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Inflammatory cytokine and C-reactive protein concentrations in dogs with systemic inflammatory response syndrome

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Abstract

Objective – To evaluate C-reactive protein (CRP), interleukin 6 (IL-6), and tumor necrosis factor alpha (TNF-α) kinetics in dogs with a systemic inflammatory response syndrome (SIRS) presented to an emergency service. We hypothesized serum CRP concentrations would increase and vary during hospitalization, and would correlate with plasma IL-6 and TNF-α concentrations, vary in magnitude according to the underlying disease, and predict survival.
Design – Prospective, observational, clinical study.
Setting – University emergency department.
Animals – Sixty-nine dogs with SIRS weighing over 5 kg who could tolerate the blood sampling.
Interventions – Serum and plasma were collected (and stored at –80°C) at presentation (T0), after 6 (T6), 12 (T12), 24 (T24), and 72 (T72) hours, and at a follow-up visit at least 1 month after discharge (T1m). Underlying diseases were categorized as infection (I), neoplasia (N), trauma (T), gastric-dilation and volvulus (GDV), other gastrointestinal (GI), renal (R), and miscellaneous (M) disease.
Measurements and Main Results – Serum CRP concentration was measured using a canine-specific immunoturbidimetric assay. Biologically active plasma IL-6 and TNF-α concentrations were assessed using bioassays. Forty-four dogs survived, 8 died, and 17 were euthanized. Nineteen dogs had follow-up visits. At T0, serum CRP concentration was above the reference interval in 73.1% (49/67), and was within the reference interval (0–141.9 nmol/L) throughout hospitalization in only 6% (4/67). Serum CRP concentrations were significantly higher ($P < 0.0001$) at T0 (882.9 ± 1082.9 nmol/L) and at all time points during hospitalization ($P < 0.0001$) compared to T1m, with highest concentrations observed at T24 (906. 7 ± 859.0 nmol/L). At T1m, serum CRP concentrations were within the reference interval (22.9 ± 42.9 nmol/L) in 95% (18/19) of dogs. Logarithmic concentrations of serum CRP and plasma IL-6 were significantly correlated ($P < 0.001$, $r = 0.479$). None of the measured cytokines were associated with disease category or outcome.
Conclusions – Serum CRP concentration is increased in dogs with SIRS, and decreases during treatment and hospitalization. Serum CRP, plasma IL-6, and plasma TNF-α concentrations cannot predict outcome in dogs with SIRS.

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Keywords: acute phase proteins, canine, CRP, cytokines, SIRS

Abbreviations

APP	acute phase proteins
CRP	C-reactive protein
GDV	gastric-dilation and volvulus
IL	interleukin
MTT	dimethylthiazol-diphenyl tetrazolium bromide
SIRS	systemic inflammatory response syndrome
TNF-α	tissue necrosis factor-alpha

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The authors declare no conflict of interest.

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Introduction

The systemic inflammatory response syndrome (SIRS) describes the systemic repercussions resulting from a generalized state of inflammation, which may occur secondary to infectious or noninfectious inflammatory disease. A diverse range of conditions including infection, trauma, and sterile inflammation (eg, pancreatitis) may provoke SIRS in dogs.[1] The clinical diagnosis of SIRS is based on defined changes in clinical (body temperature, heart rate, and respiratory rate) and hematologic (leukocyte counts, presence of a left shift) parameters (Table 1). The current criteria for the clinical diagnosis of SIRS describe the presence of clinical signs compatible with systemic inflammation, but are unfortunately considered overly sensitive and poorly specific (in essence many patients with a clinical diagnosis may in reality not suffer from SIRS, while some SIRS patients may remain undetected based on the current criteria for the clinical diagnosis).[2,3]

SIRS is largely mediated by proinflammatory cytokines, and a SIRS-like clinical picture can be induced by injection of cytokines such as tissue necrosis factor α (TNF-α).[4] Infusion of lipopolysaccharide to dogs causes a rapid increase in TNF-α concentrations that peaks within 3 hours of the start of administration.[5] Serum concentrations of TNF-α have been correlated with morbidity and mortality in humans with meningococcal meningitis and septicemia.[6]

Interleukin (IL)-6 is another major proinflammatory cytokine, which is produced by monocytes, macrophages, and fibroblasts in response to TNF-α, pathogen-, and damage-associated molecular pattern molecules.[7,8] Blood IL-6 concentrations display a greater and more sustained increase compared to TNF-α in inflammatory conditions, making it a more useful parameter for the diagnosis and monitoring of human patients that have underlying disease resulting in SIRS.[9] Indeed, some studies have identified IL-6 as a good diagnostic and prognostic marker in people with SIRS,[10,11] and this has also been confirmed in dogs with SIRS.[12]

Systemic inflammation due to any immune-mediated, neoplastic, infectious, or traumatic challenge can trigger an acute phase response.[13] Acute phase proteins (APPs) are predominantly glycoproteins synthesized by hepatocytes in response to IL-6, TNF-α, and other proinflammatory cytokines.[13–16] Measurement of serum APP concentrations can be used to assess the systemic acute phase response.[13] APPs can have both pro- and anti-inflammatory effects, and can act to regulate the immune response, control inflammation, or stimulate protection and repair of tissues.[13]

