Plasma interleukin-6 response is predictive for severity and mortality in canine systemic inflammatory response syndrome and sepsis

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Background: Sepsis is still a major cause of death in both human and veterinary medicine. Early diagnosis is essential for appropriate treatment. Identification of patients at risk for developing sepsis is already possible in human medicine through the measurement of plasma interleukin-6 (IL-6) levels. In veterinary medicine, however, this has been investigated only in canine experimental models. **Objectives:** The purpose of this study was to measure IL-6 plasma levels in dogs with naturally occurring systemic inflammatory response syndrome (SIRS) and sepsis and to analyze the value of IL-6 as a predictive parameter for severity and mortality. **Methods:** Included in the study were 79 dogs that had been admitted to the small animal clinics of Munich and Berlin from July 2004 to July 2005 and that satisfied the diagnostic criteria for SIRS and sepsis as defined using established parameters. Measurement of plasma IL-6 levels on days 0, 1, and 2 was performed by the use of a colorimetric bioassay based on IL-6–dependent cell growth. **Results:** Septic foci were identified in 43 patients (septic group), and 36 patients were enrolled in the SIRS group. The frequency of positive blood cultures was 11%. The overall mortality rate was 48%. Higher plasma IL-6 levels on the day of admission were significantly correlated with a more severe degree of disease, increased mortality rate, and earlier fatality. **Conclusions:** Plasma IL-6 concentration is predictive of outcome in canine SIRS and sepsis and may be a valuable laboratory parameter for assessing critically ill dogs. (*Vet Clin Pathol.* 2007;36:253–260)

Key Words: Dog, IL-6, inflammation, sepsis, SIRS

According to recent reports, the incidence rate for sepsis has been increasing over the past few decades, being now the 10th leading cause of death in human beings in the United States.[1–2] Unfortunately, no such information is available regarding sepsis and systemic inflammatory response syndrome (SIRS) in dogs. Data collected by the University of Pennsylvania Veterinary Teaching Hospital suggest a similar increase in the incidence of sepsis in dogs from 1 per 1000 hospital cases in 1988 to 3.5 in 1998.[3] This increase needs to be further investigated, however, as it may be due to an increased clinical suspicion of sepsis and subsequently increased likelihood of diagnosis, rather than a true increase in the frequency of sepsis. Mortality rates in dogs with sepsis range from 31% to 50% in various veterinary studies.[4–9] Therapeutic intervention in both dogs and people consists mainly of supportive care and antibiotics and, of course, treating the underlying cause, as specific anti-inflammatory strategies have failed in human clinical trials.[10–13] (The only promising exception, that of drotrecogin alfa, is rapidly eliminated, has antigenic potential,[14] and is currently far too expensive to use in companion animals.) Successful treatment depends frequently on early diagnosis. Therefore, identification of animals at risk for sepsis is an urgent current research goal. A presumptive diagnosis of sepsis is based on the criteria for the identification of SIRS, as defined by the American College of Chest Physicians/Society of Critical Care Medicine consensus conference[15] and modified for veterinary use[4,16] and either histologic or microbiologic confirmation of infection. At the time of initial clinical presentation and examination, only classification by the SIRS criteria[4] is available. Bacterial culture takes several days and false-negative results are not uncommon.

SIRS and sepsis are characterized by activation of the cytokine network. The proinflammatory cytokine interleukin-6 (IL-6) has a longer plasma half-life than does tumor necrosis factor alpha (TNF-α) or interleukin-1-beta and its concentration has proven to be significantly elevated in the plasma of septic human patients. A correlation between high levels of IL-6 on admission and mortality was found in most studies.[17–32] IL-6 thus appears to be a good marker of the severity of systemic bacterial infection when measured at the time of admission. Canine experimental models produced by administering infusions of either live *Escherichia coli* or lipopolysaccharide

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This study was presented in part at the 14th Meeting of the Sections of Internal Medicine and Clinical Laboratory Diagnostics (InnLab) of the German Veterinary Society DVG, Munich, Germany, May 13–14, 2006; and at the European Congress of Veterinary Internal Medicine – Companion Animals (ECVIM-CA), Amsterdam, The Netherlands, September 14–16, 2006.
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(LPS) or by causing artificial inflammation with turpentine oil have also shown the induction of high levels of IL-6.[33–37] However, changes in the plasma concentration of this cytokine have not, to the authors' knowledge, been investigated in naturally occurring septicemia in the dog. Additionally, the experimental studies are mostly short-term (up to 7 hours), and little information is available about IL-6 levels in dogs over several days of clinical illness. Here we provide the first evidence that plasma IL-6 levels can be used as a prognostic marker in septic canine patients.

