Clinical and immunologic assessment of sepsis and the systemic inflammatory response syndrome in cats

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Objective—To compare clinical findings and inflammatory mediator production among cats with sepsis, cats with noninfectious systemic inflammatory response syndrome (SIRS), and healthy cats.

Design—Case-control study.

Animals—Cats with sepsis (n = 16) or SIRS (19) and 8 healthy control cats.

Procedures—Clinical variables were recorded for each cat, and plasma tumor necrosis factor (TNF) and interleukin (IL)-1β activities and IL-6 and CXC chemokine ligand (CXCL)-8 concentrations were determined at initial evaluation.

Results—Clinicopathologic abnormalities associated with sepsis in cats included a high band neutrophil percentage, eosinopenia, hyponatremia, hypochloremia, hypoalbuminemia, hypocalcemia, and hyperbilirubinemia. When the sepsis and SIRS groups were compared, the only significant differences in the CBC and plasma biochemical findings were band neutrophil percentage and albumin concentration. Cats with sepsis had significantly greater plasma TNF activity than did healthy cats and were more likely to have detectable concentrations of IL-6 than were cats with SIRS or healthy cats. Plasma IL-1β activity did not differ among groups, and CXCL-8 was not detectable in most (32/43) cats. Mortality rate was not significantly greater for cats with sepsis (7/16) than for cats with SIRS (5/19). Plasma IL-1β activity and IL-6 and chloride concentrations were the only variables correlated with nonsurvival in the sepsis group.

Conclusions and Clinical Relevance—Cats with sepsis may have various clinicopathologic abnormalities but are more likely to have a high band neutrophil percentage and hypoalbuminemia than cats with noninfectious SIRS. Plasma interleukin-1β activity and plasma IL-6 and chloride concentrations may be useful prognostic biomarkers for septic cats. (*J Am Vet Med Assoc* 2011;238:890–897)

Sepsis, previously defined as a blood-borne bacterial infection, has recently been redefined as the systemic inflammatory response to any type of infectious organism, including bacteria, viruses, fungi, parasites, and protozoa.[1,2] Sepsis is a serious problem in cats that is associated with substantial disease effects and a mortality rate ranging from 29% to 79%.[3–7] The condition has been associated with many diseases in cats, including septic peritonitis, hepatic abscessation, pyothorax, bacteremia, pneumonia, endocarditis, pyelonephritis, and pyometra.[3–7] Although affected cats develop many of the classic clinical findings associated with sepsis in other animal species, they also develop relatively unique manifestations, including bradycardia, hypothermia, and abdominal pain.[3,5,6] Information regarding the pathophysiology of sepsis in cats, particularly the pathophysiology of these unique manifestations, is lacking.

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Abbreviations

ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
CXCL	CXC chemokine ligand
IL	Interleukin
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
SIRS	Systemic inflammatory response syndrome
TNF	Tumor necrosis factor

Whereas there are many inflammatory mediators involved in sepsis, TNF, IL-1β, IL-6, and CXCL-8 (previously referred to as IL-8) have been singled out as being particularly important in other species.[8–13] Of these, TNF-α and IL-1β are prominent in the early proinflammatory phase of sepsis, and IL-6 and CXCL-8 are involved in maintenance of inflammation. In experimental models, production of TNF, IL-6, and CXCL-8 can be induced by administration of endotoxin in cats.[14–16] However, to the authors' knowledge, no studies have been conducted to evaluate inflammatory mediator production in cats with naturally developing sepsis. Understanding the inflammatory response to infection in cats with naturally developing infection may help with diagnosis and treatment in cats with sepsis and in the determination of their prognosis.

Previous studies[3–7,17,18] of sepsis in cats have been retrospective in nature and focused on particular pathogens (ie, bacteria) or origins of sepsis. Although these studies have provided important clinical information pertaining to sepsis in cats, they did not include evaluation of the general syndrome of sepsis or the inflammatory mediator response. The purpose of the study reported here was to evaluate the clinical and immunologic response and identify prognostic indicators for sepsis in cats in a prospective fashion. We hypothesized that cats with sepsis would have higher plasma activity of TNF and IL-1β and higher plasma concentrations of IL-6 and CXCL-8 than would cats with noninfectious causes of SIRS or healthy cats and that the plasma concentrations of these inflammatory mediators would predict whether the cats would survive.

Materials and Methods

Animals—Any critically ill cat brought to the University of Missouri, Veterinary Medical Teaching Hospital and hospitalized for treatment between February 2008 and October 2009 was evaluated for inclusion into this prospective study. Additionally, healthy cats that were owned by the faculty and students of the University of Missouri were enrolled as a control group. All cats were required to have a complete history, physical examination, CBC, plasma biochemical analysis, and owner consent for phlebotomy prior to enrollment. Noncontrol cats were deemed critically ill by the attending veterinarian and were hospitalized for treatment. Patient management was at the discretion of the attending veterinarian. The study was approved by the University of Missouri Animal Care and Use Committee and was performed in compliance with institutional guidelines for research on animals.

