

Plasma Inflammatory Mediator Concentrations at ICU Admission in Dogs with Naturally Developing Sepsis

A.E. DeClue, C.R. Sharp, and M. Harmon

Background: Identifying biomarkers to aide in the diagnosis and prognostication of sepsis in dogs would be valuable to veterinarians.

Objective: To compare plasma inflammatory mediator concentrations among dogs with sepsis, noninfectious systemic inflammatory response syndrome (NSIRS), and healthy dogs.

Animals: Dogs with sepsis (n = 22), NSIRS (n = 23), and healthy dogs (n = 13) presenting to the intensive care unit (ICU) at a veterinary teaching hospital.

Methods: Prospective observational study. Clinical parameters were recorded for each dog and plasma tumor necrosis factor (TNF) bioactivity and concentrations of interleukin (IL)-6, CXC chemokine ligand (CXCL)-8 and IL-10 were determined at ICU presentation.

Results: Dogs with sepsis and NSIRS were significantly more likely to have measurable TNF activity (sepsis 20/22; NSIRS 19/20; healthy 0/13) and IL-6 concentration (sepsis 12/22; NSIRS 15/23; healthy 2/13), than healthy dogs. Healthy dogs (9/13) were significantly more likely to have measurable plasma IL-10 concentrations than dogs with sepsis (4/19), but not NSIRS (7/20). None of the inflammatory mediators evaluated had optimal sensitivity or specificity for the diagnosis of sepsis. Twelve of 22 dogs with sepsis and 15/23 dogs with NSIRS survived to discharge; none of the measured biomarkers correlated with survival to discharge.

Conclusions and Clinical Importance: Sepsis and NSIRS are associated with increased production of the proinflammatory cytokines TNF and IL-6. In addition, sepsis is associated with decreased production of the anti-inflammatory cytokine IL-10. Despite this, plasma TNF, IL-6, CXCL-8, and IL-10 measured at ICU presentation do not appear to be valuable biomarkers to differentiate sepsis from NSIRS, or predict hospital outcome.

Key words: Biomarker; Infection; Inflammation; Systemic inflammatory response syndrome.

Sepsis, defined as the systemic inflammatory response to infection, is associated with substantial morbidity and mortality in dogs.^{1–3} Differentiating between sepsis and noninfectious forms of the systemic inflammatory response syndrome (SIRS) can be a diagnostic challenge as the clinical presentation for dogs with sepsis and noninfectious forms of SIRS is often similar. Furthermore, clinicians have few objective prognostic indicators for dogs with sepsis. There is a need for diagnostic biomarkers that identify dogs with sepsis so that a rapid diagnosis is achieved and appropriate etiology-specific therapy initiated and prognostic biomarkers to aide in client education and clinical trial stratification. Diagnostic biomarkers and prognostic biomarkers for sepsis have been described, but no single biomarker has had optimum predictive value for both diagnosis and prognostication of sepsis in dogs.^{1,4}

Abbreviations:

| | |
|-------|---|
| CXCL | CXC chemokine ligand |
| ICU | intensive care unit |
| IL | interleukin |
| NSIRS | noninfectious SIRS |
| SIRS | systemic inflammatory response syndrome |
| TNF | tumor necrosis factor |

Plasma inflammatory mediator concentrations have been used as biomarkers for sepsis in other species in part because inflammatory mediators, such as cytokines and chemokines, are fundamentally involved in the pathophysiology of the systemic inflammatory response syndrome that accompanies sepsis. While there are many inflammatory mediators involved in sepsis, tumor necrosis factor (TNF), interleukin (IL)-6, CXC chemokine ligand (CXCL)-8 (previously referred to as IL-8), and IL-10 have been singled out as being particularly important in multiple species.^{1,5–10}

The purpose of this study was to compare plasma inflammatory mediator concentrations among dogs with sepsis, noninfectious causes SIRS (NSIRS), and healthy control dogs, as well as to evaluate and compare the diagnostic and prognostic utility of each inflammatory mediator. We hypothesized that dogs with sepsis would have higher plasma activity of TNF, higher plasma concentrations of IL-6 and CXCL-8, and lower plasma concentrations of IL-10 compared with dogs with NSIRS and healthy control dogs, and that dogs with higher plasma TNF, IL-6, or CXCL-8 concentrations or lower IL-10 concentrations would be less likely to survive to discharge.

From the Department of Veterinary Medicine and Surgery, College of Veterinary Medicine, University of Missouri, Columbia, MO. Dr Sharp's current address: Tufts Cummings School of Veterinary Medicine, North Grafton, MA; Dr Harmon's current address: Department of Clinical Studies, Matthew J. Ryan Veterinary Hospital, University of Pennsylvania, Philadelphia, PA. Dr DeClue and Dr Sharp share the first author position.

Corresponding author: Amy E. DeClue DVM, MS, DACVIM (Small Animal Internal Medicine), Department of Veterinary Medicine and Surgery, College of Veterinary Medicine, University of Missouri, 900 E. Campus Dr., Columbia, MO 65211; e-mail: decluea@missouri.edu.

Submitted August 18, 2011; Revised November 1, 2011; Accepted January 19, 2012.