Materials and Methods

Patients

Included in the study were 79 dogs with clinical suspicion of sepsis that had been admitted to the small animal clinics of Munich (n = 35) and Berlin (n = 44) from July 2004 to July 2005. Criteria for the diagnosis of SIRS were defined in accordance with the study of de Laforcade et al.[4] Systemic illness was considered to be present if the patient met 2 or more of the following criteria: hypo- or hyperthermia (<37.8°C or >39.4°C), tachycardia (heart rate >140 beats/min), tachypnea (respiratory rate >20 breaths/min), leukocytosis (>16 × 10^3 cells/μL), and leukopenia (<6 × 10^3 cells/μL) or >3% bands. Time point of application was either on the day of admittance or during hospitalization of the patient, when development of sepsis was suspected. Animals were subsequently classified as septic if additional histologic or microbiologic confirmation of infection was available. Septic patients were divided into 3 subgroups: sepsis, severe sepsis (sepsis associated with organ dysfunction, hypoperfusion, or hypotension), and septic shock (hypotension defined by a systolic arterial pressure <90 mm Hg or a reduction in systolic blood pressure of >40 mm Hg from baseline, despite adequate volume resuscitation) as proposed by the Consensus Conference.[15] Mortality was defined as death or euthanasia during hospitalization. Survival was defined as being discharged from the clinic.

Blood sampling

Blood for bacteriologic cultures was obtained from the jugular vein following disinfection with povidone iodine and isopropyl alcohol. At least 1 culture was taken in 64 dogs; in selected cases, a second culture from either the other jugular or a peripheral vein was obtained. Blood samples for CBC and IL-6 measurement were acquired on the day of admission (day 0) and the following 2 days (days 1 and 2) in case of survival. Whole blood anticoagulated with EDTA was used to obtain a CBC using an automated analyzer (Cell-Dyn 3500 R, Abbott Diagnostics, Abbott Park, IL, USA). The differential WBC count was performed on Wright's-stained blood smears by counting 100 cells. For the measurement of IL-6, blood was collected in sodium citrate tubes (Sarstedt Monovette, Nuembrecht-Rommelsdorf, Germany) and centrifuged at 1570*g* for 5 minutes. The supernatant plasma was aliquoted and stored at –70°C within 1 hour of collection for up to 1 year until analyzed.

Assay for IL-6

The IL-6 activity in plasma was measured with the 7TD1 bioassay first described by Van Snick et al[38] and later modified by Pechumer et al[39] and Schneider et al.[40] The bioactivity of canine IL-6 is expressed by its ability to stimulate proliferation of the IL-6–dependent murine hybridoma cell line 7TD1 (kindly provided by Van Snick, Ludwig Institute for Cancer Research, Brussels, Belgium). Cells were maintained at 37°C and 5% CO_2 in Rosewell Park Memorial Institute (RPMI) 1640 medium with GlutaMAX Media (GIBCO BRL Life Technologies, Karlsruhe, Germany) containing 10% fetal bovine serum, 2% penicillin and streptomycin, 1 ng/mL recombinant human IL-6, and 0.55 mM 2-mercaptoethanol (Sigma-Aldrich, Taufkirchen, Germany). For the assay, plasma samples were first diluted to 1:20, because our earlier measurements indicated toxicity of the canine plasma at higher concentrations, which resulted in an inhibition of growth of the 7TD1 cells. A serial dilution was performed for each sample to a maximum of 1:2560 and run in duplicates on a 96-well flat-bottomed microtiter plate (Nunc Inc, Wiesbaden, Germany). The cells were then harvested and washed 3 times in phosphate buffered saline before resuspension in the same medium without IL-6 and adjusted to a density of 1×10^5 cells/mL. One hundred microliters of the cell suspension were added to the diluted samples. For each assay, a standard curve was produced with recombinant human IL-6 with serial twofold dilutions starting at 1 ng/mL. Medium without added IL-6 served as a negative control. Plates were incubated at 37°C and 5% CO_2 for 4 days and proliferation was then measured using the XTT colorimetric assay (Sigma-Aldrich).[41] XTT was diluted in RPMI 1640 medium to a final concentration of 1 mg/mL, and phenazine methosulfate was added to a final concentration of 0.025 mM. Fifty microliters of this solution were applied to each well, and the plates were further incubated at 37°C and 5% CO_2 for 4 hours. The plates were vigorously shaken in order to solubilize the formazan crystals formed. The optical density was measured using an ELISA reader (Tecan Inc, Kirchheim, Germany) at 450 nm as a test and 620 nm as reference wavelengths. The standard curve of recombinant human IL-6 was used to calculate a best-fit regression of the rising portion of the curve in the linear range. IL-6 activity of the plasma samples could then be determined by the equation for this best-fit line for each assay and expressed in pg/mL. To validate the assay, we measured IL-6 in a control group of 12 client-owned clinically healthy dogs; all of these dogs had undetectable IL-6 values.

