J Vet Intern Med 2011;25:453–459

Evaluation of Serum NT-pCNP as a Diagnostic and Prognostic Biomarker for Sepsis in Dogs

A.E. DeClue, K. Osterbur, A. Bigio, and C.R. Sharp

Background: There is a need for diagnostic biomarkers that can rapidly differentiate dogs with sepsis from dogs with noninfectious forms of systemic inflammatory response syndrome (NSIRS).

Objectives: To compare serum NT-pCNP concentrations among dogs with various forms of sepsis, NSIRS, and healthy controls and to evaluate the use of serum NT-pCNP for the diagnosis of various forms of sepsis in dogs.

Animals: One hundred and twelve dogs including 63 critically ill dogs (sepsis n = 29; NSIRS n = 34) and 49 healthy control dogs.

Methods: Prospective clinical investigation. Serum samples were collected for NT-pCNP measurement from dogs with sepsis or NSIRS within 24 hours of intensive care unit admission or at the time of presentation for healthy dogs. Dogs with sepsis were subclassified based on the anatomic region of infection. Serum NT-pCNP concentrations were compared among sepsis, NSIRS and healthy groups as well as among sepsis subgroups. The area under the curve (AUC), sensitivity, and specificity for identifying dogs with sepsis were determined.

Results: Using a cut-off value of 10.1 pmol/L, AUC, sensitivity, and specificity of NT-pCNP for differentiating dogs with sepsis from dogs with NSIRS or healthy control dogs were 0.71 (95% CI, 0.58–0.85), 65.5% (45.7–82.1%), and 89.2% (80.4–94.9%), respectively. Serum NT-pCNP had poor sensitivity for peritoneal sources of sepsis; AUC [0.92 (0.81–1.0)], sensitivity [94% (71–100%)], and specificity [89% (80–95%)] improved when these dogs were excluded. Serum NT-pCNP concentration was not associated with survival in the sepsis group.

Conclusions and Clinical Importance: Serum NT-pCNP is a promising diagnostic biomarker for sepsis but is a poor indicator of septic peritonitis.

Key words: Immunology; Infection; Systemic inflammatory response syndrome; Veterinary.

Sepsis, defined as the systemic inflammatory response to infection, is associated with substantial morbidity and, in many cases, death in the dog.[1–3] Differentiating between sepsis and noninfectious forms of systemic inflammatory response syndrome (NSIRS) is a diagnostic challenge in many dogs because the inflammatory response and clinical presentation for dogs with sepsis and NSIRS can be indistinguishable. The most commonly used methods of detecting the microorganism causing sepsis such as culture, cytology, serology, or histopathology are often not rapid enough to allow immediate identification of sepsis. There is a need for biomarkers that identify dogs with sepsis so that a rapid diagnosis is achieved and appropriate specific therapy initiated. Identifying prognostic biomarkers for sepsis is also important, both for management of the individual dog and the population as whole. Evaluation of prognostic biomarkers in individual dogs is helpful in guiding decision-making with the pet owner, while population-centered biomarkers allow comparison of disease severity in dogs in multicenter investigations and among investigations. Currently, diagnostic and prognostic biomarkers for sepsis in dogs are lacking.

C-type natriuretic peptide (CNP) is expressed primarily by the vascular endothelium and macrophages in response to several stimuli, including inflammatory mediators such as tumor necrosis factor, interleukin-1β, and transforming growth factor-β, that are known to be important in the pathogenesis of sepsis.[4–6] Importantly, microbial products including lipopolysaccharide directly stimulate CNP production.[5] CNP is a particularly interesting biomarker for sepsis because CNP might be important in the innate immune response to infection. CNP exhibits antimicrobial activity by both inhibiting microbial growth and by modifying the pathogenicity of microorganisms.[7,8] People with severe sepsis have

Abbreviations:

AUC	area under the curve
95%CI	95% confidence interval
CNP	C-type natriuretic peptide
ICU	intensive care unit
IMHA	immune-mediated hemolytic anemia
NSIRS	SIRS from a noninfectious source
NT-pCNP	amino-terminal portion of pro-CNP
pCNP	pro-CNP
ROC	receiver-operating characteristic
SIRS	systemic inflammatory response syndrome

From the Comparative Internal Medicine Laboratory, Department of Veterinary Medicine and Surgery, College of Veterinary Medicine, Columbia, MO. Dr Sharp is presently affiliated with Cummings School of Veterinary Medicine at Tufts University, North Grafton, MA. Dr Bigio is presently affiliated with College of Veterinary Medicine, University of Illinois, Champaign—Urbana, IL. This work was done at the College of Veterinary Medicine, Veterinary Medical Teaching Hospital, University of Missouri, Columbia, MO. A portion of these data were presented in abstract form at the 15th Annual International Veterinary Emergency and Critical Care Symposium, Chicago, IL and the 28th Annual American College of Veterinary Internal Medicine Forum, Anaheim, CA.

