

The correlation between serum calprotectin and different inflammatory response biomarkers in healthy and diseased dogs – a short communication

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ABSTRACT

Serum calprotectin, a blood biomarker of acute phase response, has not been widely investigated in veterinary medicine. However, it has potential clinical utility in several inflammatory conditions. This study aimed to compare canine serum calprotectin concentrations (c-CP) with white blood cells, canine serum C-reactive protein (c-CRP) and albumin, as inflammatory parameters in healthy dogs (n=5) and dogs with different pathologies (musculoskeletal n=5; tumours n=5; acute abdomen n=7). Blood samples were analysed within 4 hours of collection. Serum c-CP concentrations were measured using a commercial ELISA kit. There was a moderate positive correlation between c-CP and c-CRP ($r=0.7$; $P=0.025$), a strong negative correlation between c-CP and albumin ($r=-0.79$; $P=0.001$) and a moderate negative correlation between c-CRP and albumin ($r=-0.66$; $P=0.03$). There was no significant correlation between c-CP and white blood cell parameters. Since c-CP was positively correlated with positive acute-phase proteins, and negatively with negative acute-phase proteins, serum calprotectin could be used as a promising positive acute-phase biomarker in dogs.

Key words: dog; acute-phase protein; calprotectin; C-reactive protein; albumins

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Introduction

Acute-phase proteins were first identified in the early twentieth century as a result of response to inflammation. They are considered to be an integral part of the early inflammatory response that occurs in response to infection, trauma, stress or neoplastic changes ([JAIN et al., 2011](#)). So far, about 40 acute-phase proteins have been identified which, in relation to the changes in their concentration in response to stimuli, are divided into positive and negative acute-phase proteins. On the basis of the changes in their values it is possible to confirm the presence of inflammation, stress and neoplasia ([SCHRÖDL et al., 2016](#)). To optimise patients' outcomes, frequent monitoring of acute-phase proteins is required ([CRAY et al., 2012](#)). Originally, calprotectin (CP) was thought to be an antimicrobial protein in the cytoplasm of neutrophilic granulocytes, and was thus recognized as a promising marker of inflammation and infection. Calprotectin is involved in the mobilisation of inflammatory cells in the body's response by interacting with endothelial cells. Its pleiotropic role is associated with an active immune process, including defence against microorganisms. As a molecule, calprotectin has an endogenous damage-associated pattern which plays an important role in innate immune responses ([EHRCHEN et al., 2009](#)). Serum calprotectin concentrations are increased in dogs with inflammatory diseases such as inflammatory bowel disease, systemic inflammatory response syndrome and sepsis ([HEILMANN et al., 2012](#); [THAMES et al., 2019](#)), which makes calprotectin a potentially useful biomarker of inflammation in dogs. Considerable day-to-day variation of faecal calprotectin excretion exists in healthy dogs ([HEILMANN et al., 2008](#)) and dogs with chronic gastrointestinal disease ([HEILMANN et al., 2018](#)), which suggests that changes in serum concentration could also occur. In human and veterinary medicine calprotectin is most widely used in diagnosing inflammatory bowel disease (IBD) ([KALLA et al., 2016](#); [MORI et al., 2021](#)) and rheumatoid diseases ([OMETTO et al., 2016](#)). [LARSSON et al. \(2019\)](#) proved that calprotectin in human plasma is superior to procalcitonin in predicting 30-day mortality in intensive care unit patients, and that it is superior in distinguishing non-septic from septic patients. In neonatal human medicine,

calprotectin is also considered a promising, sensitive and specific biomarker of sepsis ([DECEMBRINO et al., 2015](#)). Similarly, in veterinary medicine, serum calprotectin has been described as a prognostic factor in dogs with sepsis and systemic inflammatory response syndrome (SIRS) ([THAMES et al., 2019](#)).