Copyright © 2012 by the American College of Veterinary Internal Medicine

10.1111/j.1939-1676.2012.00895.x

Materials and Methods

Animals

Sepsis and NSIRS Groups. Dogs that presented to the University of Missouri Veterinary Medical Teaching Hospital Intensive Care Unit (ICU) between July 2007 and August 2008 were eligible for inclusion in this prospective study. The study was conducted in accordance with guidelines for clinical studies from the University of Missouri Animal Care and Use Committee. Each dog was required to have a complete physical examination, complete blood count, as well as appropriate diagnostic testing, to document the presence of infection. Dogs were required to have appropriate physical parameters evaluated at the time of sample collection and laboratory parameters within 12 hours of sample collection. Dogs that were less than 6 months of age were excluded. All dogs in the sepsis or NSIRS groups were deemed critically ill by the attending veterinarian and required hospitalization for treatment. Case management was at the discretion of the attending veterinarian. A healthy control group consisted of dogs owned by the employees and students at the UM College of Veterinary Medicine. Owner consent was obtained for blood collection.

An SIRS score was determined for each dog by assigning 1 point for each of the SIRS criteria that were fulfilled for a maximum of 4 points. The SIRS criteria used for this study were hypothermia (temperature $\leq 37.8^{\circ}\text{C}$) or hyperthermia (temperature $\geq 39.7^{\circ}\text{C}$); tachycardia (heart rate ≥ 160 beats per minute); tachypnea (respiratory rate ≥ 40 breaths per minute); and leukocytosis (white blood cell count $\geq 12,000$ cells/ μL), leukopenia (white blood cell count $\leq 4,000$ cells/ μL), or left shift ($\geq 10\%$ band neutrophils).^{3,4,11-15} Each dog enrolled was required to have an SIRS score of at least 2/4. Dogs were assigned to the sepsis group if infection was suspected on clinical examination and confirmed by combinations of cytology, histopathology, culture, and antigen/antibody testing.⁴ Dogs that did not have evidence of infection were assigned to the NSIRS group. Dogs that did not meet the inclusion criteria for either the sepsis or NSIRS group or that were euthanized for reasons other than a grave prognosis were excluded from the study.

Control Group. Dogs owned by University of Missouri, Veterinary Medical Teaching Hospital employees and students were enrolled as a control group. Dogs in the healthy control group could not have had vaccination or administration of medications, with the exception of routine parasitic prevention, within the month preceding sample collection and were required to have an unremarkable history for the preceding month, physical examination, and CBC.

Clinical Data and Sample Collection

The medical records of each dog enrolled were reviewed and clinical parameters were recorded for each dog, including white blood cell count, evidence of infection, duration of hospitalization, and mortality. Blood was collected at the time of presentation to the ICU in lithium heparin tubes. Blood was centrifuged (1500 $\times g$, 7 minutes) and plasma harvested within 1 hour of sample collection. The plasma was placed in an airtight, freezer-resistant plastic tube and stored at -80°C until analysis. Tubes were coded so that the identity of the sample was not known during inflammatory mediator analysis.

Assays

All cytokine measurements were performed by an investigator blinded to the dog's group assignment and were assayed in duplicate or triplicate with appropriate controls.

TNF. Plasma TNF activity was evaluated using a cell kill bioassay.^{16,17} Briefly, murine fibroblasts (L929) were cultured on 96-well plates for 12 hours and then the samples were added. After a 20-hour incubation with minimum essential medium plus horse serum and actinomycin D, 3-[4,5-dimethylthiazol-2-yl]-2,5-di-phenyl tetrazolium bromide^a (MTT) colorimetric assay was used to quantify the number of live cells per well. Absorption was measured at 630 nm and optical density of test wells were compared with that of wells with known concentrations of canine rTNF^b for quantification. The inter- and intra-assay coefficients of variation for this assay are less than 10% and the lower limit of detection for this assay is 0.5 ng/mL (A.E. DeClue, personal communication).¹⁸

IL-6. Plasma canine-specific IL-6 concentrations were determined using a commercially available ELISA kit^c according to the manufacturer's instructions.^{17,18} The inter- and intra-assay coefficients of variation for this assay are less than 10%, and the lower limit of detection for this assay is 31.3 pg/mL.¹⁹

CXCL-8. Plasma canine-specific CXCL-8 concentrations were determined using a commercially available ELISA kit^c according to the manufacturer's instructions. The inter- and intra-assay coefficients of variation for this assay are less than 10%, and the lower limit of detection for this assay is 15.6 pg/mL.²⁰

IL-10. Plasma canine-specific IL-10 concentrations were measured using a commercially available ELISA kit^c according to the manufacturer's instructions.^{17,18} The inter- and intra-assay coefficients of variation for this assay are less than 10%, and the lower limit of detection for this assay is 15.6 pg/mL.²¹