Group assignment—On the basis of initial evaluation findings, cats that fulfilled at least 2 of 4 SIRS criteria were assigned to 1 of 2 groups: sepsis or SIRS without infection.[5] Cats in the sepsis group were judged to have an infection on the basis of cytologic, histologic, microbial, or serologic findings. Cats with SIRS without infection (SIRS group) were judged to have no evidence of infection on the basis of clinical examination or cytologic, histologic, microbial, or serologic findings. Specific SIRS criteria were rectal temperature ≤ 37.8°C (100.0°F) or ≥ 39.7°C (103.5°F), heart rate ≤ 140 or ≥ 225 beats/min, respiratory rate ≥ 40 breaths/min, WBC count ≤ 5.0×10^3 cells/μL or ≥ 19.5×10^3 cells/μL, or ≥ 5% band cells.[5]

Cats that did not fulfill the SIRS criteria were excluded. Additionally, cats with SIRS were excluded when there was a strong clinical suspicion of infection but definitive confirmation of infection was not obtained. Cats in the healthy control group were judged as such on the basis of good health history, unremarkable physical examination and CBC findings, and no evidence of illness for 1 month after study enrollment.

Data collection—Clinical variables recorded for each cat included signalment, rectal temperature, heart rate, respiratory rate, CBC and plasma biochemical results, diagnosis, duration of hospitalization, and outcome. For the healthy control group, cat health status was monitored via communication with the owner 1 month after sample collection.

Blood samples were collected from a jugular vein into lithium heparin tubes via a 22- or 20-gauge needle at the time of admission to the intensive care unit (cats in the sepsis and SIRS groups) or at the time of physical examination (cats in the control group). Plasma was harvested immediately by use of a centrifuge ($300 \times g$ for 6 minutes). A portion of each plasma sample was used for immediate biochemical evaluation, with the remainder frozen at –80°C for inflammatory mediator assays.

Plasma inflammatory mediator activities—All cytokine measurements were performed on thawed aliquots of plasma by an investigator unaware of cat group assignment and were assayed in duplicate or triplicate with appropriate control specimens for production of standard curves.

Plasma TNF activity was evaluated with a cell kill bioassay as described elsewhere.[14,15,19,20] Briefly, cells from a mouse fibroblast cell line (L929)[a] were cultured on 96-well plates. After 12 hours, plasma samples were added to the wells in triplicate. After 20 hours of cell incubation with minimum essential medium plus horse serum and actinomycin D,[b] MTT[b] was added and the cells were incubated for an additional 2.5 hours to allow for formazan crystal formation. The formazan crystals were then solubilized in a solution containing dimethylformamide[b] and SDS.[b] Color development after 1 hour was measured at 630 nm with a spectrophotometer.[c] Feline recombinant TNF[d] was used to construct a standard curve to determine the concentration of TNF activity in the test wells. The lower limit of detection for this assay is 0.5 U/L (0.5 ng/mL).

Plasma IL-1β activity was measured by use of a modified cytotoxicity bioassay as described elsewhere.[21,22] Briefly, A375S2 cells[e] were cultured on 96-well plates in minimum essential medium with fetal bovine serum and penicillin-streptomycin.[f] Plasma samples were added to the wells in triplicate. After 72 hours of cell incubation, MTT was added and the cells incubated for an additional 2 hours. The formazan crystals were solubilized in a solution containing dimethylformamide[b] and SDS.[b] Color development after 1 hour was measured at 630 nm with a spectrophotometer.[c] Feline recombinant IL-1β[g] was used to construct a standard curve to determine the concentration of IL-1β activity in the test wells. The lower limit of detection for this assay is 0.156 U/L (0.156 ng/mL).

Plasma inflammatory mediator concentrations—Concentrations of feline-specific IL-6[h] and feline-specific CXCL-8[i] in plasma samples were determined with commercially available assay kits in accordance with the manufacturer's instructions. Plasma samples were diluted 1:2 with 1% bovine serum albumin[b] in PBS solution prior to analysis.[14] The lower limit of detection for the IL-6 assay is 62.5 pg/mL, and that for the CXCL-8 assay is 125 pg/mL.

Statistical analysis—Statistical analysis was performed by use of commercially available software.[j] A Shapiro-Wilk test was used to determine whether the data were normally distributed. One-way ANOVA with a post hoc Tukey test or ANOVA on ranks with a post hoc Dunn multiple comparison procedure was used to evaluate the differences in clinicopathologic variables and TNF and IL-1β activities among the 3 groups. Mortality rate and proportions of cats with detectable IL-6 and CXCL-8 con-

centrations were compared between pairs of groups by use of the Fisher exact test. Because the critically ill cats were selected on the basis of heart rate, respiratory rate, rectal temperature, and leukocyte counts, these clinical variables were not compared among groups. Spearman rank order correlation was used to assess the strength of the relationship between clinical variables or plasma inflammatory mediator concentrations and survival (yes or no). A value of $P < 0.05$ was considered significant for all analyses. Summary statistics are reported as mean ± SD except where indicated.