Corresponding author: A.E. DeClue, DVM, MS, DACVIM (SAIM), Comparative Internal Medicine Laboratory, Department of Veterinary Medicine and Surgery, College of Veterinary Medicine, University of Missouri, 900 E. Campus Drive, Columbia, MO 65211; e-mail: decluea@missouri.edu.

Submitted August 31, 2010; Revised January 24, 2011; Accepted February 7, 2011.

Copyright © 2011 by the American College of Veterinary Internal Medicine

10.1111/j.1939-1676.2011.0713.x

significantly greater serum CNP concentration than do healthy people or people with congestive heart failure, hypertension, or chronic kidney disease.[9] However, the clinical use of CNP as a biomarker for sepsis in people has been hindered by poor stability of the molecule in peripheral blood.[10]

Recently, identification and assay development for the amino-terminal (NT) portion of proCNP (pCNP) has offered a new method for evaluating CNP production. Compared with CNP, NT-pCNP is a larger molecule; it has a longer half-life in circulation and does not readily cross react with other natriuretic peptides, thus affording greater potential for clinical utility.[10–12] Because CNP and NT-pCNP are produced and secreted in equimolar amounts after intracellular proteolytic cleavage of pro-CNP, NT-pCNP is a reliable marker of CNP biosynthesis.[10,12–14] In the current study, serum NT-pCNP concentrations were evaluated within 24 hours of admission to an intensive care unit (ICU) and compared between dogs with sepsis, dogs with NSIRS, and healthy dogs to determine if NT-pCNP could be used as a biomarker for sepsis in dogs. We hypothesized that dogs with sepsis from a variety of sources would have significantly greater serum NT-pCNP concentrations compared with dogs with NSIRS or healthy controls; that serum NT-pCNP concentrations would be similar among dogs with various sources of sepsis; and that serum NT-pCNP concentration would be a sensitive and specific diagnostic and prognostic biomarker for sepsis in dogs.

Materials and Methods

Dogs that presented to the University of Missouri Veterinary Medical Teaching Hospital ICU between October 2008 and May 2010 were eligible for inclusion in this prospective observational study. Client consent was obtained. 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, CBC, as well as appropriate diagnostic testing to document the presence of infection. The 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 <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.

Sepsis and NSIRS Groups

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°C) or hyperthermia (temperature $\geq$39.7°C); tachycardia (heart rate $\geq$160 beats per minute); tachypnea (respiratory rate $\geq$40 breaths per minute); and a 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).[3,15–18] Dogs were required to have a SIRS score of $\geq$2 to be enrolled in the study. Infectious disease testing was performed as appropriate for the clinical findings in each dog. Samples for cytology, culture, histopathology, or antigen/antibody testing were collected from the appropriate anatomic region in dogs where a specific anatomic region of interest could be identified (eg, dogs with peritonitis would have collection of abdominal fluid and/or tissues; dogs with evidence of pulmonary disease would have collection of broncho-alveolar lavage fluid and/or lung tissue). For dogs with immune-mediated hemolytic anemia (IMHA), testing for vector-borne disease based on geographic exposure to vectors was performed. Dogs that presented with historical or clinical evidence of acute trauma ($<$4 hours) and no evidence of bowel or bladder rupture on examination or radiography within 72 hours of hospital admission; intoxication; or endocrinopathy without other clinical evidence of infection did not undergo testing for infectious diseases. Acute respiratory distress syndrome was defined based on the Dorothy Russell Havemeyer Working Group on ALI and ARDS in Veterinary Medicine consensus definition.[19] Dogs that had evidence of infection based on cytology, culture, histopathology, and/or antigen/antibody testing were assigned to the sepsis group. The sepsis group was further divided into subgroups (abdominal, musculoskeletal, peritoneal, pleural, pulmonary, subcutaneous, systemic, urogenital) based on the source of sepsis. The NSIRS group consisted of dogs that did not have evidence of infection. 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 proceeding sample collection and were required to have an unremarkable history for the preceding month, physical examination, CBC, and plasma biochemical profile.