Statistical Analysis

Statistical analysis was performed by using commercially available software.^d A Kolmogorov-Smirnov statistical test for normality was used to determine if data were normally distributed. For inflammatory cytokines, when the measured variable fell below the lower limit of detection for the assay, data were recorded at the lower limit of detection for statistical purposes. Due to a technical error, 3 dogs in the NSIRS group were excluded from the TNF activity analysis and 3 dogs with sepsis and 3 dogs with SIRS were excluded from the IL-10 analysis. A Mann-Whitney rank-sum test was used to compare TNF and IL-6 between the sepsis and NSIRS groups. Plasma CXCL-8 concentration data were normalized with a square root function and a one-way ANOVA used to evaluate differences among the sepsis, NSIRS and healthy groups. In situations where a large proportion of dogs failed to have detectable concentrations of the inflammatory mediator of interest, the proportions of dogs in each group with measurable concentrations were compared. A Fisher's exact test was used to compare proportions of dogs with measurable inflammatory mediator concentrations as well as survival data. A receiver operating characteristic (ROC) curve was used to determine the area under the curve (AUC) and select the optimum cut-off value that maximized the Youden's J statistic (sensitivity + specificity - 1) for sensitivity and specificity reporting. For the purposes of sensitivity and specificity reporting, dogs with sepsis and a positive test result were considered true positives. Conversely, dogs without sepsis and with a negative test result were considered true negatives. Test positivity was defined as a greater number for TNF, IL-6, and CXCL-8 and a lesser number for IL-10. Logistic regression was used to assess the strength of the relationship between the presence of measurable plasma inflammatory mediator activity or concentration and in-hospital mortality. The overall type one error rate was limited to 5%.

Results

Forty-five dogs met inclusion criteria, including 22 dogs with sepsis and 23 dogs with NSIRS. Thirteen healthy dogs were used as controls.

The sepsis group consisted of 2 sexually intact females, 8 spayed females, 6 intact males, and 6 neutered males with a median age of 7.5 (range 1–11) years and a median weight of 22.9 (range 4.5–63.1) kg. Breeds represented included Labrador Retriever (3), German Shepherd (2), Pointer (2), Border Collie (2), and mixed breed (2). Five dogs had abdominal sepsis; other sources of sepsis included subcutaneous infection (3), bacterial bronchopneumonia (3), pyothorax (2), hepatobiliary sepsis (2), and bacteremia (2). Twelve dogs had positive culture results. Six dogs cultured a single organism; 6 dogs had polymicrobial cultures. Organisms cultured included *Escherichia coli* (3), *Corynebacterium* spp (2), and alpha hemolytic *Streptococcus* sp (2). One dog had a positive fungal culture, growing *Candida* spp from a liver biopsy. Organisms were identified on cytology in 11 dogs with sepsis, from a variety of sources including peritoneal fluid (3), bronchoalveolar lavage fluid (2), and subcutaneous abscess fluid (2). Two dogs had sepsis confirmed on histopathology and one dog had a positive parvovirus antigen test. Fourteen, 6, and 2 dogs fulfilled 2, 3, and 4 criteria, respectively. Six dogs were hypothermic, 11 were febrile, 5 were tachycardic, and 13 were tachypneic at the time of sample collection. Abnormalities on CBC included leukocytosis (18), leukopenia (2), and a left shift (8; range 410–23,860 cells/ μ L; reference interval 0–260 cells/ μ L). Four dogs had >5% but <10% band neutrophils and 1 dog had >10% band neutrophils.

The NSIRS group consisted of 3 sexually intact females, 9 spayed females, 1 intact male, and 10 neutered males with a median age of 6 (range 0.5–15) years and a median weight of 18.7 (range 2.1–71.7) kg. Breeds represented in the NSIRS group included mixed breed (3), Labrador Retriever (3), and Beagle (2). Dogs in the NSIRS group had a variety of disease conditions including neoplasia (9), trauma (5), and toxicity (2). Fifteen, 6, and 2 dogs fulfilled 2, 3, and 4 criteria, respectively. Eight dogs were tachycardic, 7 were hypothermic, 6 were febrile, and 12 were tachypneic at the time of sample collection. Most dogs in this group had a leukocytosis (19), none had a leukopenia. Eight dogs had a left shift (range 290–4,016 cells/ μ L). Three dogs had >5% but <10% band neutrophils and one had >10% bands.

The healthy control group consisted of 7 spayed females and 6 neutered males with a median age of 3 (range 0.8–15) years and median weight of 23.2 (range 8.8–50.5) kg. The majority of dogs were mixed breed (10).

Plasma TNF activity was below the lower limit of detection in all of the control dogs, while 19/20 dogs in the NSIRS group and 20/22 dogs in the sepsis group had measurable TNF bioactivity in their plasma (Fig 1). Thus, dogs with sepsis and NSIRS were significantly more likely to have measurable TNF activity than healthy control dogs ($P < .001$; Fisher exact test). There was no difference in either the number of dogs with measurable TNF activity or the plasma TNF activity between sepsis and NSIRS groups ($P = 1$;

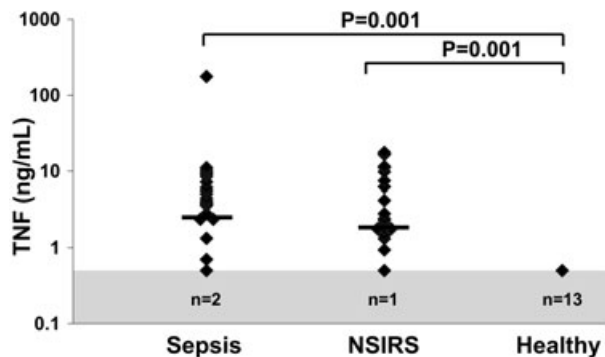


Fig 1. Comparison of the plasma TNF activity (on a logarithmic scale) in individual dogs in the sepsis ($n = 22$ total), noninfectious systemic inflammatory response syndrome (NSIRS) ($n = 20$) and healthy control ($n = 13$) groups. The horizontal line indicates the median. The number of samples that fell below the lower limit of detection of the assay is noted in the grey box. Significantly more dogs with sepsis and NSIRS had measurable TNF activity ($P < .001$; Fisher exact test) compared with the healthy control dogs.