Results

Animals—Fifty-three critically ill cats met the initial criteria for the study during the study period. Eighteen cats were excluded because they did not fulfill the SIRS criteria (n = 17) or because there was strong clinical suspicion of infection but definitive confirmation of infection was not obtained (1). Therefore, in total, 35 critically ill cats (19 with SIRS and 16 with sepsis) and 8 control cats were enrolled. Distributions of sex did not differ among groups and were as follows: control group, 5 neutered males and 3 spayed females; SIRS group, 15 neutered males and 4 spayed females; and sepsis group, 10 neutered males, 1 sexually intact male, and 5 spayed females. Groups also did not differ significantly with respect to age or body weight (**Table 1**).

Sepsis group—Breeds represented among the 16 cats with sepsis included domestic shorthair (n = 11), domestic longhair (3), Himalayan (1), and Siamese (1). One cat had been treated with corticosteroids prior to admission; another cat had received NSAIDs. Sepsis was secondary to bacterial infection in 8 cats, viral infection in 5 cats (FeLV [n = 2 cats], FIV [1], and feline coronavirus [2]), and protozoal infection in 3 cats (cytauxzoonosis [2] and toxoplasmosis [1]). The diagnosis of infection was confirmed on the basis of bacterial culture in 6 cats, cytologic evaluation in 2 cats, histologic evaluation in 3 cats, and serologic testing or immunofluorescence assay in 5 cats. The origin of sepsis in these cats was considered systemic (n = 7 cats) or the pleural space (3), heart (1), urinary tract (1), peritoneum (1), CNS (1), subcutaneous space (1), or respiratory tract (1). Bacterial culture and susceptibility testing was performed in 6 cats, yielding 7 isolates in total. Bacteria cultured included β-hemolytic *Streptococcus* spp (n = 2 cats), *Pasteurella multocida* (2), *Staphylococcus pseudintermedius* (1), *Enterococcus* spp (1), and *Clostridium* spp (1).

The mean number of SIRS criteria fulfilled in the sepsis group was 2.8; 12 of 16 cats met at least 3 of 4 SIRS criteria. Five cats were hypothermic, 8 were febrile, 1 was bradycardic, 3 were tachycardic, and 15 were tachypneic. Hematologic abnormalities included leukocytosis (n = 9 cats), > 5% band cells (6), leukopenia (4), and anemia (4). Plasma biochemical abnormalities included hyponatremia (n = 10 cats), hypoalbuminemia (9), hyperbilirubinemia (7), hypocalcemia (6), hypochloremia (6), hyperglycemia (6), hyperglobulinemia (5), high urea nitrogen concentra-

Table 1—Mean ± SD values of clinical variables in healthy cats (n = 8) and in cats with noninfectious SIRS (19) or sepsis (16).