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 samples were collected into evacuated glass tubes within 24 hours of ICU admission for sepsis or NSIRS dogs. Blood was centrifuged (1,500 × *g*, 7 minutes) and serum harvested within 1 hour of sample collection. The serum was placed in an airtight, freezer-resistant plastic tube and stored for a maximum of 9 months at −80°C for batch analysis. Tubes were coded so that the identity of the sample was only known by the investigator and not to the laboratory.

NT-pCNP Assay

Serum NT-pCNP was evaluated by a commercial laboratory.[a] The NT-pCNP assay was performed by a sandwich ELISA, which utilizes a highly purified polyclonal sheep antibody directed against amino acids 1–19 and 30–50 of human NT-pCNP (96% homologous with canine pCNP [http://www.ncbi.nlm.nih.gov/genome/guide/dog; http://blast.ncbi.nlm.nih.gov/Blast.cgi]). Interassay and intra-assay coefficients of variation for this assay using dog serum are 7–9 and 4–5%, respectively. Percent recovery is 91.3 ± 5.9% using spiked canine serum samples with 6, 18, and 30 pmol of NT-pCNP. Linearity was tested by performing serial dilutions of canine serum over a series of NT-pCNP concentrations, which gave linear regression analysis results (R^2=0.991). To assess day-to-day variation in serum NT-pCNP concentrations, serum was collected from 8 healthy dogs every 24 hours for 5 days. The day-to-day coefficient of variation for serum NT-pCNP in healthy dogs was 9.8 ± 2.5% over the 5-day period. The effects of storage at −80°C were assessed by measuring serum NT-pCNP concentration (range, 3.4–38.1 pmol/L)

in 10 serum samples evaluated immediately and again after storage at −80°C for 9 months; recovery was 110 ± 11% after storage. The lower limit of detection of this assay is 0.55 pmol/L.

Statistical Analyses

Statistical analysis was performed by commercially available software.[b] The Kolmogorov-Smirnov statistical test for normality was used to determine if data were normally distributed. When possible, data were transformed by the natural log function to fulfill normality assumptions. A Kruskal-Wallis 1-way analysis of variance on ranks with posthoc Dunn's multiple comparison procedure was used to compare serum NT-pCNP concentrations among the sepsis, NSIRS and healthy control groups. Serum NT-pCNP was compared among sepsis subgroups by a 1-way analysis of variance with posthoc Fisher least significant difference method. A Mann-Whitney rank-sum test was used to compare age, weight, duration of hospitalization and SIRS score between the sepsis and NSIRS groups. 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. Mortality was compared between the sepsis and NSIRS groups using a Fisher exact test. Logistic regression was used to assess the relationship between serum NT-pCNP concentration and mortality. A P-value of $<.05$ was considered statistically significant.

Results

Study Population

A total of 112 dogs met the criteria and were enrolled in this study, including 63 critically ill dogs and 49 healthy control dogs. Of the 63 critically ill dogs, 29 were in the sepsis group and 34 in the NSIRS group.

The sepsis group consisted of 3 sexually intact females, 7 spayed females, 8 intact males, and 11 neutered males that were aged 7.2 ± 4.1 years (range 0.66–14 years) and weighing 26.8 ± 16.6 kg (range 1.8–70 kg). Breeds represented included Labrador Retriever (4), Dachshund (3), mixed breed (2), Boxer (2), and Australian Shepherd (2). The number of dogs in each sepsis subgroup is noted in Table 1. Infection was identified based on culture (12), cytology (18), histopathology (7), and/or parvovirus antigen test (1). Infection was caused by bacterial (24), fungal (3), combined bacterial and fungal (1) or viral (1) organisms. The most commonly cultured organisms included *Escherichia coli* (4), *Enterococcus* spp. (2), *Pasteurella* spp. (2), and *Streptococcus* spp. (2). Additionally, the fungal organisms *Blastomyces dermatitidis* (2), *Histoplasma capsulatum* (1), and *Candida albicans* (1) were identified based on cytology.

The NSIRS group consisted of 1 sexually intact female, 18 spayed females, 7 intact males, and 8 neutered males that were aged 7.0 ± 3.8 years (range 1.3–14 years) and weighing 26.3 ± 16.3 kg (range 2.3–62 kg). Breeds represented included mixed breed (4), Border Collie (3), Golden Retriever (3), Labrador Retriever (3), Yorkshire Terrier (2), Bernese Mountain Dog (2), Beagle (2), and Chihuahua (2). Dogs in the NSIRS group had a variety of conditions, including neoplasia (8; hemangiosarcoma [2], lymphoma [2], hepatocellular carcinoma, malignant histiocytosis, lymphocytic leukemia, mast cell tumor), IMHA (5), acute respiratory distress syndrome (3; secondary to airway obstruction [2] and immune-mediated vasculitis [1]), trauma (2), pancreatitis (2), toxicity (2), immune-mediated polyarthritis (2), hypoadrenocorticism (1), acute renal failure (1), noninfectious aspiration pneumonia (1), chylothorax (1), gallbladder mucocele (1), gastric dilatation and volvulus (1), Shar-Pei fever (1), spontaneous pneumothorax (1), snake envenomation (1), hemoabdomen (1).