Statistical analysis

The statistical software packages SPSS and R (SPSS 13.0, SPSS Inc, Chicago, IL, USA, and R, The R Project for Statistical Computing, http://www.r-project.org/) were used to perform statistical evaluations. To determine associations between continuous and ordinal variables, Bravais-Pearson and

Table 1. General types of pre-existing disease in 79 dogs with SIRS or sepsis, as reported by their owners.*

Systems Affected by Pre-Existing Disease	No. (%) of Dogs
Musculoskeletal	15 (44%)
Cardiovascular	9 (26%)
Neoplasia	5 (15%)
Dermatologic	5 (15%)
Nervous system	4 (12%)
Endocrine	2 (6%)
Ocular	1 (3%)
Immune-mediated	1 (3%)
Other†	5 (15%)
Unknown	15 (19%)
No pre-existing disease	30 (38%)

*Nine dogs were reported to have >1 disease.

†Includes infection (2), recurrent fever (1), and undefined (2).

Spearman rank correlation coefficients were calculated, respectively. For binary variables, the association was modeled by a logistic regression model. A Cox proportional hazards regression model[42] was used to analyze the dependence between survival time and the measured levels of IL-6 on the day of admission.

Results

A total of 79 dogs were enrolled in the study. The most frequently represented breeds were Golden Retrievers (n=7) and German Shepherd dogs (n=6). Body weight ranged from 1.9 to 80.0 kg (mean, 29.6; median 30.0 kg). There were 44 (56%) male and 35 (44%) female dogs, of which 10 males and 7 females were neutered. Age at time of presentation ranged from 2 months to 16 years, with a mean age of 7.8 years (median, 9 years). Fifty-four (71%) dogs were >5 years old. A history of pre-existing disease was affirmed by the owners for 34 (43%) dogs (Table 1). Twenty-one (27%) dogs were reported to regularly receive medication. The main reasons for presentation of the dogs included gastrointestinal problems (n=65, 82%), with clinical signs including anorexia, vomiting, and diarrhea. Nonspecific signs such as weakness, polydipsia/polyuria, fever, and pain were reported in 75% (n=59) of dogs; 69 dogs had clinical signs of more than 1 disease process. Signs of illness had been recognized by the owners for a mean of 6.7 days (median, 2 days; range, 1–65 days) prior to admission.

Forty-three (54%) dogs met the criteria for sepsis, whereas 36 (46%) dogs were systemically ill without proof of infection (SIRS group). The mean hospitalization time was 5.6 days (median, 5 days; range, 1– 32 days). The overall mortality rate was 48% (n=38 nonsurvivors), of which 27 (71%) were euthanized because of a poor prognosis, and 11 (29%) died. Twenty-four (63%) of the nonsurvivors died or were euthanized within the first 3 days. Sixteen (42%) of the nonsurvivors had a necropsy performed. Of all dogs with sepsis, 20 (47%) dogs survived, and 23 (53%) died (Table 2). The most frequent disease processes were pyometra (n=10), pneumonia (n=9), intestinal or gastric perforation (n=6), prostatic abscess (n=6), pancreatitis (n=5), and parvovirus infection (n=3) (Table 3). When each criterion for SIRS was tested statistically, only body temperature at the time of admission showed a significant correlation with mortality (P = .024, proportional odds ratio (OR) = exp(β) = 0.618).