Variable	Reference interval	Healthy cats	Cats with SIRS	Cats with sepsis
Body weight (kg)	NA	5.0 ± 0.4	3.8 ± 1.1	4.6 ± 1.4
Age (y)	NA	4.4 ± 2.9	9.2 ± 5.7	6.2 ± 4.6
Temp (°C)*	NA	38.0 ± 0.3	38.4 ± 1.7	38.9 ± 1.5
Heart rate (beats/min)*	NA	179.4 ± 30.8	200.2 ± 65.8	188.8 ± 29.0
Respiratory rate (breaths/min)*	NA	46.3 ± 6.9	64.3 ± 44.3	64.9 ± 25.8
WBCs (× 10^3 cells/L)*	5.5–19.5	7.6 ± 1.4	12.3 ± 10.6	19.4 ± 14.1
Neutrophils (× 10^3 cells/μL)	2.5–12.5	5.1 ± 2.0	9.3 ± 9.3	16.4 ± 13.4
Band cells (%)	0–0.3	0.3 ± 0.4	0.1 ± 0.2	1.5 ± 3.0[a]
Lymphocytes (× 10^3 cells/μL)	1.5–7	1.7 ± 0.7	1.8 ± 1.0	0.9 ± 1.1
Monocytes (× 10^3 cells/μL)	0–0.85	0.2 ± 0.1	0.7 ± 0.8	0.3 ± 0.3
Eosinophils (× 10^3 cells/L)	0–1.5	0.4 ± 0.2	0.5 ± 1.0	0.3 ± 0.8[b]
Hct (%)	24–45	36.9 ± 4.9	27.5 ± 10.6	28.3 ± 9.2
Glucose (mg/dL)	52–153	120.4 ± 28.3	136.7 ± 43.1	139.1 ± 72.8
Urea nitrogen (mg/dL)	17–35	26.6 ± 5.9	26.3 ± 6.7	29.3 ± 15.9
Creatinine (mg/dL)	0.5–2.2	1.3 ± 0.4	1.0 ± 0.6	1.1 ± 0.5
Sodium (mEq/L)	149–159	154.3 ± 1.6	149.3 ± 6.2[b]	146.8 ± 3.7[b]
Potassium (mEq/L)	3–4.7	3.6 ± 0.4	3.7 ± 0.6	3.5 ± 0.5
Chloride (mEq/L)	114–122	120.4 ± 2.0	115.6 ± 4.7[b]	112.9 ± 7.2[b]
Total CO_2 (mEq/L)	11–22	18.6 ± 1.5	17.2 ± 2.4	18.2 ± 3.8
Albumin (g/dL)	2.6–4.2	3.4 ± 0.3	2.8 ± 0.4	2.4 ± 0.6[a]
Total proteins (g/dL)	6.4–8.8	7.1 ± 0.7	7.3 ± 1.8	7.0 ± 2.0
Globulin (g/dL)	2.6–5.9	3.7 ± 0.6	4.5 ± 1.8	5.0 ± 1.2
Total calcium (mg/dL)	8.6–10.7	9.6 ± 0.4	8.7 ± 0.5	8.6 ± 1.2[b]
Phosphorus (mg/dL)	2–5.3	3.5 ± 0.8	4.8 ± 1.6	4.4 ± 1.4
Total bilirubin (mg/dL)	0–0.3	0.1 ± 0.0	0.8 ± 1.7	1.5 ± 2.4[b]
ALT (U/L)	18–77	61.3 ± 32.6	129 ± 227.9	37.6 ± 17.5
ALP (U/L)	5–55	36.4 ± 12.6	38.3 ± 55.3	18.7 ± 16.7[b]

*Variables that were not statistically compared among groups.
[a]Value is significantly ($P < 0.05$) different, compared with values for the SIRS and healthy control groups.
[b]Value is significantly ($P < 0.05$) different, compared with value for the healthy control group.
To convert temperatures in Celsius to Fahrenheit, multiply by 9/5 and add 32.

tion (5), hyperphosphatemia (5), hypoproteinemia (5), high ALP activity (4), hyperproteinemia (4), hypokalemia (3), hyperalbuminemia (3), hyperkalemia (2), hyperchloremia (2), low urea nitrogen concentration (2), high creatinine concentration (2), hypoglobulinemia (2), high ALT activity (2), hypoglycemia (1), hypernatremia (1), hypercalcemia (1), and low ALP activity (1). Of the cats with hypochloremia, all but 1 had concurrent hyponatremia. The mean duration of hospitalization was 2.9 days. Seven of the cats in this group did not survive to hospital discharge; this included 2 cats with pyothorax, 2 cats with cytauxzoonosis, and 1 cat each with septic peritonitis, feline coronavirus infection, and toxoplasmosis.

SIRS group—Breeds represented among the 19 cats with SIRS included domestic shorthair (n = 6), domestic longhair (3), and 1 each of Maine Coon, Persian, Rag Doll, Siamese, Himalayan, and Birman. None of the cats in this group had previously received corticosteroids; 1 cat had received NSAIDs. Underlying diseases included neoplasia (n = 5 cats), trauma (2), neurologic disease (2), gastrointestinal disease (2), uroabdomen (1), pancreatitis (1), thromboembolism (1), toxicosis (1), acute respiratory distress syndrome (1), hypertension (1), lower urinary tract disease (1), and disease of undetermined diagnosis (1). The mean number of SIRS criteria fulfilled was 2.5; 8 of 19 cats met at least 3 of 4 SIRS criteria. Eight cats were hypothermic, 2 were febrile, 1 was bradycardic, 3 were tachycardic, and 15 were tachypneic. Hematologic abnormalities included leukocytosis (n = 6 cats), anemia (5), and leukopenia (4). None of the cats had > 5% band cells. Plasma biochemical abnormalities included hyperglycemia (n = 9), hyponatremia (9), hypochloremia (7), high ALT activity (7), high urea nitrogen concentration (5), hyperphosphatemia (4), hypoproteinemia (4), hyperbilirubinemia (4), hypocalcemia (3), hypoalbuminemia (3), high creatinine concentration (2), hyperglobulinemia (2), hyperproteinemia (2), low urea nitrogen concentration (2), high ALP activity (2), hypokalemia (1), hyperkalemia (1), hypoglobulinemia (1), and low creatinine concentration (1). Of the cats with hypochloremia, all but 1 had concurrent hyponatremia. The mean duration of hospitalization was 2.1 ± 2.3 days. Five cats in the SIRS group did not survive to discharge, including 1 cat each with neurologic disease, uroabdomen, neoplasia, gastrointestinal disease, and acute respiratory distress syndrome.