In dogs, canine c-reactive protein (c-CRP) is an important protein in the acute phase of inflammation in serum, and its concentration rapidly increases as a result of several conditions, including infectious diseases, immune-mediated diseases and neoplasms ([CERON et al. 2005](#)). In human patients, CRP is considered to be the most sensitive blood indicator in the detection of inflammation that can be currently applied in clinical practice ([KOENIG et al., 1999](#); [WU et al. 2024](#)).

Albumin is an important negative protein in the acute phase of inflammation in all species of animals ([TÓTHOVÁ et al., 2014](#)). Hypoalbuminemia can occur as a response to infection or inflammation due to protein loss in cases diseases of the digestive system and kidneys, reduced synthesis as a result of liver disease or malnutrition, and increased permeability of blood vessels, which leads to extravascular accumulation of albumin ([CRAY, 2012](#); [JITPEAN et al., 2014](#)).

This study aimed to determine calprotectin serum concentrations, and to compare these values with the biomarkers of early inflammation already described, in client-owned dogs. It was hypothesised that calprotectin concentrations would correlate with c-CRP and albumin. An additional aim of the study was to compare serum calprotectin values between healthy dogs and dogs with different pathologies.

Materials and methods

Animals. The dogs enrolled in this study were admitted to the University Veterinary Hospital of the Faculty of Veterinary Medicine in Zagreb, Croatia, due to reasons unrelated to this study. The dogs were randomly chosen from animals admitted to the hospital on the same day. The first 22 dogs whose blood was sampled because of their medical indications (trauma, a surgical procedure on the day of admittance, or blood analysis on a check-up) were considered acceptable candidates. To be eligible for the

study, the dogs had to have data available from the clinical examination and a working diagnosis. Considering the healthy group of dogs, the animals had to have been admitted due to elective procedures, and have an unremarkable anamnestic history and clinical examination. The exclusion criteria were a lack of available data and an insufficient amount of blood for testing. The owners provided written consent for participation in the study. The history, complete blood count (CBC), biochemistry blood analysis, and a physical examination were noted for all subjects.

Sampling. The blood was obtained from a cephalic vein using a standardized method in one haematology and one biochemistry tube. The blood in the tubes, which contained clot activators, was left at room temperature until a clot formed, after which serum was separated by centrifugation for 20 minutes at 3500 x g. Serum from each animal was then separated into Eppendorf tubes (Nuova Aptaca, Italy) and stored at 4°C.

Analysis. The analysis of blood samples was performed on the same day the participants were admitted. The serum was analysed within 4 hours after serum separation. Serum c-CP concentrations were determined by sandwich enzyme immunoassay for *in vitro* quantitative measurement of calprotectin in canine serum, plasma and other biological fluids – using a Canine Calprotectin ELISA Kit CALPRO, RK00520 (AbClonal technology, Woburn, Massachusetts, United States). The measurement was done according to the manufacturer's recommendations. Optical density was determined within 5 minutes under 450 nm with a correction wavelength set at 570 nm and 630 nm. All measurements were done with the same batch of reagents. All samples were measured in duplicate and their mean value was taken for statistical analysis. Haematological analyses were performed using a Horiba Scil Vet ABC Plus analyser (Scil, Germany). Differential leukocyte counts were determined from blood smears stained by the Pappenheim method. Briefly, air-dried blood smears were fixed in May-Grunwald solution for 3 minutes, rinsed with distilled water, and stained in Giemsa solution for 20 minutes. After staining they were rinsed with distilled water and air-dried. The stained and dried blood smears were examined un-

der an Olympus BX41 light microscope (Olympus, Japan) with a magnification of x1000. Albumin was analysed with commercially available kits from Abbott using an Architect c4000 automated biochemistry analyser (Abbott, USA). The concentration of c-CRP was determined by an automated spectrophotometric assay using a commercial Gentian kit (Gentian, Denmark) on the Architect c4000 biochemistry analyser (Abbott, USA).