Table 2. Number (percentage) of survivors and nonsurvivors for 79 dogs with SIRS with and without sepsis.*

Group	SIRS Without Confirmed Sepsis	SIRS with Confirmed Sepsis		
		Sepsis	Severe Sepsis	Septic Shock
Survivors	21 (58%)	15 (75%)	5 (24%)	0 (0%)
Nonsurvivors	15 (42%)	5 (25%)	16 (76%)	2 (100%)
Total	36 (100%)	20 (100%)	21 (100%)	2 (100%)

The frequency of positive blood cultures was 11% (9 of 64 cultures) (Table 4). Thirty-seven (47%) dogs had been pretreated with antibiotics by the referring veterinarian; 18 (49%) of these patients developed sepsis nevertheless, with 4 positive blood cultures on admission. An additional 62 samples from different tissues (eg, urine, swab samples from wounds, abscesses, or vaginal discharge) were either cultured or, in a few cases, screened for bacteria in Diff-Quik stained cytologic preparations. Of these 62 samples, 37 (60%) were positive. Seven dogs had positive results for the same bacteria from 2 separate samples (either blood culture and a separate sample or 2 samples from different locations). The most common organism isolated was *E coli* (28%, 13 of 46 isolates). Species found in mixed infections were not further identified. A positive blood culture result did not correlate with a higher number of SIRS criteria; correlation with mortality had a P value of .078 (ρ_{SP} = –0.128).

Standard curves of 7 different IL-6 assays were combined to determine interassay variability. The coefficient of variation was 17%. The detectable limit of the bioassay was 1 pg/mL. Icteric plasma was detected in some of the samples. To determine whether icteric plasma could affect the colorimetric measurement of IL-6, a highly icteric but non–IL-6-containing sample was selected from the patient samples in our laboratory (bilirubin concentration, 1.52 mg/dL) and mixed with human recombinant IL-6 (Sigma-Aldrich, Taufkirchen, Germany) to a final concentration of 1 ng/mL. The same was done with a nonicteric patient sample (bilirubin concentration <0.4 mg/dL). The same results (~1 ng/mL) were obtained in both icteric and nonicteric plasma samples as well as in a control sample containing human recombinant IL-6 alone.

IL-6 concentration was determined in dogs with SIRS, sepsis, severe sepsis, and septic shock on days 0, 1, and 2 (Table 3). Visual inspection of IL-6 data on the day of admission showed 2 outliers and a skewed distribution. Therefore, this variable was transformed to the log scale: natural logarithm (ln)-IL-6 = ln (''IL-6 day 0'' + 1) for all further analyses.

Table 3. Plasma IL-6 concentration and disease diagnoses in dogs with SIRS and sepsis on days 0, 1, and 2 after admission.

Group	Survival	Plasma IL-6 (pg/mL)								Clinical or Pathologic Diagnoses
		Day 0			Day 1			Day 2		
		Median	Min-Max	n	Median	Min-Max	n	Median	Min-Max	n	
SIRS	Survivor	0	0–6837.45	21	0	0–2048.13	19	0	0–9495.83	17	Parvovirus infection (3), prostatic abscess (2), fever of unknown origin (2), gastric bleeding (2), hemorrhagic gastroenteritis, mesenteric infarct, bile peritonitis, pyelonephritis, cellulitis, hemometra, aspiration pneumonia, discospondylitis/myositis, foreign body, steroid-responsive meningoencephalitis, juvenile cellulitis, heatstroke
	Nonsurvivor	616.21	0–19,192.17	15	305.20	0–5788.06	7	335.37	0–2261.17	5	Heart failure (3*), pancreatitis (3), pneumonia (2*), uroabdomen/peritonitis, fever of unknown origin, renal insufficiency, foreign body/giardiosis*, splenic neoplasia*, lymphosarcoma, gastric ulceration*, cellulitis,
Sepsis	Survivor	457.53	0–2723.08	15	36.46	0–2578.55	14	0	0–199.60	14	Pyometra (5), abscess (4; perianal, prostatic, neck, limb), mastitis, necrotizing tonsillitis, intra-articular empyema/discospondylitis, intestinal perforation, fever of unknown origin, foreign body, cystitis
	Nonsurvivor	91.31	0–2339.49	5	0	0–184.11	3	1.06	0–2.12	2	Pyometra, volvulus, lymphosarcoma with intestinal perforation/peritonitis*, salmonellosis, necrotizing pulmonary carcinoma*
Severe sepsis	Survivor	710.35	0–4320.72	5	0	0–3909.02	5	0	0–314.87	4	Pyometra (2), prostatic abscess/peritonitis, pneumonia, foreign body with intestinal perforation/peritonitis
	Nonsurvivor	511.77	0–46,404.7	16	437.73	0–42,911.9	11	87.33	0–1213.61	11	Aspiration pneumonia (3**), pyometra (2*), prostatic abscess (2*), intestinal perforation/peritonitis (2), gastric perforation/peritonitis, peritonitis/protein-losing enteropathy*, intra-abdominal abscess/peritonitis, parvovirus infection*, pulmonary hemorrhage*, intestinal hemorrhage/maggot infestation, pyodermatitis, cellulitis
Septic shock	Nonsurvivor	65,855.77	0–127,471.2	2	ND	ND	ND	ND	ND	ND	Aspiration pneumonia*, cellulitis*

*Diagnoses made at necropsy, with the number of asterisks representing the number of cases. ND indicates not done.