Control group—Breeds represented among the 8 cats in the control group included domestic shorthair (n = 5), domestic long hair (2), and Manx (1). None were receiving medication with the exception of routine parasitic preventives. All CBC values for all cats were within reference intervals for the laboratory. Mild biochemical abnormalities included increased ALT activity (n = 2 cats), hyperchloremia (2), hyperglycemia (1), mild hypoproteinemia despite albumin and globulin concentrations in the reference interval (1), and hypophosphatemia (1). All cats in this group remained alive and healthy 1 month after sample collection.

Clinicopathologic variables—The sepsis group had a significantly (P = 0.010) greater band cell percentage than did the SIRS or control group and a significantly (P = 0.025) lower eosinophil count than did the control group (Table 1). Plasma sodium and chloride concentrations were significantly (P = 0.002 and P = 0.007, respectively) lower in the sepsis and SIRS groups, compared with values from the control group, but there was no significant difference in sodium or chloride concentrations between the sepsis and SIRS groups. The sepsis group had a lower plasma albumin concentration than did the SIRS (P = 0.006) and control (P < 0.001) groups. In the sepsis group, plasma total calcium concentration and ALT activity were significantly (P = 0.035 and P = 0.008, respectively) lower and total bilirubin concentration significantly (P = 0.010) higher than in the control group. There was no difference among groups in monocyte count, Hct, or plasma concentrations of glucose, urea nitrogen, creatinine, potassium, total carbon dioxide, total proteins, globulins, or phosphorus.

The only 2 clinicopathologic abnormalities that were more severe in the sepsis group than in the SIRS group were a high band neutrophil percentage and hypoalbuminemia. The sepsis group mean band neutrophil percentage was significantly (P = 0.010) greater and the plasma albumin concentration was significantly (P = 0.006) less than those of the SIRS group. In the sepsis group, 6 of 16 cats had > 5% band cells and 9 of 16 cats had hypoalbuminemia, and of these 10 cats, 5 had > 5% band cells and hypoalbuminemia concurrently. In the SIRS group, none of the cats had > 5% band cells and 3 of 19 cats were hypoalbuminemic.

Plasma inflammatory mediator concentrations—Plasma TNF activity was significantly (P = 0.040) greater in the sepsis group than in the control group (**Figure 1**). There was no difference in plasma TNF activity between the sepsis and SIRS groups or the SIRS and control groups. Plasma IL-1β activity was greater in the sepsis group than in the SIRS or control group, but this difference was not significant (**Figure 2**). Plasma IL-6 concentrations were not detectable in any of the control cats and were detectable in only 4 of 19 cats with SIRS, with values ranging from 66 to 128 pg/mL (**Figure 3**). Conversely, 10 of 16 cats with sepsis had detectable

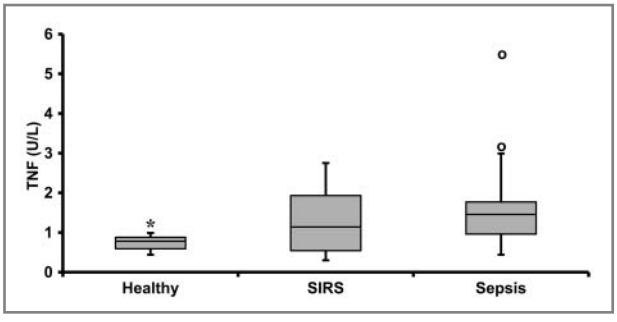

Figure 1—Box-and-whisker plots of plasma TNF activity in healthy cats (n = 8) and in cats with noninfectious SIRS (19) or sepsis (16). The upper and lower edges of the box represent the 75th and 25th percentiles, respectively, whereas the line within the box is the median value. Whiskers represent the largest and smallest values. Outliers (extreme values) are indicated with a circle. One unit per liter of bioactivity is equivalent to the bioactivity of 1 ng/mL of feline recombinant TNF. *Value is significantly (P < 0.05) different from that for the sepsis group.

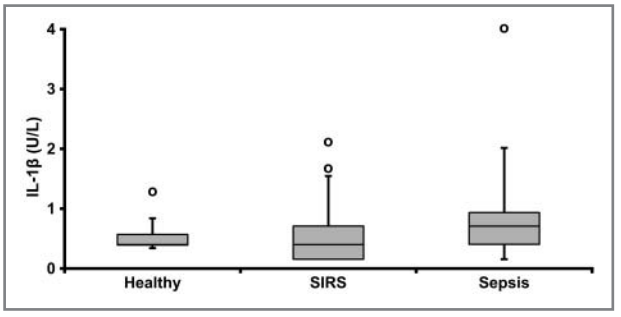

Figure 2—Box-and-whisker plots of plasma IL-1β activity in healthy cats (n = 8) and in cats with noninfectious SIRS (19) or sepsis (16). One unit per liter of bioactivity is equivalent to the bioactivity of 1 ng/mL of feline recombinant IL-1β. Values did not differ significantly (ie, $P > 0.05$) among groups. *See* Figure 1 for remainder of key.