Table 1: Clinical criteria used for the diagnosis of systemic inflammatory response syndrome in this study

Parameter	Cut-off points
Heart rate	>120/min
Respiratory rate	<20/min
Body temperature (°C)	<38 or >39
White cell count (cells/μL)	>1600 or <5000
Bands	>3%

C-reactive protein (CRP) is a major APP in dogs. The main functions of CRP are thought to be promotion of complement binding to facilitate phagocytosis of bacteria, induction of cytokine release from monocytes, inhibitory effects on chemotaxis, and modulation of neutrophil function.[15,17] Serum CRP concentration is usually less than 47.6 nmol/L in healthy dogs and reference ranges vary from 2.1 to 156.2 nmol/L.[18–21] CRP displays a rapid increase in serum concentration from <9.5 nmol/L to >952 nmol/L in response to tissue destruction or inflammatory stimulation[22] secondary to infectious,[13,23] neoplastic,[24] immune mediated,[23–25] and other conditions.[26] CRP has a short half-life in the dog[26] and serum CRP concentrations are not affected by glucocorticoid administration.[21] The administration of nonsteroidal anti-inflammatory drugs (NSAIDs) does not alter CRP concentrations.[27,28] Moreover, CRP concentrations do not have a circadian rhythm in dogs and are not affected by sex, age, or repeated venous blood sampling.[18,20,29,30]

Taking all of these facts into consideration, CRP could serve as a useful clinical marker for the presence and resolution of systemic inflammation in dogs.[31] CRP may be used to evaluate the severity of ongoing inflammation and to monitor disease progression and the response to treatment. These characteristics have been confirmed in human studies,[32,33] and in some veterinary studies evaluating dogs with pancreatitis, *Ehrlichiosis*, *Leishmaniasis*, and steroid-responsive meningitis-arteritis.[23,34–38] The kinetics and prognostic value of serum CRP in a cohort of dogs presenting to a veterinary emergency room with clinical signs of SIRS has not been evaluated.

We measured serum CRP, IL-6, and TNF-α concentration kinetics in dogs presenting to the emergency room with a clinical diagnosis of SIRS. We hypothesized that serum CRP concentrations would increase in dogs with clinical SIRS and would vary throughout hospitalization. In addition, we hypothesized that serum CRP concentrations would correlate with IL-6 and TNF-α concentrations, would be influenced by the underlying etiology, and would serve as a reliable prognostic marker for patient survival.

Materials and Methods

Dog selection

All dogs presented to the emergency room at Liège University between January and August, 2010, were eligible for inclusion in the study. A diagnosis of SIRS was based on clinical criteria and total and differential white blood cell counts. A dog with at least 2 of the diagnostic criteria (Table 1) was considered to have SIRS[2] and was included in the study once informed consent was obtained from the owner. Exclusion criteria included a body weight <5 kg and if the attending clinician considered the dog too unstable to take part. The study protocol received approval (#1709) from the institutional ethical committee.

Dogs underwent further diagnostic testing and received treatment according to their underlying conditions at the discretion of the attending veterinarian. Final diagnoses were classified into 7 disease categories for statistical comparison. These disease categories were infection (I), neoplasia (N), trauma (T), gastric-dilation and volvulus (GDV), other gastrointestinal (GI), renal (R), and miscellaneous (M) diseases.

Data collection

Baseline parameters (T0) were assessed prior to beginning treatment. Blood was sampled again after 6 hours (T6), 12 hours (T12), 24 hours (T24), and then every other day (T72, T120, etc) until the dog was discharged, died, or was euthanized. Owners of discharged dogs were asked to return for a follow-up assessment at least 1 month after discharge from the hospital for collection of an additional blood sample (T1m).

At each time point, 6 mL of blood was collected and divided into EDTA (4 mL) and serum tubes (2 mL). Blood was sampled from the jugular vein unless a coagulopathy was suspected, in which case a cephalic vein was used. Samples were centrifuged within 15 minutes at 1500 x *g* for 10 minutes, and plasma and serum were immediately separated and stored at –80°C until analysis.[39,40] All analyses were performed simultaneously on each sample.

Analyses

Serum CRP concentration was measured according to the manufacturer's instructions, using a previously validated[41] dog-specific immunoturbidimetric CRP assay[a] on a fully automated clinical chemistry analyzer.[b] In summary, the main reagent is a polyclonal chicken anti-canine CRP antibody, which generates increased sample turbidity upon reaction with canine CRP. The turbidity of the sample is then measured spectrophotometrically.[42] Calibration of the assay was performed with canine CRP.[c] Serum samples were visually semiquantitavely (absent, mild, moderate, or severe) assessed for presence of hemolysis, hyperbilirubinemia, and hyperlipemia. Samples with CRP concentrations >2857 nmol/L were diluted 1:5 with 0.9% sodium chloride and reanalyzed. Two canine control samples[d,e] were analyzed each day when analysis was performed. CRP assays were not run in duplicate.