Statistical analysis showed a moderate correlation between the concentration of IL-6 on the day of admission and the number of abnormal SIRS criteria ($P = .015$, $\rho_{SP} = -0.274$). Examining a possible connection between each of the inclusion criteria and IL-6 concentration on day 0, a statistically significant correlation was found only with the total WBC count ($P = .002$, $\rho_{SP} = -0.340$). The WBC count was either lower than or within the reference interval in those patients with an IL-6 concentration higher than 7 log-transformed units. Based on evaluation of a scatterplot (Figure 2), the observed effect was weak. A higher IL-6 concentration correlated significantly ($P = .006$, $\rho_{SP} = -0.308$) with severity of illness (SIRS < sepsis < severe sepsis < septic shock). A logistic regression model using sepsis or SIRS as a binary response and ln-IL-6 as a covariate revealed a positive association ($P = .0222$, Figure 3). A higher ln-IL-6 concentration was related to a higher proportion of sepsis and no SIRS (proportional OR = $\exp(\beta) = 1.177$). A logistic regression curve of mortality rate and ln-IL-6 demonstrated the association between a higher plasma level of IL-6 on the day of admission and higher probability for death ($P = .0549$, OR = $\exp(\beta) = 1.1456$) (Figure 4). Time of death was also significantly earlier; the higher measured level of IL-6 on the day of admission was significant ($P = .012$, $\rho_{SP} = -0.404$).

To model the relationship between ln-IL-6 and the survival time of the patients, we conducted a Cox proportional hazards regression (Figure 5). The curve describes the expected survival probabilities for a patient, with a mean value of ln-IL-6. In order to deal with the many tied death times (18 event times on 76 dogs), we chose Efron's approximation. This model detected a significant association ($P = .025$) of ln-IL-6 to the survival time. The calculated coefficient $\exp(\beta_{ln-IL-6*}) = 1.1385$ is the predicted proportional change in the hazard rate for a unit increase in ln-IL-6.

Discussion

As in human patients, SIRS and sepsis pose a major problem for patients and clinicians in small animal medicine. Of particular importance for optimal therapeutic interventions are the early diagnosis of disease and the availability of parameters that qualify as prognostic markers. Studies in animal models and human patients have shown that the massive production of proinflammatory cytokines such as TNF-α, IL-1, and IL-6 is a hallmark of sepsis and of particular importance in the pathophysiology of the disease.[17–37] Surprisingly little information is available regarding serum responses of cytokines in septic canine patients. In this study, we investigated the IL-6 response in dogs diagnosed with sepsis on the basis of established parameters and concluded that the plasma IL-6 response qualifies as an potentially valuable laboratory marker of prognosis in these patients.

We chose to analyze the plasma IL-6 response in our canine patients, since this parameter has been studied most intensively in septic human patients, was shown to be of prognostic value in several studies,[17–32] and can be measured

Table 4. Bacterial organisms isolated from dogs with SIRS and sepsis.

Group	Organism	Tissue Source						
		Blood	Respiratory Tract	Reproductive Tract	Urine	Wound	Abscess	GI Tract
Gram-positive (n=14)	*Staphylococcus* sp (*S epidermidis*-group)	1	—	—	—	—	—	—
	Staphylococcus sp (*S intermedius*)	—	—	1	—	1	—	—
	Staphylococcus sp (β-hemolytic *S intermedius*)	1	—	—	—	2	1	—
	Staphylococcus aureus (methicillin-resistant)	1	—	—	—	—	—	—
	Streptococcus canis	—	—	1	—	1	—	—
	Streptococcus sp (β-hemolytic)	—	—	—	—	—	1	—
	Streptococcus sp (α-hemolytic)	—	1	—	—	—	—	—
	Coccoid (unspecified)	—	—	1	—	1	—	—
Gram-negative (n=19)	*Escherichia coli*	4	1	6	2	—	—	—
	Salmonella enterica sp, *S enterica* group B (*Salmonella typhimurium*)	1	—	—	—	—	—	—
	Acinetobacter baumanii	1	—	—	—	1	—	—
	Prevotella sp	—	—	—	—	—	1	—
	Klebsiella sp	—	—	1	—	—	—	—
	Proteus mirabilis	—	—	—	1	—	—	—
Mixed (n=13)		—	2	1	1	6	1	2
Total		9	4	12	4	12	4	2