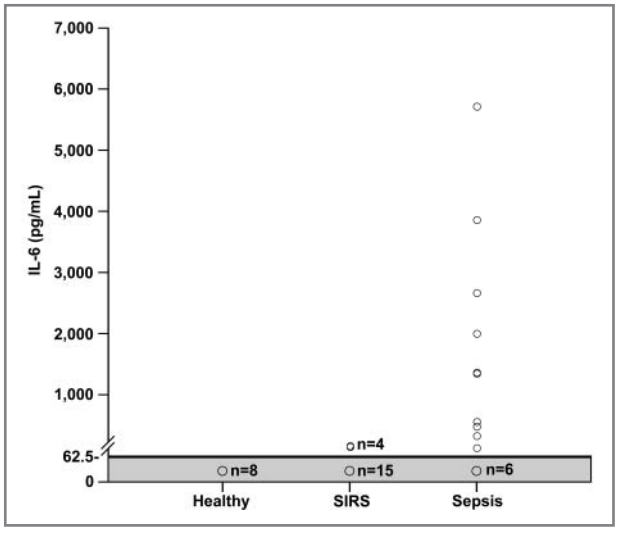

Figure 3—Plasma IL-6 concentrations in individual healthy cats (n = 8) and cats with noninfectious SIRS (19) or sepsis (16). The lower limit of detection of the assay used was 62.5 pg/mL, which is indicated by the shaded box. All 8 control cats, 15 cats with noninfectious SIRS, and 6 cats with sepsis had undetectable concentrations.

IL-6 in their plasma, with values ranging from 94 to 5,738 pg/mL. Plasma CXCL-8 concentrations were less than the lower limit of detection for the assay in all of the control cats, 14 of 19 cats with SIRS, and 10 of 16 cats with sepsis (**Figure 4**). Values for cats with detectable concentrations ranged from 374 to 874 pg/mL for the SIRS group and 252 to 4,227 pg/mL for the sepsis group. Because of the limited number of cats with detectable concentrations of IL-6 and CXCL-8, group mean concentrations were not statistically compared. However, there were significantly more cats in the sepsis group with detectable IL-6 than in the SIRS group ($P = 0.018$) and control group ($P = 0.006$). There was no significant difference in the number of cats with detectable CXCL-8 concentrations among groups.

Prognostic indicators—Mortality rate was greater for cats with sepsis (7/16) than for cats with noninfectious SIRS (5/19), but this difference was not significant. In the sepsis group, there were moderate, positive correlations between nonsurvival and plasma IL-1β activity ($r = 0.70$; $P = 0.002$) and IL-6 concentration ($r = 0.52$; $P = 0.038$). There was also a moderate, negative correlation ($r = 0.55$; $P = 0.037$) between plasma chloride concentration and nonsurvival. When all of the critically ill cats were evaluated together (ie, sepsis and SIRS groups), plasma IL-1β activity ($r = 0.56$; $P < 0.001$), had a moderate, positive correlation with nonsurvival and total lymphocyte count ($r = 0.40$; $P = 0.017$), plasma chloride concentration ($r = 0.41$; $P = 0.019$), and total protein concentration ($r = 0.41$; $P = 0.018$) had moderate, negative correlations with nonsurvival. None of the other variables evaluated, including the total number of SIRS criteria fulfilled, were significantly correlated with nonsurvival.

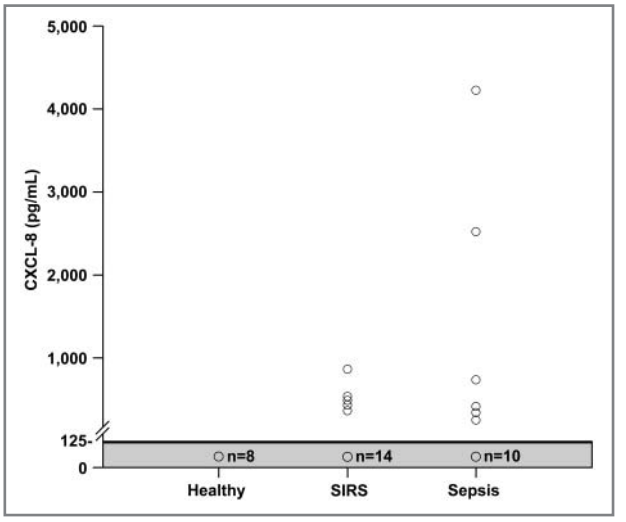

Figure 4—Plasma CXCL-8 concentrations in individual healthy cats (n = 8) and cats with noninfectious SIRS (19) or sepsis (16). Open circles represent plasma CXCL-8 concentration in individual cats. The lower limit of detection of the assay used was 125 pg/mL, which is indicated by the shaded box. All 8 control cats, 14 cats with noninfectious SIRS, and 10 cats with sepsis had undetectable concentrations.