Statistical analysis. Statistical and exploratory data analyses were performed using R v4.0.4 (R: A language and environment for statistical computing, R Core Team (2021). R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>). The Shapiro-Wilk test was used to test normal distribution, and c-CP and band neutrophils were the only variables with non-normal distribution. In order to present the data uniformly, the median, inter-quartile range and overall range (minimal – maximal) were used. Likewise, the strength of the association between all variables was tested using Spearman's correlation test and presented as a correlogram. For differences between healthy and diseased dogs, the t test and Wilcoxon rank sum test were used for normal and non-normal data, respectively. In all tests, values of $P < 0.05$ were considered significant.

The Shapiro-Wilk test was used to test the normality of distribution of all data analysed in serum and whole blood. Calprotectin and neutrophil values were not normally distributed. All data were presented as minimal and maximal values and medians with the interquartile range (IQR). Concentrations of c-CP, c-CRP and albumin measured in serum were compared with each other and with WBC and neutrophil counts and band percentage in whole blood. Results were presented graphically and values of $P < 0.05$ were considered significant.

Results

Whole blood was analysed in only 15 dogs since some samples were excluded from further analysis due to clotting. There were eight different dog breeds included in this study (four west highland terriers, three German shepherds, three French bulldogs, two dachshunds, two border collies, one husky,

one ridgeback, and one Bernese mountain dog) with the most common being mixed breed ($n=5$). Eight dogs were females and 14 dogs were males. Gonadectomy status was not known. The median age of the dogs was 7 years. Of the 22 enrolled dogs, there were 5 healthy dogs and seventeen dogs with different pathological conditions (musculoskeletal $n=5$; tumours $n=5$; acute abdomen $n=7$). Considering musculoskeletal disorders, two dogs were admitted with acute femoral fracture, two with degenerative intervertebral disk disease, and one with chronic stifle arthrosis. There was one dog each with splenic hemangiosarcoma, lipoma and lymphoma, as well as two dogs without a histopathological examination performed (one with a skin mass and the other with a hepatic tumour). The dogs presenting with acute abdominal pain had the following pathological conditions: two gastric volvulus, three enteritis, one urinary obstruction due to urethral calculi, and one with a foreign body in the stomach. The intra-assay coefficient of variation was $<10\%$ and for inter-assay $<12\%$ respectively. Concentrations of c-CP, c-CRP, and serum albumin and WBC, neutrophil and band neutrophil counts are shown in Table 1.

The correlation was calculated between c-CP and all other parameters (c-CRP, albumin, WBC, neutrophils and band neutrophils). A moderate positive correlation between c-CP and c-CRP ($r=0.7$; $P=0.025$), and a strong negative correlation between c-CP and albumin ($r=-0.79$; $P=0.001$) were observed. Also, c-CRP and albumin ($r=-0.66$; $P=0.03$) had a moderate negative correlation. There was no significant correlation between c-CP and white blood cell

parameters (Fig. 1). Spearman's correlation coefficient between values of analysed blood biomarkers in dogs is shown in Table 2.

Considering the differences in the values of the blood parameters studied in healthy dogs and diseased dogs with different pathological conditions, significant differences between groups were noted for WBC ($P<0.001$) and neutrophils ($P<0.01$). These are shown in Fig. 2.

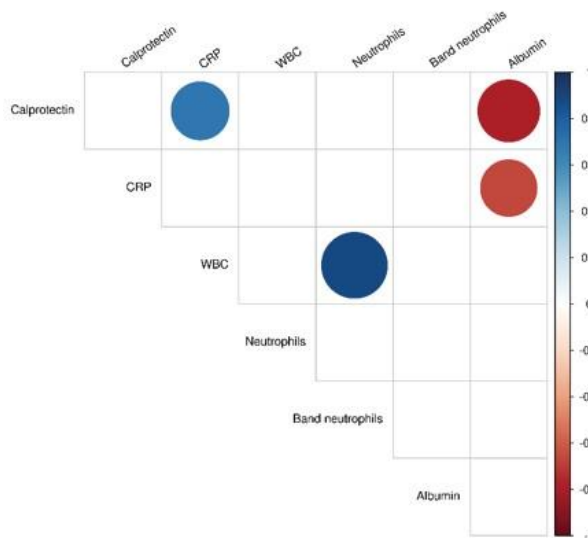


Fig. 1. Correlogram showing the correlation between haematological and serum biochemical parameters in 22 dogs