Fisher Exact test and $P = .71$ Mann–Whitney rank-sum test, respectively).

Plasma IL-6 was below the limit of detection in 11/13 dogs in the control group, 8/23 dogs in the NSIRS group, and 10/22 dogs in the sepsis group (Fig 2). Dogs with sepsis ($P = .034$; Fisher exact test) and NSIRS ($P = .012$; Fisher exact test) were significantly more likely to have measurable IL-6 concentration than healthy control dogs; however, there was no difference in the number of dogs with measurable plasma IL-6 or the plasma concentration of IL-6 between the sepsis and NSIRS groups ($P = .76$; Fisher Exact test and $P = .66$; Mann–Whitney rank-sum test, respectively).

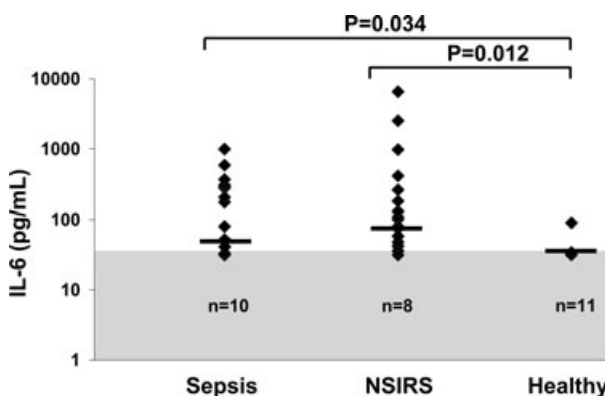


Fig 2. Comparison of plasma IL-6 concentrations (on a logarithmic scale) in individual dogs in the sepsis ($n = 22$), noninfectious systemic inflammatory response syndrome (NSIRS) ($n = 23$), and healthy control ($n = 13$) groups. The horizontal line indicates the median. The number of samples that fell below the lower limit of detection of the assay is noted in the grey box. Dogs with sepsis and NSIRS were significantly more likely to have measurable IL-6 concentrations ($P = .034$ and $P = .012$, respectively; Fisher exact test) than healthy control dogs.

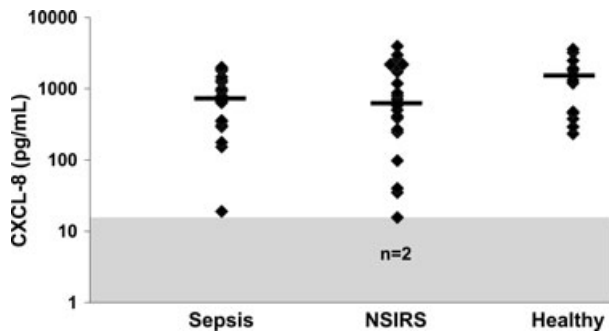


Fig 3. Comparison of plasma CXCL-8 concentrations (on a logarithmic scale) in individual dogs in the sepsis ($n = 22$), noninfectious systemic inflammatory response syndrome (NSIRS) ($n = 23$), and healthy control ($n = 13$) groups. The number of samples that fell below the lower limit of detection of the assay is noted in the grey box. There were no significant differences among groups (ANOVA).

There was no significant difference in plasma CXCL-8 concentrations among groups ($P = .45$; ANOVA) (Fig 3).

Healthy dogs (9/13) were significantly more likely to have measurable plasma IL-10 concentrations than dogs with sepsis (4/19; $P = .011$; Fisher exact test), but not NSIRS (7/20; $P = .08$; Fisher exact test) (Fig 4). There was no difference in the number of dogs with measurable IL-10 between the sepsis and NSIRS groups ($P = .48$; Fisher exact test).

The usefulness of each inflammatory biomarker for differentiating dogs with sepsis from dogs with NSIRS was evaluated using an ROC curve (Fig 5). Sensitivity, specificity, positive likelihood ratio, and negative likelihood ratio were calculated for each biomarker (Table 1). None of the inflammatory mediators evaluated had optimal sensitivity and specificity for the diagnosis of sepsis. Plasma TNF bioactivity had the

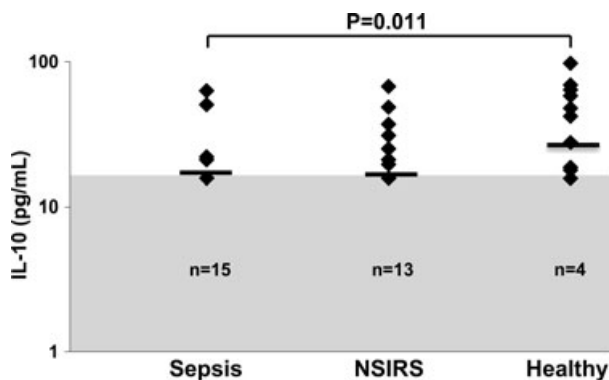


Fig 4. Comparison of plasma IL-10 concentrations (on a logarithmic scale) in individual dogs in the sepsis ($n = 19$), noninfectious systemic inflammatory response syndrome (NSIRS) ($n = 20$), and healthy control ($n = 13$) groups. The number of samples that fell below the lower limit of detection of the assay is noted in the grey box. Healthy dogs were significantly more likely to have measurable plasma IL-10 concentrations than dogs with sepsis ($P = .011$; Fisher exact test).

best combined sensitivity (81.8%) and specificity (55%) for differentiating dogs with sepsis from dogs with NSIRS (cut-off value 2.8 ng/mL).