Previously described bioassays were used to measure biologically active plasma TNF-α and IL-6 concentrations.[43] Both assays have been used to assess these plasma cytokines in dogs, in experimental and clinical studies.[44,45] Determination of plasma TNF-α concentration was achieved using a cell-kill bioassay based on the cytotoxic effect of TNF-α on the mouse fibrosarcoma cell line, WEHI 164 subclone 13.[46] This assay has high sensitivity and detects only bioactive TNF-α, in contrast to antibody-based immunoassays, which can detect TNF-α that may not be biologically active.[47,48] The assay was performed in sterile, 96-well microtiter plates. Serial dilutions of biological samples, which were run in duplicate, or different concentrations of a murine TNF-α standard[f] were incubated for 24 hours in wells that had each been seeded with 50,000 actinomycin D-treated mouse fibrosarcoma cells. The number of surviving cells after 24 hours was measured by use of the dimethylthiazol-diphenyl tetrazolium bromide (MTT) colorimetric assay.[49] Plasma samples were prediluted in order to obtain parallel serial dilutions of samples and standard dilution curves. The detection limit of the assay, after considering the dilution of samples, was 6 ng/L of TNF-α.

Interleukin-6 concentrations were determined using a bioassay based on the IL-6-dependent growth stimulation of the B9 hybridoma cell line.[50,51] This cell line requires IL-6 for survival and proliferation. The assay was performed in sterile, 96-well microtiter plates. In each well, 5000 B9 cells were incubated for 72 h with duplicate serial dilutions of biological samples, or with different concentrations of a human IL-6 standard.[g] Plasma samples were prediluted so that serial dilutions of samples and standard dilution curves were parallel. The number of cells in each well was measured by use of the MTT assay. The detection limit of the assay, after considering the dilution of samples, was 3 IU/mL of IL-6.

Statistical analysis

Statistical analysis was performed using a commercially available statistics program.[h] Shapiro–Wilk, Kolmogorov–Smirnov (univariate procedure), and normality QQplots analyses were performed to assess the data for a normal distribution. As the distribution of CRP, IL-6, and TNF-α concentrations was skewed, a logarithmic transformation of these values was performed prior to statistical analysis. A mixed procedure on a

generalized linear model was used to assess the effect of time, age, sex, reproductive status, and disease category on the logarithmic concentrations of CRP, IL-6, and TNF-α simultaneously. A mixed procedure was applied to evaluate whether presence and severity of hemolysis, hyperbilirubinemia, or hyperlipemia had an effect on logarithmic CRP concentrations. As the data were taken repeatedly over time on the same animals, there is a correlation between successive data. This correlation structure is reflected in the linear-mixed model used (MIXED procedure, repeated by time). Correlation between CRP, IL-6, and TNF-α was performed (CORR procedure). A logistic analysis (LOGISTIC procedure) was performed in order to evaluate the effect of CRP, IL-6, and TNF-α concentrations on survival to discharge. Only dogs that survived, died of natural causes, or were euthanized for prognostic reasons were included for the assessment of prognostic value of the evaluated parameters. Statistical significance was reached at a P value <0.05.

Results

Dogs

Fifty-eight pure bred and 11 mixed-breed dogs (69 dogs in total) were included in the study. The most commonly represented breeds were Bernese Mountain Dog ($n = 8$), German Shepherd ($n = 6$), Great Dane ($n = 4$), Jack Russell Terrier ($n = 4$), and Belgian Shepherd ($n = 3$). There were 38 male (29 intact and 9 neutered) and 31 female (17 intact and 14 neutered) dogs with a median age of 6.5 years (ranging between 7 months and 15.2 years) and with a median weight of 30.3 kg (ranging from 5.5 to 75 kg). Dogs were also grouped into disease category ($N = 13$; I = 12; GDV = 11; GI = 5; T = 6; R = 3; and M = 19). Forty-four of 69 dogs (63.8%) were discharged from the hospital, 8/69 (11.6%) died during hospitalization, and 17/69 (24.6%) dogs were euthanized (8/17 due to perceived poor prognosis, 7/17 for financial reasons, and 2 for reasons not specified). Thirty-four of 69 dogs were confirmed to be alive and considered healthy at least 1 month after discharge (5 died [both euthanasia and natural cause of death] from related causes such as persistent diarrhea, aspiration pneumonia secondary to a mega esophagus, worsening hepatocutaneous syndrome, and tumor recurrence with secondary hemoperitoneum, and 5 were lost to follow-up). Nineteen of 34 returned for a follow-up visit. Sex, reproductive status, and age did not have a significant effect on blood CRP, IL-6, or TNF-α concentrations, or prognosis ($P > 0.05$).