Spearman's test was used with colour coding of rho (correlation coefficient) for significant correlations observed ($P<0.05$) and empty cells representing correlations that were not significant ($P>0.05$); c CRP (canine c-reactive protein), WBC (white blood cell

Table 1. Summary of results of measured serum and haematological parameters in 22 dogs

	Calprotectin ($\mu\text{g/L}$)	C-reactive protein (mg/L)	Albumin (g/L)	WBC ($\times 10^9/\text{L}$)	Neutrophils ($\times 10^9/\text{L}$)	Band neutrophils (%)
n	22	10	13	16	15	15
Minimal	18.1	1.4	24	4.7	2.88	0
Maximal	366.96	70.9	38	23	18.4	6
Median	75.66	21.95	30	11.55	8.38	0
IQR	117.22	32.48	7	7.35	6.81	1

*n – number of animals, IQR – interquartile range

Table 2. Spearman's correlation test results of analysed blood biomarkers* in dogs (n=22) with correlation coefficient (rho) in the lower and respective P values in the upper triangle

	c-CP	c-CRP	WBC	Neutrophils	Band neutrophils	Albumin
c-CP	-	0.03	0.17	0.17	0.4	0
CRP	0.7	-	0.32	0.14	0.16	0.04
WBC	0.36	0.35	-	<0.001	0.93	0.45
Neutrophils	0.38	0.5	0.9	-	0.37	0.18
Band neutrophils	0.24	0.48	0.02	0.25	-	0.28
Albumin	-0.79	-0.66	-0.23	-0.39	-0.32	-

*c-CP- canine calprotectin, c-CRP- canine c-reactive protein; WBC-white blood cell count

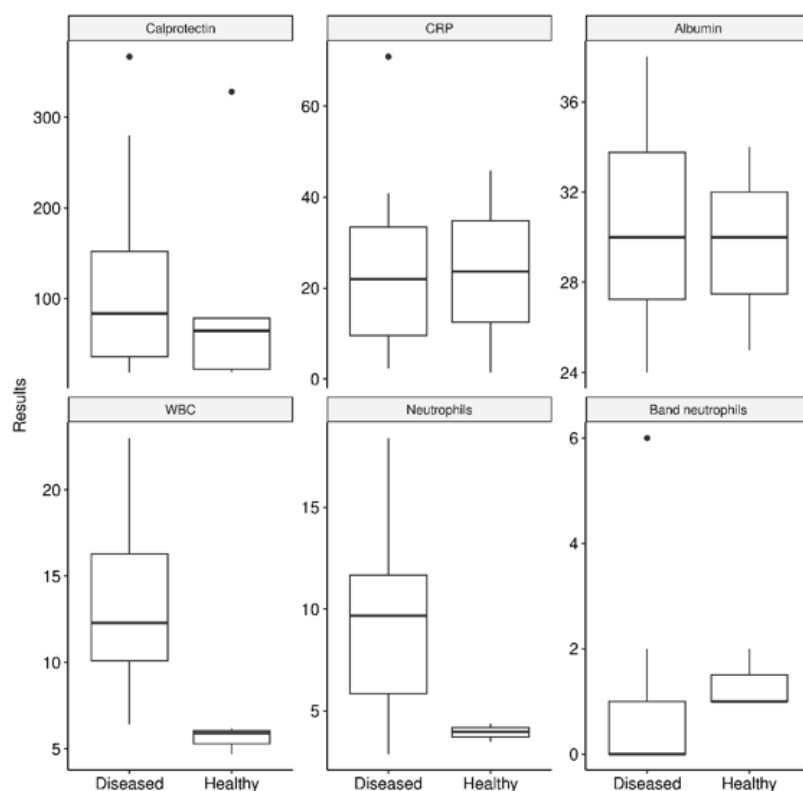


Fig. 2. Boxplot representing values of studied parameters in diseased (n=17) and healthy dogs (n=5)
c-CRP- canine c-reactive protein; WBC-white blood cell count