Twelve of 22 dogs (54.5%) in the sepsis group and 15/23 dogs (65.2%) in the NSIRS group survived to hospital discharge. Survival to discharge was not significantly different between sepsis and NSIRS groups (Fisher exact test). All dogs that did not survive to discharge were euthanized due to a grave prognosis; none died naturally in the hospital. Based on logistic regression analysis, plasma TNF bioactivity and concentrations of IL-6, CXCL-8, and IL-10 were not significantly associated with survival to discharge in the sepsis or NSIRS groups (Table 2).

Discussion

Here, we describe the plasma concentrations of several pro- and anti-inflammatory cytokines in dogs with naturally developing sepsis at the time of ICU admission with the aim of evaluating their utility as diagnostic and prognostic indicators for sepsis at the time of ICU presentation. Dogs with sepsis and NSIRS were more likely to have measurable TNF activity and IL-6 concentrations than healthy control dogs. Healthy dogs were more likely to have measurable plasma IL-10 concentrations than dogs with sepsis, but not NSIRS. None of the measured biomarkers performed well in differentiating dogs with sepsis from NSIRS nor did they correlate with survival to discharge. While these results suggest that TNF, IL-6, and IL-10 play a role in both infectious and noninfectious causes of SIRS in dogs, they do not appear to be useful stand-alone diagnostic or prognostic biomarkers for sepsis in dogs when measured at ICU admission.

We used a TNF bioassay as previous reports suggest that this method might be more successful at detecting TNF in dog plasma compared with the currently available ELISA assays.^{9,c} Using a bioassay, TNF activity was identified in 39/42 dogs with systemic inflammatory response syndrome (sepsis + NSIRS). However, we found no difference in the number of dogs with measurable plasma TNF activity between the sepsis and NSIRS groups, and plasma TNF activity had a poor specificity for differentiating sepsis from NSIRS. This differs from our group's previous findings in cats where cats with sepsis were significantly more likely to have measurable plasma TNF activity than cats with NSIRS.²² In addition, we found no correlation between plasma TNF bioactivity and survival to discharge in the sepsis group. Given the dynamic nature of TNF production and its short half-life, serial measurement could be more useful for prognostication of sepsis. Indeed, the change in TNF activity over time rather than the baseline activity was predictive of death in dogs with parvoviral enteritis.⁹

Interleukin-6 was measured via ELISA methodology that has been previously used in dogs.^{10,23} While dogs with sepsis in our study were more likely to have measurable IL-6 than healthy dogs, IL-6 concentrations were not different in dogs with sepsis compared

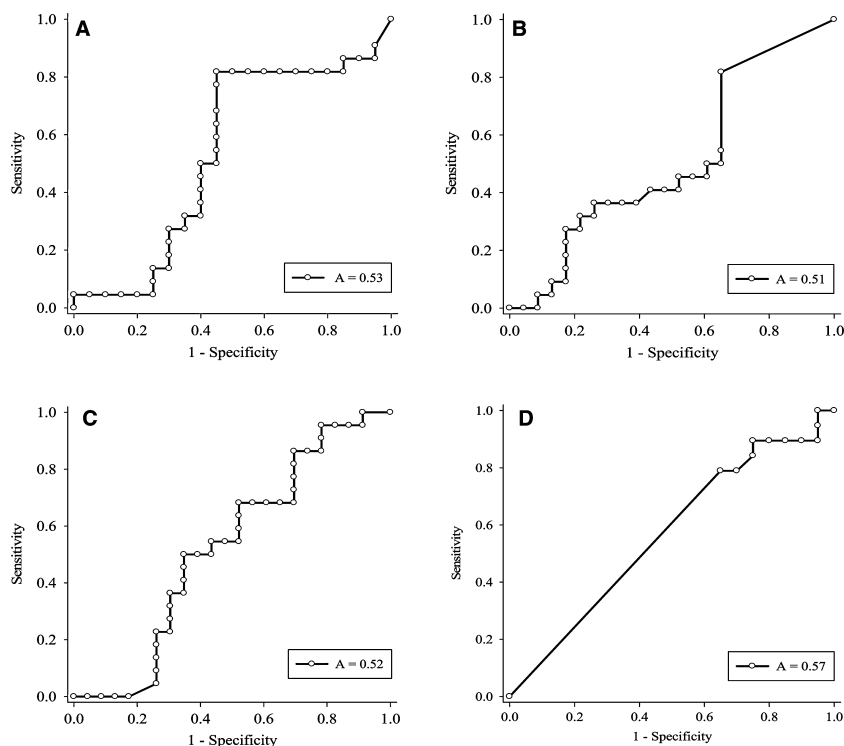


Fig 5. Receiver operating characteristic curves comparing the diagnostic sensitivity and 1-specificity of plasma TNF (A), IL-6 (B), CXCL-8 (C), and IL-10 (D) concentrations for differentiating dogs with sepsis from dogs with noninfectious systemic inflammatory response syndrome (NSIRS) (black line). A = Area under the curve.

with dogs with NSIRS, nor did IL-6 concentrations predict survival. Previously, plasma IL-6 concentrations measured by ELISA have been shown to be a poor predictor of survival in dogs with sepsis or NSIRS.¹⁰ Conversely, plasma IL-6 activity measured using a bioassay was significantly correlated with survival in dogs with sepsis.¹ These differences could be explained by variation in the cohort of dogs enrolled in each study or the different assay methodologies used for IL-6 measurement. The possible prognostic superiority of IL-6 bioactivity versus protein concentration should be considered by investigators when designing future studies.