CRP analysis

In 19 dogs, serum CRP concentrations were significantly increased ($P < 0.0001$) at all time points during hospitalization compared to values from T1m (Figure 1A). Serum CRP concentrations were above the upper limit (142 nmol/L) in 73.1% (49/67) of dogs at T0 with a mean concentration of 882.9 ± 1082.8 nmol/L. CRP was only assessed in 67/69 patients at T0 as 2 samples contained insufficient amounts of serum for analysis. Serum CRP concentrations at T6, T12, and T24 were significantly higher than at T120 ($P = 0.0135$, $P = 0.0085$, and $P = 0.0356$, respectively), with highest mean serum concentrations observed at T24 (906.7 ± 859 nmol/L). Concentrations at T0, T72, and T120 remained significantly higher than values at T1m ($P < 0.001$ at all time points), but mean values at T72 had decreased to 677.2 ± 729.5 nmol/L, and were not significantly different from values at T120 ($P = 0.2136$). Dogs that survived to discharge also demonstrated significantly higher concentrations at T6, T12, and T24 compared to T120 ($P = 0.0196$, $P = 0.0035$, and $P = 0.0415$, respectively), and a lack of significant differences ($P = 0.3275$) between samples at T72 and T120 (Figure 1B). Due to the small amount of patients in disease categories, we did not perform further statistical analysis regarding the effect of disease category on CRP kinetics. However, we did observe that in 11 dogs with GDV, serum CRP concentration was measured within the reference interval in 6, 2, 1, and 0 dogs at T0, T6, T12, and T24, respectively, while in dogs with trauma ($n = 6$), serum CRP concentration was within the reference interval in 4, 1, and 0 dogs at T0, T6, and T12, respectively. CRP concentrations remained within the reference interval throughout hospitalization in only 4/67 (6.0%) dogs. Diagnoses for these 4 dogs included wound dehiscence following elective ovariohysterectomy, acute paralysis due to suspected fibrocartilaginous embolism, status epilepticus, and GDV. Concentrations of serum CRP were within the reference intervals at T1m in 18/19 dogs, with a mean serum concentration of 22.9 ± 42.9 nmol/L. The only dog with a value above the reference interval (173.3 nmol/L) was one dog with serum CRP concentrations that had been within reference intervals during hospitalization for wound dehiscence after ovariohysterectomy. This dog had no other abnormalities detected and remained clinically normal after the visit. Hemolysis, hyperlipemia, and hyperbilirubinemia was detected in 85, 50, and 18 serum samples respectively, and was considered severe in 22, 3, and 0 of these samples, respectively. Presence and severity of hemoglobinemia, hyperlipemia, and hyperbilirubinemia did not significantly influence CRP concentrations ($P > 0.05$). Although not significantly different, mean serum CRP concentrations at T0 were highest in dogs with SIRS caused by an infectious etiology (1870.5 ± 1790.5 nmol/L) compared to the other disease groups combined (663.8 ± 723.8 nmol/L; $P = 0.12$).