Table 1. Anatomic subgroup classification based on the source of sepsis for dogs in the sepsis group.

Source of Sepsis	Final Diagnosis	Number of Dogs
Peritoneal (n = 12)	Gastrointestinal tract rupture	7
	Primary septic peritonitis	2
	Urosepsis	1
	Prostatic abscess rupture	1
	Iatrogenic	1
Pulmonary (n = 6)	Pneumonia	6
Abdominal (n = 5)	Hepatic abscess	2
	Bacterial splenitis	1
	Bacterial gastroenteritis	1
	Parvoviral enteritis	1
Urogenital (n = 2)	Pyometra	1
	Prostatitis	1
Subcutaneous (n = 2)	Abscess	2
Systemic (n = 1)	Bacterial myocarditis and brain abscess	1
Musculoskeletal (n = 1)	Bacterial myositis	1

The healthy control dogs included 25 spayed females and 24 castrated males that were aged 4.6 ± 3.6 years (range, 0.5–16 years) and weighed 22.5 kg (range, 6.7–50.5 kg). Breeds represented included mixed breed (28), Golden Retriever (3), Labrador Retriever (3), Belgian Malinois (2), Border Collie (2), and Weimaraner (2).

The duration of hospitalization was 5.5 days (mean, range 1–41 days) for the sepsis group and 3.3 days (range 1–28 days) for the NSIRS group. The sepsis group SIRS score was 2.5 ± 0.6 (mean ± SD); 15 dogs had a score of 2, 13 dogs had a score of 3, and 1 dog had a score of 4. The NSIRS group SIRS score was 2.3 ± 0.5; 24 dogs had a score of 2, 9 dogs had a score of 3, and 1 dog had a score of 4. There was no significant difference in age, weight, duration of hospitalization, or SIRS score between groups nor was there a difference in rectal temperature [median (range); sepsis, 39.3°C (35.9–40.7); NSIRS, 38.8°C (35.5–41.1)], heart rate [sepsis, 120 (65–210); NSIRS 116 (60–102), beats per minute], respiratory rate [sepsis, 38 (10–80); NSIRS, 42 (12–102), breaths per minute] or white blood cell count [sepsis, 16.1 (0.8–54.4); NSIRS, 15.9 (0.9–74.2), 10^3 cells/μL] between groups.

Comparison of Serum NT-pCNP among Groups and Sepsis Subgroups

Serum NT-pCNP concentration was significantly greater in the sepsis group (median, Q1, Q3; 11, 5.2, 32.8 pmol/L) compared with the NSIRS (5.2, 3.3,