The chemokine CXCL-8 has not been previously investigated in dogs with naturally developing sepsis to our knowledge. The authors chose to evaluate CXCL-8 in this study, given data suggesting its prognostic utility in people with sepsis, with increased circulating

concentrations associated with poor outcomes.^{24–26} In addition, endotoxin and lipoteichoic acid induce CXCL-8 release from canine neutrophils and CXCL-8 is involved in neutrophil recruitment, suggesting that CXCL-8 probably plays a role in sepsis.^{27,28} We found a large range of CXCL-8 concentrations in all 3 study groups, and a lack of association between CXCL-8 concentration and survival to discharge. Healthy dogs had plasma CXCL-8 concentrations that were similar to the concentrations measured in dogs with sepsis and NSIRS, which was unexpected as circulating concentrations of CXCL-8 are low in healthy people.²⁹ Serum CXCL-8 concentrations have been reported to range from 913 to 6,003 pg/mL in healthy dogs.³⁰ The highest concentration of CXCL-8 recorded in our study was 3,953 pg/mL, well within this range. This suggests that either peak plasma CXCL-8 concentration was missed at the time of sample collection in our study or

Table 1. Sensitivity, specificity, positive likelihood ratio, and negative likelihood ratio for each of the cytokine biomarkers for differentiating sepsis from noninfectious systemic inflammatory response syndrome (NSIRS) in dogs.

| Cytokine (units) | AUC | Cut-off | Se | 95% CI | Sp | 95% CI | Positive LR | Negative LR |
|------------------|-------|---------|------|-----------|------|-----------|-------------|-------------|
| TNF (ng/mL) | 0.534 | 2.8 | 81.8 | 59.7–94.8 | 55 | 31.5–77 | 1.82 | 0.33 |
| IL-6 (pg/mL) | 0.509 | 31.4 | 81.8 | 59.7–94.8 | 34.8 | 16.3–57.2 | 1.25 | 0.52 |
| CXCL-8 (pg/mL) | 0.524 | 125.5 | 95.4 | 77.1–99.8 | 21.7 | 7.4–43.7 | 1.22 | 0.21 |
| IL-10 (pg/mL) | 0.566 | <17.6 | 79 | 54.4–93.9 | 35 | 15.3–59.2 | 1.21 | 0.60 |

NSIRS, noninfectious systemic inflammatory response syndrome; LR, likelihood ratio; AUC, area under the ROC curve; Se, sensitivity (%); Sp, specificity (%); CI, confidence interval.

Table 2. Result of multivariate analysis evaluating factors associated with death in dogs with sepsis and noninfectious systemic inflammatory response syndrome (NSIRS) groups.

| Factor | Odds Ratio | 95% Confidence Interval | P-Value |
|--------------|------------|-------------------------|---------|
| Sepsis group | | | |
| TNF | 1.025 | 0.94–1.11 | .567 |
| IL-6 | 0.995 | 0.99–1.00 | .260 |
| CXCL-8 | 1.00 | 0.99–1.00 | .638 |
| IL-10 | 0.898 | 0.70–1.13 | .374 |
| NSIRS group | | | |
| TNF | 1.05 | 0.85–1.30 | .655 |
| IL-6 | 1.00 | 0.99–1.00 | .577 |
| CXCL-8 | 0.99 | 0.99–1.00 | .122 |
| IL-10 | 0.92 | 0.75–1.11 | .398 |

that dogs with sepsis do not have a dramatic increase in plasma concentrations of CXCL-8.

Healthy dogs in this study were more likely to have measurable IL-10 concentrations than dogs with sepsis and NSIRS. Because dogs with sepsis were less likely to have measurable concentrations of IL-10 (an anti-inflammatory cytokine), but did have measurable TNF activity and IL-6 concentrations (both proinflammatory cytokines), this may suggest an imbalance between proinflammatory and anti-inflammatory mediators. Our findings are consistent with a previous study that documented an imbalance in pro- and anti-inflammatory cytokines in the most severely ill dogs with sepsis and NSIRS.¹⁰ In that study, survivors of sepsis and NSIRS were more likely to have a high ratio of high-mobility group box -1 (a late proinflammatory mediator) to IL-10, compared with nonsurvivors.¹⁰