Discussion

In this report, the clinical findings and inflammatory mediator response were characterized for cats with sepsis induced by various microorganisms. Clinicopathologic abnormalities associated with sepsis included high band neutrophil percentage, eosinopenia, hyponatremia, hypochloremia, hypoalbuminemia, hypocalcemia, and hyperbilirubinemia. Hyponatremia and hypochloremia were detected in cats with noninfectious SIRS as well. Cats with sepsis had a higher percentage of band cells and lower plasma albumin concentration than did cats with noninfectious SIRS, whereas cats with noninfectious SIRS had a higher ALP concentration. Cats with sepsis had significantly greater plasma TNF activity than did healthy cats and were more likely to have detectable concentrations of IL-6 than were cats with noninfectious SIRS or healthy cats. The mortality rate did not differ significantly between cats with sepsis (7/16) and cats with noninfectious SIRS (5/19). Plasma IL-1β activity and IL-6 and chloride concentrations were the only variables correlated with nonsurvival in the sepsis group.

Infection in the study cats could be attributed to multiple classes of microorganisms including bacterial, viral, and protozoal. Previous retrospective studies[3–7,17,18] of cats with sepsis attributable to bacterial infections identified neutrophilia with a left shift, lymphopenia, eosinophilia, anemia, hypoalbuminemia, hypoglycemia or hyperglycemia, hyperphosphatemia, hyponatremia, hypocalcemia, hypokalemia or hyperkalemia, hyperbilirubinemia, azotemia, and high ALT and ALP activity in affected cats. In the present study, cats with sepsis had various clinicopathologic abnormalities, and a left shift and hypoalbuminemia were the only 2 clinicopathologic findings that were more severe in the cats with sepsis than in those with noninfectious SIRS. However, these 2 abnormalities were not associated with a poorer prognosis.

Tumor necrosis factor is an early phase proinflammatory mediator that is produced in response to various stimuli. This cytokine activates secondary inflammatory cascades, resulting in a prolonged inflammatory response, endothelial cell permeability, neutrophil release from bone marrow, expression of endothelial leukocyte adhesion molecules, lymphocyte apoptosis, acute phase protein production, and pyrexia.[23] Experimentally, endotoxin induces TNF production in cats.[14] In the present study, cats with sepsis had significantly greater plasma TNF activity than did healthy control cats but there was no difference in plasma TNF activity between cats with sepsis and those with noninfectious SIRS. Plasma TNF activity at admission to the hospital was not associated with a poorer prognosis in septic cats. In humans, plasma TNF concentrations are correlated with death,[8,10,24–26] and in dogs, increasing plasma TNF activity over time is associated with death among those with naturally occurring canine parvoviral enteritis.[13] Our study involved evaluation of TNF activity at hospital admission only, and dynamic changes in TNF production over time may be more important than TNF values at 1 time point for predicting prognosis in cats.

In other species, IL-1β increases the production of other proinflammatory mediators and expression of adhesion molecules and plays a role in the development of organ dysfunction.[27,28] Although plasma IL-1β activity was greater in cats with sepsis than in cats with noninfectious SIRS and healthy cats in the present study, the differences were not significant. In experimentally induced endotoxemia in cats, plasma IL-1β concentrations are not detectable in most affected cats.[14] However, endotoxin, lipoteichoic acid, and peptidoglycan can stimulate IL-1β production from feline whole blood ex vivo.[22] Plasma IL-1β activity in our study was correlated with nonsurvival in septic cats, with higher concentrations of IL-1β being associated with a poorer outcome. Thus, IL-1β deserves additional attention as a possible prognostic biomarker for sepsis in cats.

Interleukin-6 and CXCL-8, 2 late phase inflammatory mediators in sepsis, are produced in response to early cytokines (eg, TNF- and IL-1β) and bacterial products (eg, lipopolysaccharide)[23,29,30] and may persist in the plasma for a longer period, compared with other inflammatory mediators, thereby representing potentially useful markers of inflammatory cell activation. Interleukin-6 is involved in many of the manifestations of sepsis, including pyrexia, synthesis of acute phase proteins, differentiation of B cells to produce immunoglobulins, dysfunction of gastrointestinal barriers, and hemostatic derangement.[23] As a chemokine, the main role of CXCL-8 is recruitment and activation of neutrophils. High serum concentrations of CXCL-8 and IL-6 are associated with poor outcomes in humans with sepsis.[23,25,30–32] In fact, there is a direct correlation between plasma IL-6 concentration and the risk of death in humans with intra-abdominal sepsis, with IL-6 measurement alone predicting outcome with > 80% accuracy.[33] Plasma IL-6 concentration is also predictive of poor outcome in dogs with sepsis.[12] In our study, plasma IL-6 concentration had a moderate but significant association with nonsurvival in cats with sepsis, and cats with sepsis were more likely to have detectable concentrations of IL-6 than were cats with noninfectious SIRS or healthy cats. Additional studies involving a large sample size are needed to determine whether IL-6 could be a prognostic biomarker for sepsis in cats. Only 6 of 16 cats with sepsis and 4 of 18 cats with noninfectious SIRS had detectable concentrations of CXCL-8 in our study, so it is difficult to formulate a conclusion pertaining to the importance of CXCL-8 in prognostic decision making.