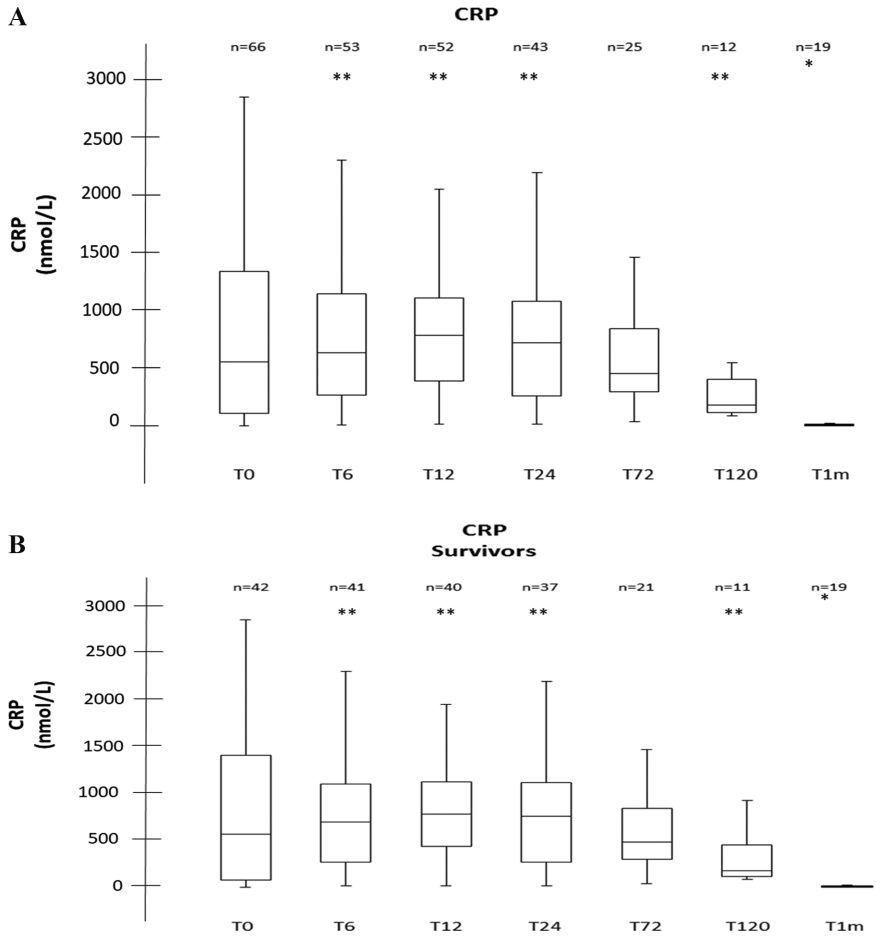

Figure 1: (A) Box plots displaying the mean, median, and distribution of C-reactive protein concentrations (mg/L) in canine systemic inflammatory response syndrome patients at different time points during hospitalization. The central line of the box plot indicates the median value, the upper, and lower line of the box plot illustrate the range of the 25% and 75% of the values, the outer lines at the end of the vertical lines indicate the 95% and 5% range of the recorded values. *Values at T1m were significantly lower than values at all other time points; **Values at T120 were significantly lower than values at T6, T12, and T24. (B) Box plots displaying the mean, median, and distribution of C-reactive protein concentrations (mg/L) in canine systemic inflammatory response syndrome patients that survived until discharge at different time points during hospitalization. The central line of the box plot indicates the median value, the upper, and lower line of the box plot illustrate the range of the 25% and 75% of the values, the outer lines at the end of the vertical lines indicate the 95% and 5% range of the recorded values. *Values at T1m were significantly lower than values at all other time points; **Values at T120 were significantly lower than values at T6, T12, and T24.

IL-6 analysis

There were no significant differences in plasma IL-6 concentrations between different time points during the study, with the exception of a significant decrease in the concentrations measured at T1m compared to T0 (Figure 2, $P < 0.0001$). Mean plasma IL-6 concentrations at T0 were 3758 ± 11,395 IU/mL, decreasing to 508 ± 430 IU/mL at T72 ($P = 0.1686$). At T1m, mean plasma IL-6 concentration had dropped to 274 ± 135 IU/mL.

TNF-α analysis

Logarithmic plasma TNF-α concentrations did not change significantly ($P = 0.167$) throughout hospitalization (Figure 3) with a mean TNF-α concentration at T0 of 64 ± 188 ng/L. Only 20/69 dogs (29.0%) had detectable plasma TNF-α at any time point. TNF-α was detectable at one or more time points during hospitalization in 5/11 (45%) dogs with GDV and 3/6 (50%) dogs presented for trauma. TNF-α was detectable in 2 dogs at T72 (384 and 478 ng/L) and in 1 dog at T120 (422 ng/L). None of the dogs had detectable plasma concentrations of TNF-α at T1m. Twelve of the 20 (60%) dogs that had detectable plasma TNF-α concentrations survived to discharge, 5 were euthanized for prognostic reasons, and 3 died during hospitalization. Plasma TNF-α concentrations were not significantly associated with outcome ($P = 0.2985$).

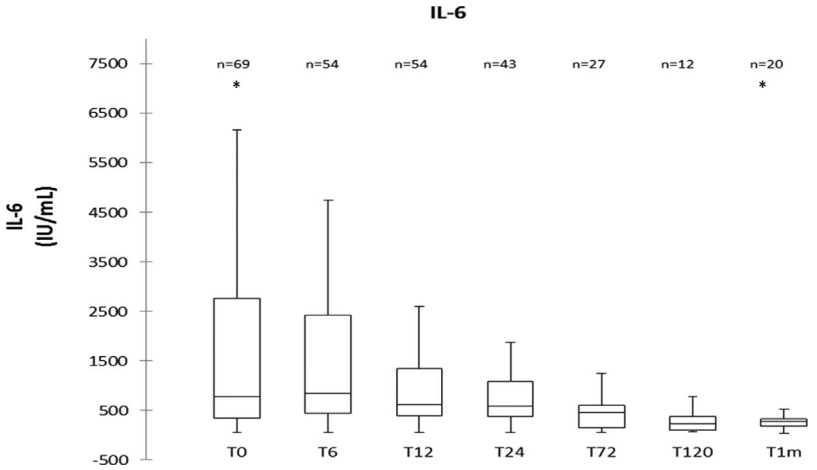

Figure 2: Box plots displaying the mean, median, and distribution of IL-6 concentrations (IU/mL) in canine systemic inflammatory response syndrome patients at different time points during hospitalization. The central line of the box plot indicates the median value, the upper and lower line of the box plot illustrate the range of the 25% and 75% of the values, the outer lines at the end of the vertical lines indicate the 95% and 5% range of the recorded values. *Values at T0 were significantly higher than values at T1m.

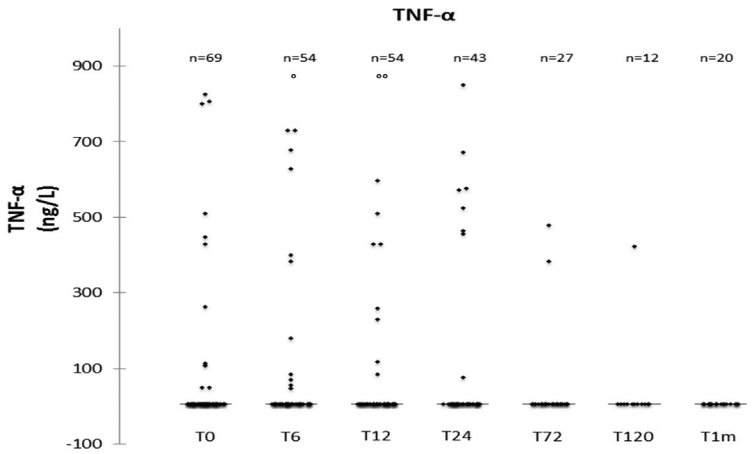

Figure 3: Scatterplot displaying the mean, median, and distribution of TNF-α concentrations (ng/L) in canine systemic inflammatory response syndrome patients at different time points during hospitalization. Dots represent obtained values for TNF-α, outliers are represented by black circles. The horizontal line indicates the median value (which always equals 0 ng/L).