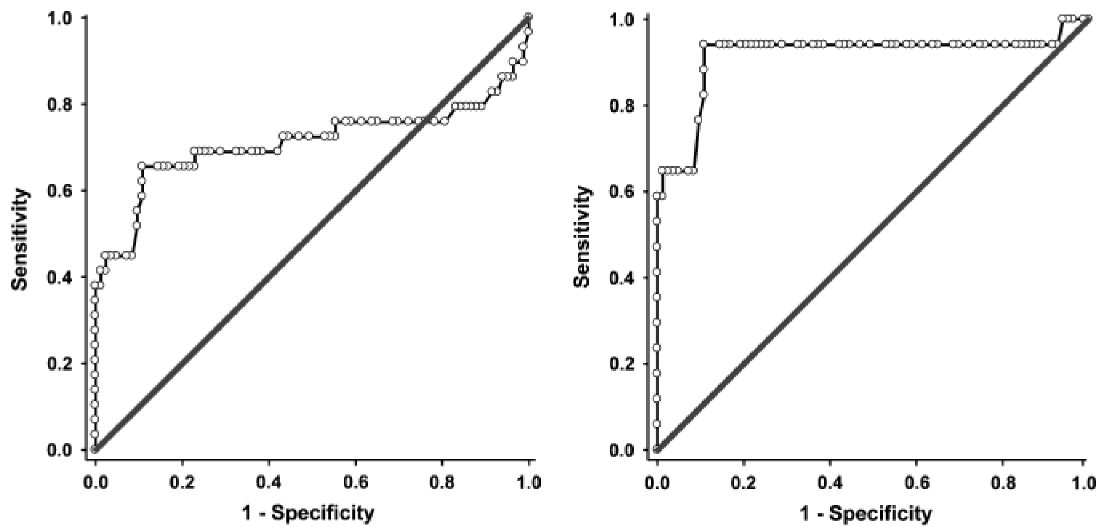

Fig 1. Receiver-operating characteristic (ROC) curves comparing the diagnostic sensitivity and 1-specificity of serum amino-terminal portion of pro-CNP concentration for differentiating dogs with sepsis from dogs with systemic inflammatory response syndrome and healthy dogs (black line). The gray line represents an area under the curve of 0.5. The ROC curve on the left includes all dogs with sepsis. The ROC curve on the right includes only dogs with nonperitoneal sources of sepsis.

7.6 pmol/L; $P < .001$) and healthy control groups (6.4, 4.8, 8.9 pmol/L; $P < .001$). There was no significant difference in the serum NT-pCNP concentration between the NSIRS and healthy control groups. Based on evaluation of an ROC curve, the AUC for NT-pCNP for differentiating dogs with sepsis from dogs with NSIRS or healthy control dogs was 0.71 (95% confidence interval [CI], 0.58–0.85) (Fig 1). The sensitivity was 65.5% (95% CI, 45.7–82.1%) and the specificity was 89.2% (95% CI, 80.4–94.9%) for differentiating dogs with sepsis from dogs with NSIRS or healthy control dogs at a cut-off value of 10.1 pmol/L. Using this cut-off value, there were 9 false positives and 10 false negatives (Fig 2). The positive and negative likelihood ratios were 6.06 and 0.386, respectively.

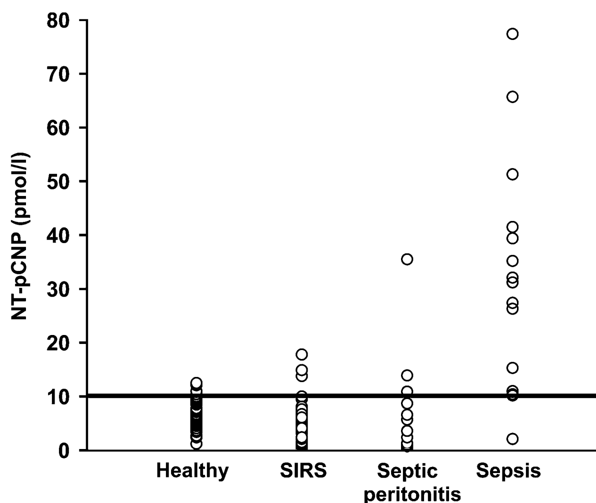

Fig 2. Comparison of serum amino-terminal portion of pro-CNP (NT-pCNP) concentrations among healthy dogs and dogs with noninfectious systemic inflammatory response syndrome (NSIRS), septic peritonitis and dogs with nonperitoneal sources of sepsis (Sepsis). The black line indicates the cut-off value for a positive test result. Open circles below the black line represent negative test results while open circles above the black line indicate positive test results.

The false positives consisted of 2 dogs with IMHA, 1 dog with a toxicosis (because of suspected tremorgenic mycotoxin ingestion), and 6 healthy dogs (Fig 2). There were 10 false negatives, which included 1 dog in the pulmonary subgroup with bacterial bronchopneumonia and 9 dogs in the peritoneal subgroup (septic peritonitis). Nine of 12 dogs in the peritoneal sepsis subgroup had a false-negative test. There were various inciting causes of peritoneal sepsis in the dogs with false-negative test results. These included perforated gastric ulcer (2), perforated duodenal ulcer, gun shot wound with bowel perforation, perforated intestinal mass (lymphoma), colonic perforation, bacterial contamination from repeated abdominocentesis, dehiscence of 2 enterotomy and a jejunostomy tube sites, and both bacterial and fungal (Candida) peritonitis post-laparotomy.

