeLa cells induced a dose-dependent increase Cys-C secretion in culture after 40 hours. Authors suggested that the increase observed in Cys-C was due to a corticoid-related stimulatory effect on the Cys-C gene promoter, thus increasing the transcription of the Cys-C gene. In methylprednisolone-treated asthma patients, the raise observed in sCysC has also been related to the pathogenesis of the process, as it is actively secreted by macrophages in the alveolus. Although the pathologic causes, dosages, and administration schedule vary between veterinary and human medicine, and with the ones used in the present study, these results show that sCysC significantly increases after exogenous glucocorticoid administration in dogs. These results showed that sCysC significantly increased at day 7 (4 mg/kg/24 hours) and decreased to basal values at day 14 when the dose was reduced to half (2 mg/kg/24 hours and further decreased after treatment withdrawal (Fig. 1). These results parallel those of Risch et al. who demonstrated that in humans subjected to kidney transplantation sCysC was higher in patients treated with corticosteroids and raised with increasing glucocorticoid doses. Similar results were reported by Pöge et al. who described that, in patients subjected to kidney transplantation treated with 500 mg of methylprednisolone, sCysC peaked after 24 hours and that this rise was dose-dependent. Interestingly, these results demonstrate that in dogs diagnosed with PDH in which

Fig 1. Serum cystatin C values in the three groups of dogs included in the study. sCysC values were determined after a 12-hour fasting in dogs affected with steroid responsive meningitis arteritis (SRMA; group A at days 1, 7, 14, and 21 after prednisone administration for 7 days at 4 mg/kg/24 hours; this finding is not related to impaired glomerular filtration as serum creatinine, urea and UP/C remain within reference ranges in all groups (Table 2). As previously mentioned, sCysC has been demonstrated to be an earlier indicator of decreased glomerular function compared to creatinine. However, exogenous corticosteroid administration does not seem to induce a raise in sCysC by decreasing the GFR. Instead, other mechanisms have been proposed, for example Bjarnadottir et al., in 1995 demonstrated that in vitro addition of dexamethasone to HeLa cells induced a dose-dependent increase Cys-C secretion in culture after 40 hours. Authors suggested that the increase observed in Cys-C was due to a corticoid-related stimulatory effect on the Cys-C gene promoter, thus increasing the transcription of the Cys-C gene. In methylprednisolone-treated asthma patients, the raise observed in sCysC has also been related to the pathogenesis of the process, as it is actively secreted by macrophages in the alveolus. Although the pathologic causes, dosages, and administration schedule vary between veterinary and human medicine, and with the ones used in the present study, these results show that sCysC significantly increases after exogenous glucocorticoid administration in dogs. These results showed that sCysC significantly increased at day 7 (4 mg/kg/24 hours) and decreased to basal values at day 14 when the dose was reduced to half (2 mg/kg/24 hours and further decreased after treatment withdrawal (Fig. 1). These results parallel those of Risch et al. who demonstrated that in humans subjected to kidney transplantation sCysC was higher in patients treated with corticosteroids and raised with increasing glucocorticoid doses. Similar results were reported by Pöge et al. who described that, in patients subjected to kidney transplantation treated with 500 mg of methylprednisolone, sCysC peaked after 24 hours and that this rise was dose-dependent. Interestingly, these results demonstrate that in dogs diagnosed with PDH in which
endogenous corticosteroid production is altered (mean pre-ACTH cortisol of 8.7 ± 1.2 μg/dL; n = 20), and sCysC values are not significantly different than those obtained in the control group (P > 0.05; Fig. 1). These results are in agreement with a recent report by Marynissen et al., who demonstrated that in dogs affected with hyperadrenocorticism followed for 12 months, sCysC values were not significantly different compared to healthy dogs. In view of these results and previous publications, it can be concluded that exogenous administration of corticosteroids increases sCysC in a dose-dependent fashion, but impaired endogenous production of corticosteroids does not alter sCysC. Hence, these results suggest that in dogs affected with PDH, the endogenous corticosteroid production is not sufficiently high to induce a raise in sCysC and that there is a threshold below which glucocorticoids do not alter this parameter (Fig. 1). In view of these results, the high immunosuppressive corticosteroid doses used in dogs (4 mg/kg) induce significant sCysC rise, while low immunosuppressive doses (2 mg/kg) do not. However, it remains to be studied if corticosteroid influence over sCysC is transitory in dogs, as previously observed in human patients affected with lupus nephritis chronically treated with corticoids.

In conclusion, oral prednisone administration increases sCysC in the canine species, and this rise seems to be dose-dependent; however, altered sCysC was not observed in dogs showing impaired endogenous corticosteroid production due to PDH. Hence, these results need to be considered when interpreting sCysC values in dogs receiving corticosteroid therapy.

Footnotes

a Prednisona Alonga; sanofi-aventis, S.A., Barcelona, Spain
b Nuvacthen Depot; Sigma-Tau Laboratory, Madrid, Spain

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Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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