Correlation of biomarkers

Logarithmic concentrations of serum CRP and plasma IL-6 were weakly but significantly correlated ($P < 0.001$, $r = 0.479$). Logarithmic plasma concentrations of TNF-α were not correlated with the logarithmic concentrations of serum CRP ($P = 0.126$, $r = –0.093$) or plasma IL-6 ($P = 0.739$, $r = –0.020$). Statistical analysis failed to identify any significant influence of the underlying disease category on serum or plasma concentrations of CRP, IL-6, and TNF-α ($P = 0.9870$, $P = 0.7498$, and $P = 0.0651$, respectively).

Blood concentrations of CRP, IL-6, and TNF-α in survivors did not significantly differ (P-values of 0.3381, 0.8586, and 0.2985, respectively) from concentrations in nonsurvivors (Figure 4).

Discussion

Clinical signs of SIRS frequently accompany diseases requiring emergency treatment in dogs. The present study demonstrates that the majority of dogs presenting on an emergency basis with SIRS have, or will soon develop, increased serum CRP concentrations. High serum CRP concentration is consistent with an acute phase response, and the majority of this cohort of dogs also displayed additional indicators of an active inflammatory process (ie, increased plasma concentrations of pro-inflammatory cytokines) at T0.

The focus of our study was to evaluate the prognostic value and kinetics of blood CRP and major proinflammatory cytokines in dogs admitted to an emergency