by a well-established bioassay.[38–40] In addition, the plasma IL-6 response to different inflammatory stimuli has been studied to some extent in models of canine sepsis (summarized in Table 5), providing an experimental basis for data analysis. From studies in human patients, it appears that the increase in plasma IL-6 concentration is both delayed and sustained in comparison with other cytokines and is therefore more easily detectable than, for example, TNF-α, which shows peak activity early but vanishes within hours after induction.[20] Comparable kinetics have been described in dogs that received fever-inducing doses of LPS, where TNF-α returned to baseline values within 6 hours after fever induction. In contrast, IL-6 values remained high for at least 7 hours and showed a clear dose response,[35] making this cytokine the preferable parameter. Unfortunately, no data are available from experimental models for IL-6 serum kinetics beyond the 7-hour values.

Patients were enrolled in this study if they fulfilled inclusion criteria previously established by de Laforcade et al.[4] Our general patient statistics were, for the most part, consistent with those reported in the literature for naturally occurring cases of sepsis.[4,7–9,16,43–46] The mortality rate among our patients was at the higher end of the described range of 31–50%,[4–9] which might be a consequence of many factors, including the large number of pretreated dogs with severe disease and the suspected referral of many patients at a later stage of disease than in the US-based studies, where primary accession emergency clinics are more common and hold a higher public profile. In the majority (54%) of our patients, sepsis was confirmed through positive bacterial cultures of

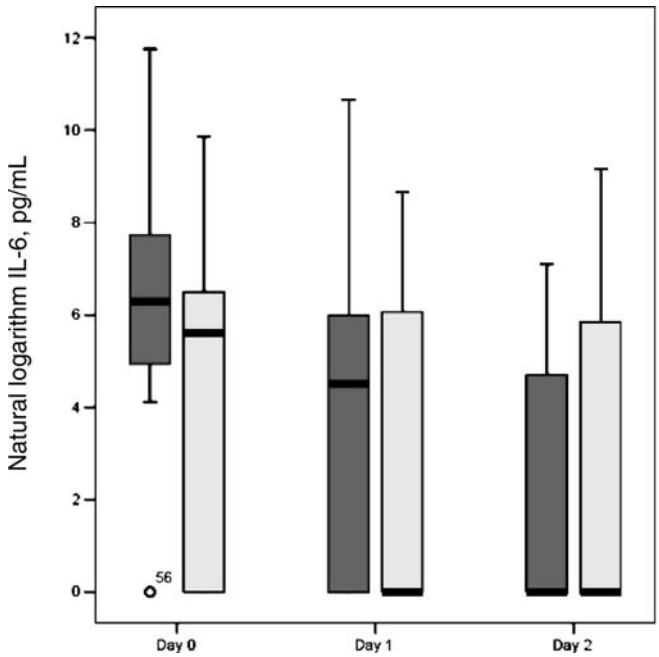

Figure 1. Boxplots of IL-6 concentration in 43 dogs with sepsis (dark bars) and 36 dogs with SIRS (light bars) on the day of admission (day 0) and the following 2 days. Boxes indicate interquartile range, whiskers indicate outliers, and horizontal lines within boxes indicate median. Plasma IL-6 concentration returned to near baseline levels on day 2. Case number 56 (lower left) is an extreme outlier.

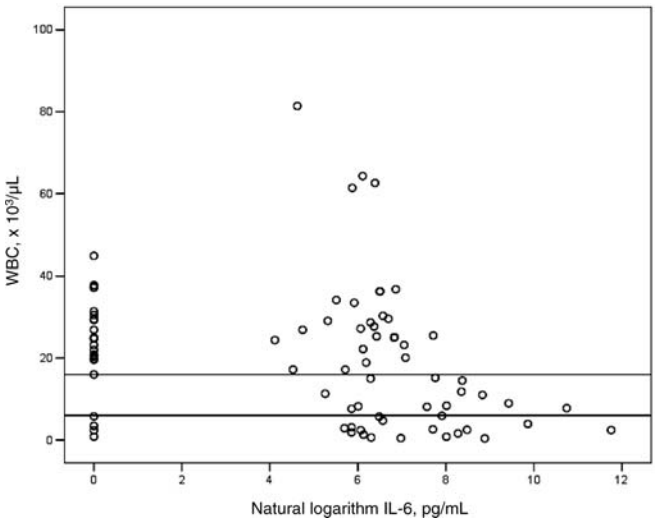

Figure 2. Scatterplot of IL-6 concentration and total WBC count on the day of admission in 79 dogs with sepsis and SIRS. Horizontal lines indicate the reference interval (6–16 $\times$ 10^3 cells/µL) in our laboratory.