As with any clinical study, there are several limitations that should be considered when interpreting these data. Plasma cytokine concentrations were only evaluated at 1 time point in this study, given that the aim of the study was to evaluate their utility as diagnostic and prognostic indicators for sepsis upon ICU admission. Cytokine production is dynamic in nature and it is feasible that peak production was missed by a single sampling point. Serial evaluation may have resulted in a significant association between cytokine concentration and mortality or a statistical difference between the sepsis and NSIRS groups. Unfortunately, inflammatory mediator concentrations were frequently below the lower limit of detection of the assays used. This highlights the importance of developing more sensitive canine-specific immunologic assays. We chose to include a heterogeneous population of critically ill dogs instead of a more targeted population, such as dogs with gram-negative septic peritonitis, in the hope of finding biomarkers for sepsis that would be applicable to the general population of dogs with sepsis; studies targeting a specific disease subset may allow the detection of site- or organism-specific biomarkers. In addition, although dogs in the NSIRS group were carefully screened for infection, it is possible that infection went undetected. This may have decreased our

ability to differentiate dogs with and without infection. Finally, while the proportion of dogs that survived to be discharged from the hospital in the sepsis group was similar to that in many previous studies, euthanasia was the ultimate cause of death in all dogs that suffered mortality.^{31–34} Great care was taken to only include animals that were euthanized because of a grave prognosis in an attempt to exclude nonillness-related factors. It is possible that this approach induced a bias in our survival statistics and in the association between plasma cytokines at presentation and survival.

Sepsis is a frequent condition in dogs and is associated with substantial morbidity and mortality. This study evaluated the potential utility of circulating concentrations of cytokines at ICU admission as diagnostic and prognostic biomarkers for sepsis in dogs. Dogs with sepsis and NSIRS were more likely to have measurable activity and concentrations of proinflammatory cytokines and less likely to have measurable concentrations of the anti-inflammatory cytokine IL-10, which suggests that sepsis is associated with an imbalance in pro- and anti-inflammatory mediators. Unfortunately, TNF activity and the concentrations of IL-6, CXCL-8, and IL-10 did not effectively differentiate between dogs with sepsis and NSIRS, nor did they predict survival to discharge in dogs with sepsis. The authors suggest that future studies should be conducted to elucidate the importance of the proinflammatory and anti-inflammatory mediator balance, and include dynamic evaluation of cytokine production in sepsis in dogs.

Footnotes

^a Sigma-Aldrich, St. Louis, MO

^b Endogen, Rockford, IL

^c Canine-specific (either IL-6, CXCL-8 or IL-10 as appropriate), Quantikine Assay, R&D Systems, Inc, Minneapolis, MN

^d SigmaPlot, Systat Software Inc, San Jose, CA

^e Humm KR, Boag AK, House AK. TNF-alpha levels in the serum and abdominal fluid of dogs with septic peritonitis. In: 14th IVECCS. Phoenix, AZ: 2008:819 (abstract)

Acknowledgments

The authors thank Dr Leona Rubin for providing L929 cells for this study, and Dr Chee-hoon Chang for her technical assistance.

This study was performed at the Veterinary Medical Teaching Hospital and Comparative Internal Medicine Laboratory at the University of Missouri.

Funding for this research was provided by a grant from the University of Missouri Research Council.

A portion of the data from this study were presented at the 2010 American College of Veterinary Internal Medicine Forum, Anaheim, California and the 16th Annual International Veterinary Emergency and Critical Care Symposium, San Antonio, Texas.