Serum NT-pCNP was compared among the sepsis subgroups. Because the urogenital, systemic, subcutaneous, and musculoskeletal groups had 2 or less dogs per category, they were combined. Therefore, peritoneal, abdominal, pulmonary, and a combined subgroup were used for analysis. Dogs in the peritoneal subgroup (mean $\pm$ SD, 7.6 $\pm$ 9.8 pmol/L) had significantly lower serum NT-pCNP concentrations than dogs in the abdominal (32.2 $\pm$ 14.4 pmol/L; $P = .001$), pulmonary (19 $\pm$ 23.3 pmol/L; $P = .04$), and combined (37.2 $\pm$ 22.5 pmol/L; $P < .001$) subgroups. There were no other significant differences among subgroups. Because the peritoneal subgroup had significantly lower serum NT-pCNP concentrations than the other subgroups, we evaluated the sensitivity and specificity of this diagnostic test when the peritoneal subgroup was removed. When all dogs in the peritoneal subgroup were removed from the analysis, the AUC was 0.92 (95% CI, 0.81–1.0), the sensitivity was 94% (95% CI, 71–100%) and the specificity was 89% (95% CI, 80–95%) for differentiating

dogs with sepsis from dogs with NSIRS or healthy control dogs (Fig 1). The positive and negative likelihood ratios were 9.4 and 0.067, respectively.

There was no significant difference in survival to hospital discharge between the sepsis group (58.6%) and the NSIRS group (46.9%). Serum NT-pCNP concentration did not significantly correlate with survival in the sepsis group (odds ratio 0.995; 95% CI 0.959–1.032; $P = .792$).

Discussion

In this investigation we found that serum NT-pCNP had a sensitivity of 65.5%, specificity of 89.2%, positive likelihood ratio of 6.06, and negative likelihood ratio of 0.386 for differentiating dogs with sepsis from dogs with NSIRS or healthy control dogs in this population overall and serum NT-pCNP concentrations at ICU admission were not associated with survival in the sepsis group. Serum NT-pCNP identified sepsis in dogs with a variety of infection types including bacterial, fungal, and viral infections involving a variety of organ systems. In comparing sepsis subgroups, NT-pCNP had poor sensitivity in dogs with a peritoneal source of sepsis. Septic peritonitis might be a unique form of sepsis in dogs as it is in other species which results in altered concentrations of inflammatory markers in the blood compared with the localized area of infection.[20–29] Excluding the peritoneal source of sepsis subgroup, NT-pCNP had a sensitivity, specificity, positive likelihood ratio, and negative likelihood ratio of 94, 89%, 9.4, and 0.067, respectively, for differentiating dogs with sepsis from dogs with NSIRS or healthy control dogs. These data suggest that serum NT-pCNP could be a valuable diagnostic test for dogs with nonperitoneal sources of sepsis.

The quest to find more rapid, accurate means to identify and prognosticate sepsis has resulted in evaluation of a variety of potential biomarkers. Endotoxin activity, tumor necrosis factor, interleukin-6, nitric oxide, interleukin-10, procalcitonin mRNA, markers of coagulation, von Willebrand factor antigen concentration, C-reactive protein and a variety of clinical findings including heart rate, respiratory rate, white blood cell count, or markers of organ or metabolic dysfunction have been evaluated as possible diagnostic, prognostic, or both, biomarkers for sepsis in dogs.[1,3,30–42] Many of these studies either focused on the prognostic importance of the particular parameter or did not evaluate the sensitivity and specificity of the marker of interest. Therefore, it is difficult to compare the performance of NT-pCNP to each of these biomarkers.

Clinical parameters such as heart rate, respiratory rate, rectal temperature, and white blood cell count are often used in the diagnosis of sepsis. Investigators have evaluated the sensitivity and specificity of these parameters for the diagnosis of sepsis in dogs. Hauptman et al[3] reported sensitivity and specificity for rectal temperature (sensitivity, specificity; 63, 72%), heart rate (47, 81%), respiratory rate (85, 12%), and white blood cell count (87, 69%) in differentiating sepsis from other forms of noninfectious illness. We found no difference between the sepsis and NSIRS groups in regard to rectal temperature, heart rate, respiratory rate, white blood cell count. While SIRS criteria might help the clinician identify illness, they do not accurately differentiate infectious from noninfectious causes of that illness. This highlights the importance of identifying diagnostic biomarkers for sepsis in dogs.