blood, urine, and vaginal discharge or tissue samples. The frequency of positive blood cultures was relatively low in comparison with other studies, in which positive results were reported in 100%,[4,9] 81%,[46] 23%,[47] 25%,[43] and 49%[7] of patients. This can be explained by the high percentage (47%) of dogs in our study that were pretreated with antibiotics by the referring clinicians or during emergency service.

Higher IL-6 plasma levels on the day of admission were significantly correlated with a more severe degree of disease,

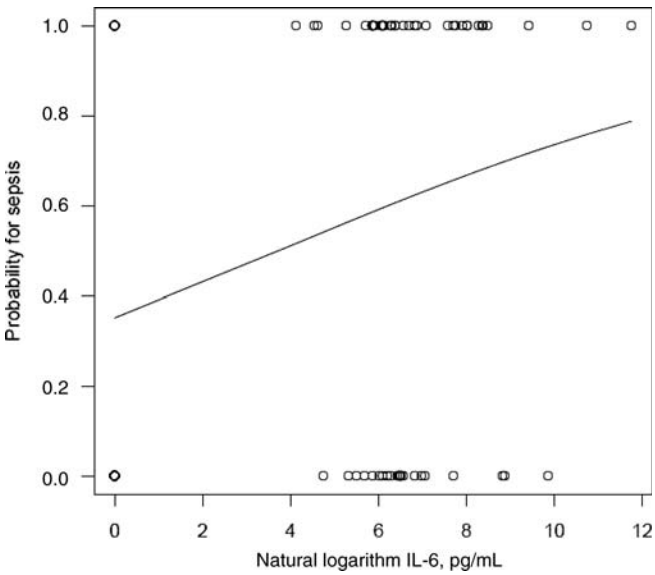

Figure 3. Logistic regression curve of probability for sepsis IL-6 concentration. On the *y* axis, ''1'' (100%) is defined as the event ''sepsis'', and ''0,'' as ''no sepsis'' (ie, SIRS). Circles indicate individual dogs. A higher concentration of IL-6 is related to a higher proportion of sepsis and not SIRS.

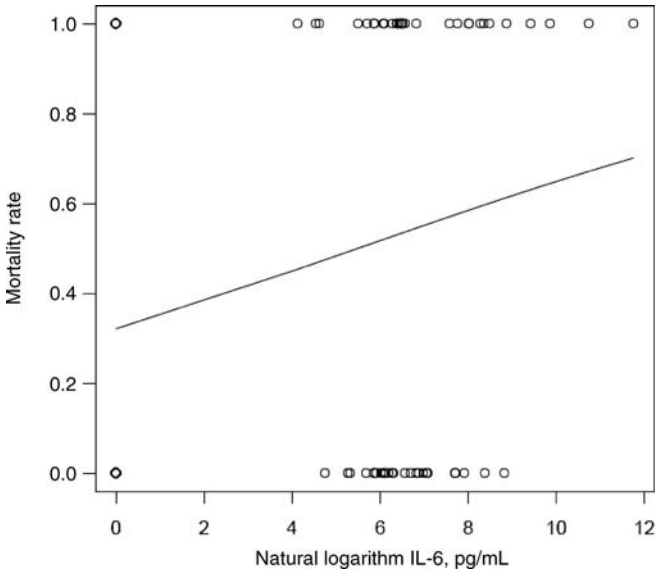

Figure 4. Logistic regression curve of mortality rate and IL-6 concentration on the day of admission (day 0). A higher plasma IL-6 concentration on the day of admission is correlated with a higher probability for death.

increased mortality rates, and earlier fatality. As summarized in Table 3, higher IL-6 concentrations in some dogs in the SIRS group may well be explained by missing confirmation of sepsis. Pneumonia and prostatic abscesses are usually of bacterial origin and were probably septic, but microbiologic cultures either were not possible or were negative. Our results were in good agreement with observations made in human patients, where IL-6 has been proven to predict mortality.[17–32] However, the majority of the animals in our study were euthanized rather than dying naturally. Even though this decision was based on clinical status and probability of