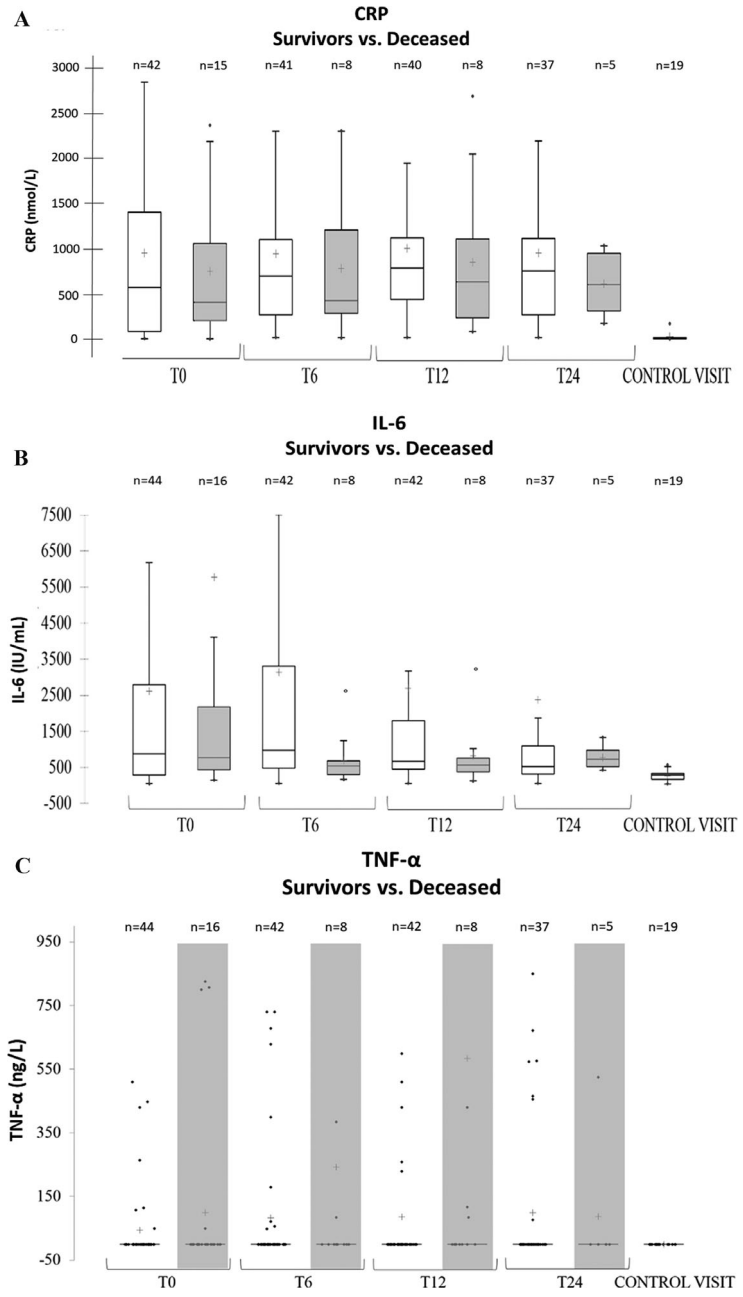

Figure 4: (A) Box plots displaying the mean, median, and distribution of C-reactive protein concentrations (mg/L) in survivors and deceased systemic inflammatory response syndrome (SIRS) patients at different time points during hospitalization. White box plots represent values obtained in survivors, gray boxes reflect values obtained in deceased SIRS patients. The central line of the box plot indicates the median value, the upper and lower line of the box plot illustrate the range of the 25% and 75% of the values, the outer lines at the end of the vertical lines indicate the 95% and 5% range of the recorded values. Crosses represent the mean value while outliers are represented by black circles. (B) Box plots displaying the mean, median, and distribution of IL-6 concentrations (IU/mL) in survivors and deceased SIRS patients at different time points during hospitalization. White box plots represent values obtained in survivors, gray boxes reflect values obtained in deceased SIRS patients. The central line of the box plot indicates the median value, the upper and lower line of the box plot illustrate the range of the 25% and 75% of the values, the outer lines at the end of the vertical lines indicate the 95% and 5% range of the recorded values. Crosses represent the mean value while dots indicate outliers. (C) Scatter plots displaying the mean, median, and distribution of TNF concentrations (ng/L) in survivors and deceased SIRS patients at different time points during hospitalization. Values in the white columns represent values obtained in survivors, values in gray columns reflect values obtained in deceased SIRS patients. Dots represent obtained values for TNF-α. Crosses represent the mean value, the line indicates the median value (which always equals 0 ng/L).

room for treatment. Emergency cases that did not fulfill the SIRS criteria were excluded and we can therefore not comment on the sensitivity of the SIRS criteria to identify dogs with increased CRP, nor on the agreement between a clinical diagnosis of SIRS and serum CRP concentrations. A previous report in dogs with pyometra did demonstrate that serum CRP concentration is associated with the presence of SIRS.[52] Identification of SIRS in a dog presenting as an emergency suggests the presence of systemic inflammation, and may justify measurement of serum CRP to confirm the presence of an acute phase response.[53]

Only 71.2% of dogs in this report had increased serum CRP concentrations at T0. The absence of an increased serum CRP concentration at presentation to an emergency room may, at least in part, be explained by the kinetics of APPs. Typically, major APPs increase before the onset of clinical signs[13]; in experimental studies, serum CRP concentration has been found to increase within 4 to 6 hours of stimulation, reaching a peak concentration after 36 hours.[54] In this study, some dogs were presented for peracute conditions such as GDV and trauma. Although the small sample size prevented meaningful statistics from being performed, serum CRP concentration in dogs with these peracute conditions was often within normal limits at T0 (6/11 GDV and 4/6 trauma patients) but increased above reference range during the initial hours of hospitalization (8/9 GDV and 6/6 trauma patients). It is interesting to note that diagnosis of SIRS may precede changes in APPs in dogs presenting to the emergency room.

Reference intervals for plasma IL-6 concentrations have not been established in dogs, and IL-6 is one of the few cytokines that is detectable in the plasma of healthy dogs.[45] A previous report did not detect significant differences in plasma IL-6 concentrations between healthy control dogs and dogs with pyometra.[52] Experimental models have demonstrated that both plasma IL-6 and TNF-α concentrations can rapidly rise exponentially in dogs given intravenous lipopolysaccharide.[45] In our study on patients with SIRS, a significantly higher logarithmic plasma IL-6 concentration was detected at T0 compared to T1m.

In this study, biologically active serum TNF-α was only detected in 20/69 dogs (29.0%) at at least one time point during their hospitalization. It has previously been established in a group of dogs with pyometra that serum TNF-α concentrations are not correlated with the presence of SIRS.[52] Several factors can explain this low proportion of detectable TNF-α concentrations. In experimental models, serum TNF-α peaks within 2 hours but often becomes unmeasurable 6 hours after the initiation of an inflammatory stimulus, and rarely remains detectable for longer than 24 hours, although sustained increases have been described in patients with sepsis.[45,55] In agreement with these findings, only 2/69 dogs in the present study had measurable plasma TNF-α concentrations for longer than 24 hours. Most of the dogs with detectable plasma TNF-α concentrations at T0 were diagnosed with peracute conditions such as GDV and trauma. Plasma TNF-α concentrations decrease rapidly due to the inhibitory effects of IL-6 on TNF-α production.[56] It is possible that a rise in serum TNF-α concentrations occurred in some of the other dogs in the study prior to T0 and thus was not identified. Furthermore, increases in TNF-α concentration may be relatively mild or even absent in man and dogs with localized inflammation, or following some elective surgeries or trauma.[14,57] It is therefore possible that some of the dogs of this report had disease conditions that failed to provoke an increase in serum TNF-α concentrations despite clinical signs of SIRS.