Based on our data, serum NT-pCNP is a promising biomarker for nonperitoneal sources of sepsis in dogs. Serum NT-pCNP had a sensitivity of 65.5%, specificity of 89.2%, and AUC of 0.71, indicating that this test had moderate accuracy for discriminating between dogs with and without sepsis.[43] Likewise, serum NT-pCNP concentrations > 10.1 pmol/L predict sepsis with a positive likelihood ratio of 6.06 and a serum NT-pCNP concentration < 10.1 pmol/L excludes sepsis with a likelihood ratio of 0.386, indicating moderate to poor impact on posttest probability of disease.[43] However, when the peritoneal sepsis subgroup was removed, the sensitivity improved dramatically from 65.5 to 94%. The AUC also markedly increased to 0.92, which is in the high discrimination accuracy range. Positive and negative likelihood ratios were 9.4 and 0.067, indicating that serum NT-pCNP substantially improved the posttest probability estimates for the diagnosis of nonperitoneal sources of sepsis.

Serum NT-pCNP performed poorly in dogs with peritoneal sources of sepsis. Of the 10 dogs with false-negative NT-pCNP test results, 9 were in the peritoneal subgroup and 1 dog was in the pulmonary subgroup (bacterial bronchopneumonia likely secondary to aspiration of food). The dog with pneumonia had been treated with antibiotics for several days before presentation and was clinically improving. In this instance, the NT-pCNP concentration could have been higher in the days proceeding presentation to our hospital and the low concentration of NT-pCNP might have been evidence of resolving infection.

It is unclear why 75% of dogs in the peritoneal sepsis subgroup had a false-negative test result. One possible explanation is that the anatomic region of sepsis alters the immune response and physiologic manifestation of disease.[21–25] Variation in organ and tissue-specific response (ie, compartmentalization) is a well-described phenomenon in multiple species, particularly in relation to peritoneal sepsis.[26–29] Compartmentalization results in greater concentrations of inflammatory markers in peritoneal fluid than in the blood and a lack of correlation between peritoneal fluid and serum inflammatory marker concentrations in people with septic peritonitis.[29] This is in contrast to other organs or anatomic regions where compartmentalization is not recognized and inflammatory markers in the blood could be more exaggerated than in the individual tissue.[20] Additionally, the peritoneum might act as a barrier decreasing absorption of molecules and thus absorption of NT-pCNP into the systemic circulation.[44] In dogs with septic peritonitis, tumor necrosis factor concentrations are greater in the abdominal fluid than in the serum suggesting that compartmentalization occurs in this species as well.[45] It is possible that the lower than expected serum concentrations of NT-pCNP in dogs in the peritoneal sepsis

subgroup relates to these unique features of septic peritonitis. Future investigations comparing NT-pCNP concentrations in peritoneal fluid and serum could help investigate this hypothesis. Nevertheless, based on these data, serum NT-pCNP is not a sensitive means to diagnose septic peritonitis and should not be used in this subset of dogs.

In the NSIRS group, there were 3 false-positive test results. There are several plausible explanations for these results. All 3 dogs with false-positive test results had conditions that might have resulted in gastrointestinal barrier dysfunction and bacterial or endotoxin translocation. Of these cases, 2/3 had clinical signs that suggested gastrointestinal barrier dysfunction. Thus, it is possible that there was an infectious component secondary to the primary disease process and the increased serum NT-pCNP concentration was actually a true-positive result. Additionally, CNP could have been induced through a pathway unrelated to infection in these dogs. In bovine endothelial cells, human peripheral blood leukocytes, and mouse peritoneal macrophages, CNP is induced by several inflammatory mediators and it is possible that similar pathways for CNP induction are present in the dog.[4–6] However, most of the dogs in the NSIRS group had pathologic processes known to induce inflammatory mediator production in dogs.[46–50] If the false-positive results were simply because of activation of inflammatory pathways unrelated to infection, it would be rational to expect a greater number of false-positive test results in the NSIRS group. This hypothesis could be further evaluated by concurrent measurement of NT-pCNP and key proinflammatory mediators in dogs and through ex vivo work evaluating induction pathways for CNP in canine vascular endothelium and macrophages. There were also 6 false positives in the healthy control dogs. The dogs with false positives all had serum NT-pCNP concentrations $\leq$12.5 pmol/L. The false positives in the healthy group could represent normal biologic variation.

There are several limitations to this study that should be noted. There was a small sample size and there were relatively few dogs with fungal or vial infections included in this study. Additionally, it is possible that some of the dogs placed in the noninfectious NSIRS category might have had an infection thus skewing our results. Investigation of larger, more diverse population of dogs is needed to confirm our findings. Some of the dogs in this investigation had treatments administered before sample collection and this could have influenced the results. Serum NT-pCNP concentrations were only evaluated at a single time point, with in 24 hours of admission to the ICU. It is possible that evaluation of NT-pCNP at a different time point or serially would have improved diagnostic sensitivity. Because serum NT-pCNP concentrations at ICU admission did not correlate with survival in this study, serial evaluation of NT-pCNP could be a more effective means to predict outcome. Finally, to optimize the clinical usefulness of this biomarker, a cage-side test should be developed and evaluated.