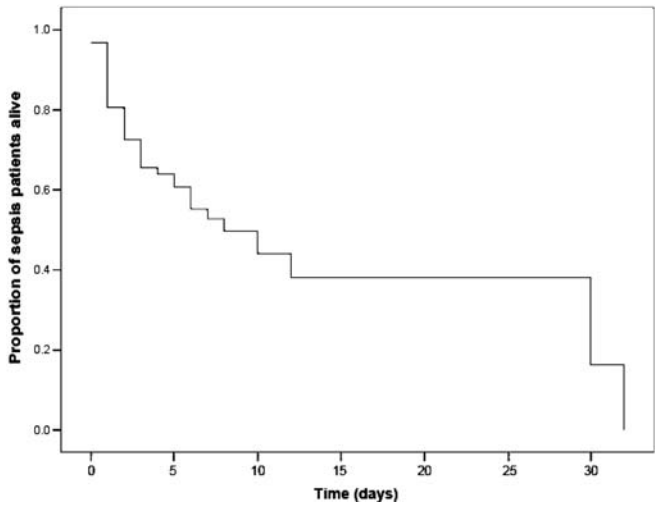

Figure 5. Survival function using a Cox proportional hazards regression. The curve describes the expected survival probabilities for a patient with a mean value of 1000 pg IL-6/mL.

Table 5. A comparison of IL-6 measurement in dogs after experimentally induced sepsis.*

Reference No.	Method of Inducing Inflammation	IL-6 Concentration	Time Point of Maximum Concentration	Method of IL-6 Measurement
37	1 mL turpentine oil, IM	148.9 ± 55.5 U/mL†	12 hours	Bioassay with MH60.BSF2 (murine hybridoma clone)
33	10^9 live *E coli* organisms (B15:0125)/kg, IV over 10 min	110,000 pg/mL	180 min	Bioassay with B9
34	500 μg/kg LPS (*E coli* 055:B5), IV	1.6 optical density at 540 nm	2 hours	Bioassay with MH60.BSF2 (murine hybridoma clone)
35	40, 10, 1 μg/kg LPS (*E coli* 0111:B4), IV	>1,000,000 U/mL with highest dose	2 hours	Bioassay with B9
36	1 mL turpentine oil or 40 μg/kg LPS (*E coli* 055:B5), IM	17,545.5 ± 5545.5 cpm	2 hours	Bioassay with MH60.BSF2 (murine hybridoma clone)

*LPS indicates lipopolysaccharide; cpm, counts per minute.

†One unit (U) was defined as the amount that caused half-maximal proliferation in the standard curve.

survival, euthanasia is still an unavoidable source of considerable bias and may serve as a potential source of error.

We conclude that routine analysis of plasma IL-6 levels may be an important laboratory parameter of sepsis in canine medicine and should be further investigated in detail. Several studies in humans have shown that IL-6 kinetics during the course of sepsis plays a role in prognosis. Survivors were characterized by a rapid decrease in plasma IL-6 concentration, whereas nonsurvivors had constantly high levels of IL-6.[17,19,21,24,31] IL-6 was undetectable in 6 dogs with sepsis and 2 dogs with severe sepsis, which may be explained by cytokine kinetics. As confirmed by the experimental studies, peak levels of IL-6 are known to occur after onset of the septic incident. These patients could have been sick for a longer time, and other cytokines may already have replaced IL-6 as the main cytokine. We did not perform kinetic studies in our patients as it was unclear if IL-6 would be of prognostic value at all. Interestingly, more detailed data analysis revealed that although elevated plasma IL-6 concentrations were found in dogs with both SIRS and sepsis, significantly higher levels were found in septic patients. Again, this has also been shown in human patients[48] and further supports the hypothesis that the IL-6 response follows a highly conserved pattern in humans and dogs. An interesting aspect for further studies could be the investigation of IL-6 levels in sick dogs with various illnesses, in contrast to those with sepsis.

In summary, our study establishes the basis for future work aimed at a better understanding of SIRS and sepsis in canine patients. Subsequent studies should further scrutinize both the value of plasma IL-6 quantification and IL-6 kinetics as a prognostic marker. A rapid test will have to be developed and evaluated in order to increase practicability; in fact, an ELISA canine IL-6 kit (R&D Systems, Minneapolis, MN, USA) recently became available, unfortunately only after the completion of this study. This study could also help to establish the canine patient as a model for further research into the pathophysiology of SIRS and sepsis and the development and validation of new prognostic markers and therapeutic concepts.

Acknowledgments

The authors would like to thank Dr. Bettenay, BVSc (hons), MACVSc, Dipl ECVD, for revising the manuscript and for correcting language errors.

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