Discussion

The results of the present study showed a correlation between serum c-CP values with both serum c-CRP (positive correlation) and albumin (negative correlation) in dogs with different pathological conditions. Since serum calprotectin was correlated with other indicators of systemic inflammation (CRP, Alb), it could be considered a potential biomarker in the assessment of different inflammatory conditions. There were no differences in c-CP values between healthy and diseased dogs.

C-reactive protein is an acute-phase protein whose concentration rises during the acute stage of inflammation in humans ([AGRAWAL, 2005](#)). As well as CRP, the concentration of CP also increases during inflammation that usually originates inside the gastro-intestinal system. The correlation of these two parameters, whose concentration increases during inflammation, was expected and is in accordance with research done by [KOPI et al. \(2019\)](#), where a positive correlation was established between serum calprotectin concentrations and serum CRP concentrations in people with irritable bowel disease (IBD). Both CP and CRP were elevated in patients with IBD. In the same study, there was a negative correlation between serum c-CP and serum albumin. In our study, the results were similar, with a positive correlation between serum c-CP and c-CRP. Similarly, [HEILMANN et al. \(2012\)](#) noted a mild positive correlation between serum c-CP and c-CRP in dogs with chronic inflammatory enteropathies, which could be attributed to concurrent usage of non-steroid anti-inflammatory drugs and antibiotics in their studied population.

Albumin is a negative acute-phase protein due to its decreasing concentrations during inflammation. Hypoalbuminemia during inflammations is common, and it is considered that monocytes play an important role in reducing the production of albumin in the liver ([SHEINENZON et al., 2021](#)), which leads to a decrease in their concentration in the serum. Our results showed a negative correlation between c-CP and albumin, and c-CRP and albumin. A correlation between these biomarkers was also evident in the research done by [SHEINENZON et al. \(2021\)](#), where a negative correlation was noted between CRP and albumin in hu-

man patients. [KALLA et al. \(2016\)](#) showed that a correlation between serum c-CP and white blood cells and neutrophils was also evident in people with IBD. A possible reason why the correlation between c-CP and white blood cells was not evident in the present study was the heterogeneity of our population primarily considering the different pathological conditions of the dogs. Calprotectin is an acute-phase protein that is usually released within a couple of hours after exposure to microorganisms ([LIPCSEY et al., 2019](#)) and after the death of neutrophils. Given the patients enrolled in this study, there is a high possibility that some of the dogs were admitted in the acute phase of the disease before adequate white blood cell release, or after therapy had already started, when the number of white blood cells could have decreased. In the study done by [THAMES et al. \(2019\)](#), c-CP was used to assess dogs with sepsis and systemic inflammatory response syndrome (SIRS). Their results showed that calprotectin concentrations in both dogs with sepsis and SIRS were significantly higher than in the control group. Interestingly, in that study, calprotectin only correlated with WBC on day one. In the present research, no correlation was observed between WBC count (leucocyte, neutrophil, band neutrophil) and serum calprotectin concentrations. Similar results were obtained in the research done by [SHI et al. \(2020\)](#) on patients affected with severe pulmonary disease due to COVID-19. In their study, there was no correlation between absolute lymphocyte count and serum calprotectin, while a positive correlation was noticed between serum CRP, absolute neutrophil count, and serum calprotectin.