References

- Rau S, Kohn B, Richter C, et al. Plasma interleukin-6 response is predictive for severity and mortality in canine systemic inflammatory response syndrome and sepsis. *Vet Clin Pathol* 2007;36:253–260.
- Luschini MA, Fletcher DJ, Schoeffler GL. Incidence of ionized hypocalcemia in septic dogs and its association with morbidity and mortality: 58 cases (2006–2007). *J Vet Emerg Crit Care (San Antonio)* 2010;20:406–412.
- Hauptmann J, Walshaw R, Olivier N. Evaluation of the sensitivity and specificity of diagnostic criteria for sepsis in dogs. *Vet Surg* 1997;26:393–397.
- DeClue AE, Osterbur K, Bigio A, et al. Evaluation of serum NT-pCNP as a diagnostic and prognostic biomarker for sepsis in dogs. *J Vet Intern Med* 2011;25:453–459.
- Calandra T, Baumgartner JD, Grau GE, et al. Prognostic values of tumor necrosis factor/cachectin, interleukin-1, interferon-alpha, and interferon-gamma in the serum of patients with septic shock. Swiss-Dutch J5 Immunoglobulin Study Group. *J Infect Dis* 1990;161:982–987.
- de Werra I, Jaccard C, Corradin SB, et al. Cytokines, nitrite/nitrate, soluble tumor necrosis factor receptors, and procalcitonin concentrations: Comparisons in patients with septic shock, cardiogenic shock, and bacterial pneumonia. *Crit Care Med* 1997;25:607–613.
- Gogos CA, Drosou E, Bassaris HP, et al. Pro- versus anti-inflammatory cytokine profile in patients with severe sepsis: A marker for prognosis and future therapeutic options. *J Infect Dis* 2000;181:176–180.
- Martin C, Boisson C, Haccoun M, et al. Patterns of cytokine evolution (tumor necrosis factor-alpha and interleukin-6) after septic shock, hemorrhagic shock, and severe trauma. *Crit Care Med* 1997;25:1813–1819.
- Otto C, Drobatz K, Soter C. Endotoxemia and tumor necrosis factor activity in dogs with naturally occurring parvoviral enteritis. *J Vet Intern Med* 1997;11:65–70.
- Yu DH, Nho DH, Song RH, et al. High-mobility group box 1 as a surrogate prognostic marker in dogs with systemic inflammatory response syndrome. *J Vet Emerg Crit Care* 2010;20:298–302.
- Okano S, Yoshida M, Fukushima U, et al. Usefulness of systemic inflammatory response syndrome criteria as an index for prognosis judgement. *Vet Rec* 2002;150:245–246.
- Giunti M, Peli A, Battilani M, et al. Evaluation of CALC-I gene (CALCA) expression in tissues of dogs with signs of the systemic inflammatory response syndrome. *J Vet Emerg Crit Care (San Antonio)* 2010;20:523–527.
- Iris K, Leontides LS, Mylonakis ME, et al. Factors affecting the occurrence, duration of hospitalization and final outcome in canine parvovirus infection. *Res Vet Sci* 2010;89:174–178.
- Matijatko V, Kis I, Torti M, et al. Septic shock in canine babesiosis. *Vet Parasitol* 2009;162:263–270.
- Osterbur K, Whitehead Z, Sharp CR, et al. Plasma nitrate/nitrite concentrations in dogs with naturally developing sepsis and non-infectious forms of the systemic inflammatory response syndrome. *Vet Rec* 2011;169:554.
- DeClue AE, Cohn LA, Lechner ES, et al. Effects of subanesthetic doses of ketamine on hemodynamic and immunologic variables in dogs with experimentally induced endotoxemia. *Am J Vet Res* 2008;69:228–232.
- Deitschel SJ, Kerl ME, Chang CH, et al. Age-associated changes to pathogen-associated molecular pattern-induced inflammatory mediator production in dogs. *J Vet Emerg Crit Care (San Antonio)* 2010;20:494–502.
- Fowler BL, Axiak SM, DeClue AE. Blunted pathogen-associated molecular pattern motif induced TNF, IL-6 and IL-10 production from whole blood in dogs with lymphoma. *Vet Immunol Immunopathol* 2011;144:167–71.
- R and D Systems. Quantikine canine IL-10 immunoassay. Available at: <http://www.rndsystems.com/pdf/CA6000.pdf>. Accessed August 13, 2009.
- R and D Systems. Quantikine canine CXCL-8/IL-8 immunoassay. Available at: <http://www.rndsystems.com/pdf/CA8000.pdf>. Accessed August 13, 2009.
- R and D Systems. Quantikine canine IL-10 immunoassay. Available at: <http://www.rndsystems.com/pdf/ca1000.pdf>. Accessed October 12, 2009.
- DeClue AE, Delgado C, Chang CH, et al. Clinical and immunologic assessment of sepsis and the systemic inflammatory response syndrome in cats. *J Am Vet Med Assoc* 2011;238:890–897.
- Martins TL, Kahvejian MA, Noel-Morgan J, et al. Comparison of the effects of tramadol, codeine, and ketoprofen alone or in combination on postoperative pain and on concentrations of blood glucose, serum cortisol, and serum interleukin-6 in dogs undergoing maxillectomy or mandibulectomy. *Am J Vet Res* 2010;71:1019–26.
- Gosain A, Gamelli RL. A primer in cytokines. *J Burn Care Rehabil* 2005;26:7–12.
- Marty C, Misset B, Tamion F, et al. Circulating interleukin-8 concentrations in patients with multiple organ failure of septic and nonseptic origin. *Crit Care Med* 1994;22:673–679.
- Wong HR, Cvijanovich N, Wheeler DS, et al. Interleukin-8 as a stratification tool for interventional trials involving pediatric septic shock. *Am J Respir Crit Care Med* 2008;178:276–282.
- Inoue H, Hara M, Massion PP, et al. Role of recruited neutrophils in interleukin-8 production in dog trachea after stimulation with *Pseudomonas* in vivo. *Am J Respir Cell Mol Biol* 1995;13:570–577.
- Inoue H, Massion PP, Ueki IF, et al. *Pseudomonas* stimulates interleukin-8 mRNA expression selectively in airway epithelium, in gland ducts, and in recruited neutrophils. *Am J Respir Cell Mol Biol* 1994;11:651–663.
- Martin K, Viera K, Petr C, et al. Simultaneous analysis of cytokines and co-stimulatory molecules concentrations by ELISA technique and of probabilities of measurable concentrations of interleukins IL-2, IL-4, IL-5, IL-6, CXCL8 (IL-8), IL-10, IL-13 occurring in plasma of healthy blood donors. *Mediators Inflamm* 2006;2006:65237.
- Zois NE, Moesgaard SG, Kjelgaard-Hansen M, et al. Circulating cytokine concentrations in dogs with different degrees of myxomatous mitral valve disease. *Vet J* 2011; Epub Jun 20.
- de Laforcade AMRE, Freeman LM, Li W. Serial evaluation of protein C and antithrombin in dogs with sepsis. *J Vet Intern Med* 2008;22:26–30.
- de Laforcade AMFL, Shaw SP, Brooks MB, et al. Hemostatic changes in dogs with naturally occurring sepsis. *J Vet Intern Med* 2003;17:674–679.
- Burkitt J, Haskins S, Nelson R, et al. Relative adrenal insufficiency in dogs with sepsis. *J Vet Intern Med* 2007;21:226–231.
- Webb CMK, Dow S. Neutrophil function in septic dogs. *J Vet Intern Med* 2007;21:982–989.