Other studies in dogs with SIRS and sepsis have identified a higher proportion of dogs with detectable serum TNF-α concentrations.[52,58] These difference may be explained by differences in assay methodologies and variations in the enrolled cohort of dogs. ELISA techniques measure all circulating TNF-α, including the portion that is clinically inactivated by TNF-α soluble receptors, while bioassays only measure the active TNF-α.[56] Besides variations between assays, differences in the studied population may play an important role. Another report using a different bioassay found measurable serum TNF-α concentrations in 39/42 dogs with SIRS or sepsis.[58] This study, however, studied dogs at admission to an intensive care unit, regardless of the presenting signs and previous history, and can therefore not be easily compared with this cohort of emergency patients with SIRS. The role of TNF-α as an early mediator of the acute phase response with rapid down-regulation makes it a poor diagnostic and prognostic tool in human critical care patients as well.[59–61]

Although logarithmic concentrations of serum CRP and plasma IL-6 were correlated, none of the evaluated parameters in this study were associated with the underlying disease category or with survival. The large proportion of enrolled dogs with non-detectable concentrations of biologically-active TNF-α may explain the lack of correlation between logarithmic concentrations of plasma TNF-α and IL-6 and serum CRP. APPs are sensitive markers of inflammation but lack specificity regarding the underlying disease process.[23] The magnitude of the increase in CRP depends on multiple factors including the initiating cause, disease severity, and the extent of tissue damage.[13,18,36] The greatest serum CRP concentrations in specific patients may occur at different time points depending on the type of insult.[26,36] The dogs studied in this investigation suffered from SIRS due to a

wide variety of etiologies, and may also have been presented to the hospital at different points in the disease process.

Serum CRP concentrations at T0 were higher in dogs with SIRS caused by an infectious cause, but the difference was not significant compared to other etiologies. The use of CRP to discriminate septic from nonseptic human patients with SIRS has met with variable results, and serum procalcitonin concentrations have a higher sensitivity and can also have prognostic value in human patients.[62–67]

The finding in this study that CRP was not related to prognosis differs from findings in several previous reports of human and canine SIRS.[24,68,69] In a group of dogs with a fluid-filled uterus and presumptive diagnosis of pyometra, serum CRP concentrations was useful, with other parameters, to predict the presence of pyometra (versus cystic endometrial hyperplasia).[53] In a report of dogs with leptospirosis, which has a more variable clinical presentation, serum CRP concentrations were not useful to predict prognosis.[70] A previous study on CRP in dogs with SIRS found that while initial CRP concentrations were unhelpful, the 3-day change in CRP predicted survival, with survivors experiencing a greater drop in CRP concentrations than nonsurvivors.[68] In the study reported here, serum CRP concentrations remained higher than the reference interval during the initial 24 hours and were only mildly decreased (and still above the reference interval) by day 3 in survivors, and therefore were not useful to evaluate treatment efficacy.

In the present study, plasma IL-6 concentration at any point of hospitalization was not related to outcome. Mean plasma IL-6 concentrations for survivors were not significantly higher at T0 compared to nonsurvivors, and were not significantly lower as hospitalization and treatment progressed. These findings are in agreement with 2 other clinical canine studies that failed to detect significant prognostic differences in plasma IL-6 and TNF-α concentrations.[58,71] Another clinical study in dogs and research in human medicine did suggest a prognostic value for plasma IL-6 concentrations.[9,12,72] The clinical canine study did however include dogs that were hospitalized and dogs with chronic conditions (the mean duration of illness was 6.7 days, with a range from 1 to 65 days), and lacked trauma cases or dogs with GDV.[12] Population characteristics therefore differed significantly from the population evaluated here.

The inherent characteristics of a veterinary clinical study account for some limitations of this study. The ability for the attending veterinarian to exclude dogs considered too unstable for blood sampling was an important ethical consideration; however, this may have introduced an important bias to this study, as the most severely affected animals would be more likely to be excluded. In order to avoid an effect of financial considerations on the evaluation of prognostic information, all dogs that were euthanized for financial or unspecified reasons were removed from the prognostic analysis. The inclusion of dogs that were euthanized for prognostic reasons might still have had an influence on our findings; however, all of these dogs had a deteriorating clinical condition or had suffered life-threatening complications. Categorizing clinical cases to disease categories is sometimes complicated, resulting in a high proportion of animals in the miscellaneous group, and low numbers of dogs in each separate disease category. For these reasons, the findings of this study should ideally be tested in larger, multicenter studies.

A previous study demonstrated that age can have a significant effect on the immune response, with older animals expressing a higher capacity for TNF-α production.[7] We did not identify any effect of age on our findings, but such an effect could have been missed as we investigated a relatively small number of young dogs and did not attempt to subdivide patients by age. Hemolysis, lipemia, and hyperbilirubinemia can influence CRP measurements[73] but we did not find a significant effect of these factors on CRP measurements. According to previously reported data,[41] interference would indeed only be expected at very high concentrations of hemoglobin (5 g/L), intralipid (10 g/L), and bilirubin (800 mg/L).

Serum CRP concentration is increased in dogs with clinical signs of SIRS that are presented to the emergency room, and is positively correlated with increased plasma concentrations of IL-6. However, neither absolute values nor changes in blood CRP, IL-6, or TNF-α concentrations helped to identify the underlying disease or to predict outcome in this cohort of dogs.

Footnotes

[a] Gentian cCRP; Gentian AS, Moss, Norway.
[b] ABX Pentra 400; Horiba ABX SAS, Montpellier, France.
[c] cCRP calibrator; Gentian AS.
[d] cCRP low control; Gentian AS.
[e] cCRP high control; Gentian AS.
[f] code 88/532; National Institute for Biological Standards and Control, South Mimms, UK.
[g] code 89–548; National Institute for Biological Standards and Control.
[h] SAS; Statistical Analysis Software, Cary, NC.

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