The lack of difference in c-CP concentrations between healthy and diseased dogs in the present study could be attributed to our heterogenic study population. While the diseased group differed from the healthy dogs in terms of WBC and neutrophils, this is typical for numerous clinical conditions. The lack of upregulation of serum c-CP in the diseased group of dogs could be attributed to the more advanced stage of pathology (particularly for the subgroup of dogs with tumours) since blood calprotectin is said to be a valuable biomarker of early sepsis in humans ([DIEHL-WIESENECKER et al. 2024](#)).

The present study does have certain limitations that need to be emphasized. This research was done on a relatively small number of samples taken from a heterogenic population (there were variations in age, sex and health status between patients). The number of samples was not based on a power analysis but on our financial possibilities. It is unknown whether gonadectomy status can influence calprotectin values or not. Another limitation is that c-CP values were not compared with other inflammation biomarkers (such as interleukin 6 and haptoglobin) but only with WBC, c-CRP, and serum albumin, due to our financial limitations. The dogs' histories and their pathological conditions at the time of research could have influenced our results. The lack of available data regarding the duration of illness and previous treatments, as well as the heterogenicity of the pathological conditions of the diseased animals made it impossible to conclude the role of c-CP as an indicator of a disease stage and/or pathology type. The role of blood calprotectin as a protein during systemic inflammation in dogs has not yet been thoroughly studied. In future research, specific conditions should be assessed considering the correlation between serum calprotectin and other acute-phase proteins and inflammatory cells.

Conclusions

We report a significant correlation between serum c-CP/c-CRP, c-CP/albumin and c-CRP/albumin. The correlation between c-CP/c-CRP was positive, while the correlation between c-CRP/Alb and c-CP/Alb was considered negative. This confirms that calprotectin is a positive systemic acute-phase protein, and that its concentrations change in the same direction as c-CRP during inflammation, but opposite to albumin concentration.

Ethics approval

All procedures performed in this study involving animals were approved under the ethical standards of the Animal Ethics Committee of the Faculty of Veterinary Medicine, University of Zagreb, approval No: 640-01/20-02/09; 251-61-01/139-20-27.

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Declaration of competing interest

The authors declare no conflict of interest

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SAŽETAK

Serumski kalprotektin, krvni biomarker akutne faze upalnog odgovora, malo je istraživao u veterinarskoj medicini. Uvažavajući potencijal kalprotektina za kliničku primjenu pri različitim upalnim stanjima, cilj rada bio je usporediti koncentracije psećeg kalprotektina (c-CP) s bijelim krvnim stanicama, psećim serumskim C-reaktivnim proteinom (c-CRP) i albuminima u zdravih pasa (n=5) i u pasa s različitim promjenama zdravstvenog stanja (patološka stanja lokomotornog sustava n=5; tumori n=5; akutni abdomen n=7). Pokazatelji upale mjereni su u serumu (c-CP, c-CRP i albumini) i u punoj krvi (bijele krvne stanice). Uzorci krvi analizirani su unutar četiri sata od njihova prikupljanja. Koncentracije c-CP-a u serumu mjerene su ELISA-om, a koncentracija C-reaktivnog proteina (c-CRP) određena je automatiziranim spektrofotometrijskim testom. Utvrđena je umjerena pozitivna korelacija između c-CP-a i c-CRP-a ($r=0,7$; $P=0,025$), jaka negativna korelacija između c-CP-a i albumina ($r=-0,79$; $P=0,001$) i umjerena negativna korelacija c-CRP-a i serumskih albumina ($r=-0,66$; $P=0,03$). Nije bilo znakovite korelacije između koncentracije c-CP-a i bijelih krvnih stanica. Budući da su vrijednosti c-CP-a u pozitivnoj korelaciji s vrijednostima c-CRP-a, a u negativnoj korelaciji s koncentracijama albumina, kalprotektin bi mogao poslužiti kao pokazatelj akutne faze upale u pasa.

Ključne riječi: pas; proteini akutne faze upale; kalprotektin; C-reaktivni protein; albumini