The mortality rate was greater for cats with sepsis (7/16) than for cats with noninfectious SIRS (5/19) as was duration of hospitalization, but not significantly so. Of the variables routinely evaluated in a clinical setting, only plasma chloride concentration was significantly associated with nonsurvival in the sepsis group, with hypochloremia associated with a poorer prognosis. When the sepsis and SIRS groups were combined, plasma chloride concentration was also negatively correlated with outcome. In all but 2 of the cats with hypochloremia in the sepsis and SIRS groups, hyponatremia was a concurrent finding. This suggested that circulating sodium and chloride concentrations were changing in parallel and thus may have been associated with gastrointestinal, renal, or third-space loss of sodium chloride.[34] Additionally, corrected hypochloremia can be associated with dilutional acidosis.[34] Because hypochloremia was associated with outcome in both the sepsis and SIRS groups, plasma chloride concentration may be a prognostic indicator in critically ill cats, regardless of the cause.

In dogs with critical illness, higher SIRS scores are associated with a poorer prognosis,[35] and it was believed that a similar scoring system could be used to predict prognosis in cats with sepsis. However, the number of SIRS criteria fulfilled was not correlated with outcome in cats with sepsis. This observation is similar to findings in humans with sepsis, in which high SIRS scores are not predictive of death.[36,37] The SIRS criteria used in our study were based on criteria established by a retrospective, necropsy-based study[5] of severe sepsis in cats. Because these criteria were based on cats that died from sepsis, their applicability to critically ill cats in general may be limited. Additional studies of larger numbers of cats with critical and noncritical illness are needed to confirm or refute our findings and to determine whether a different clinical scoring system exists that can be used for diagnostic and prognostic decision making in cats with sepsis.

Several limitations to the present study should be considered. Although, to the authors' knowledge, this is the first study to involve evaluation of inflammatory mediator production in cats with naturally developing sepsis, markers of inflammation were only evaluated at 1 time point. To fully understand the true importance of TNF, IL-1β, IL-6, and CXCL-8 production in response to infection in cats, longitudinal studies are needed to evaluate the production dynamics of these mediators over time. Additionally, establishing appropriate cutoff values for inflammatory mediator concentrations by use of receiver operating characteristic curves to obtain the greatest sensitivity, specificity, and predictive values for these markers will help clarify their clinical usefulness as biomarkers. Another limitation is that the concentrations of IL-6 and CXCL-8 were less than the lower limit of detection for many cats in this study. At the time the study was performed, the availability of feline-specific inflammatory mediator assays was limited. There is a need to develop feline-specific assays with greater analytic sensitivity for these mediators. Furthermore, as with any clinical study of noninfectious SIRS, it is possible that some of the cats in the SIRS group actually had subclinical infections. Although the investigators attempted to ensure that the cats in the SIRS group did not have evidence of infection, the possibility remains and may have biased the results.

The present study revealed that cats with sepsis were more likely to have band cells and hypoalbuminemia than were cats with noninfectious causes of SIRS. Cats with sepsis had significantly greater plasma TNF activity and were more likely to have detectable concentrations of IL-6 than were cats with noninfectious SIRS or healthy cats. The number of SIRS criteria fulfilled did not predict outcome; thus, the prognostic value of SIRS scoring in cats should be evaluated further prior to routine clinical use. Plasma IL-1β activity and IL-6 and chloride concentrations may be useful prognostic biomarkers for sepsis in cats.

a. Courtesy of Dr. Leona Rubin, Comparative Internal Medicine Laboratory, University of Missouri, Columbia, Mo.
b. Sigma-Aldrich, St Louis, Mo.
c. SpectraMax Plus 384 Microplate Spectrophotometer, Molecular Devices, Sunnyvale, Calif.
d. Endogen, Rockford, Ill.
e. American Type Culture Collection, Manassas, Va.
f. GIBCO, Invitrogen Corp, Carlsbad, Calif.
g. R and D systems, Minneapolis, Minn.
h. Feline IL-6 Duoset, R and D systems, Minneapolis, Minn.
i. Feline CXCL-8/IL-8 Duoset, R and D systems, Minneapolis, Minn.
j. SigmaPlot, Systat Software Inc, San Jose, Calif.

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