Serum NT-pCNP has good specificity (89%) for the diagnosis of sepsis in dogs. However, NT-pCNP had a sensitivity of only 25% in dogs with a peritoneal source of sepsis, which reduced the overall sensitivity of this diagnostic test to 65.5%. For nonperitoneal source sepsis, serum NT-pCNP has a good sensitivity (92%). Serum NT-pCNP has promise as a new diagnostic test for sepsis in dogs, but caution should be used in interpreting negative test results from dogs with clinical signs suggesting septic peritonitis. Additional investigations are needed to confirm these data and investigate if peritoneal fluid would be a better sample type for NT-pCNP analysis for the identification of septic peritonitis in dogs.

Footnotes

[a] NT-proCNP ELISA, Biomedica Medizinprodukte GmbH & Co KG, Vienna, Austria. Assay performed by Veterinary Diagnostics Institute, Simi Valley, CA

[b] SigmaStat, Systat Software Inc, Chicago, IL

Acknowledgment

Funding: Veterinary Diagnostics Institute provided NT-pCNP analysis and partially funded technician salary for this study.

References

1. 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.
2. 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.
3. 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.
4. Suga S, Nakao K, Itoh H, et al. Endothelial production of C-type natriuretic peptide and its marked augmentation by transforming growth factor-beta. Possible existence of "vascular natriuretic peptide system." J Clin Invest 1992;90:1145–1149.
5. Suga S, Itoh H, Komatsu Y, et al. Cytokine-induced C-type natriuretic peptide (CNP) secretion from vascular endothelial cells—evidence for CNP as a novel autocrine/paracrine regulator from endothelial cells. Endocrinology 1993;133:3038–3041.
6. Kubo A, Isumi Y, Ishizaka Y, et al. C-type natriuretic peptide is synthesized and secreted from leukemia cell lines, peripheral blood cells, and peritoneal macrophages. Exp Hematol 2001;29:609–615.
7. Veron W, Lesouhaitier O, Pennanec X, et al. Natriuretic peptides affect *Pseudomonas aeruginosa* and specifically modify lipopolysaccharide biosynthesis. FEBS J 2007;274:5852–5864.
8. Veron W, Orange N, Feuilloley MG, et al. Natriuretic peptides modify *Pseudomonas fluorescens* cytotoxicity by regulating cyclic nucleotides and modifying LPS structure. BMC Microbiol 2008;8:114.
9. Hama N, Itoh H, Shirakami G, et al. Detection of C-type natriuretic peptide in human circulation and marked increase of plasma CNP level in septic shock patients. Biochem Biophys Res Commun 1994;198:1177–1182.
10. Del Ry S, Cabiati M, Stefano T, et al. Comparison of NT-proCNP and CNP plasma levels in heart failure, diabetes and cirrhosis patients. Regul Pept 2011;166:15–20.

11. Prickett TC, Yandle TG, Nicholls MG, et al. Identification of amino-terminal pro-C-type natriuretic peptide in human plasma. Biochem Biophys Res Commun 2001;286:513–517.

12. Palmer SC, Prickett TC, Espiner EA, et al. Regional release and clearance of C-type natriuretic peptides in the human circulation and relation to cardiac function. Hypertension 2009;54: 612–618.

13. Tawaragi Y, Fuchimura K, Tanaka S, et al. Gene and precursor structures of human C-type natriuretic peptide. Biochem Biophys Res Commun 1991;175:645–651.

14. Wu C, Wu F, Pan J, et al. Furin-mediated processing of pro-C-type natriuretic peptide. J Biol Chem 2003;278:25847–25852.

15. 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.

16. 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.

17. 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 89:174–178.

18. Matijatko V, Kis I, Torti M, et al. Septic shock in canine babesiosis. Vet Parasitol 2009;162:263–270.

19. Wilkins P, Otto C, Baumgardner J, et al. Acute lung injury and acute respiratory distress syndromes in veterinary medicine: Consensus definitions: The Dorothy Russell Havemeyer working group on ALI and ARDS in veterinary medicine. J Vet Emergency Crit Care 2007;17:333–339.

20. Nemec A, Pavlica Z, Svete AN, et al. Lack of soluble tumor necrosis factor alpha receptor 1 and 2 and interleukin-1beta compartmentalization in lungs of mice after a single intratracheal inoculation with live *Porphyromonas gingivalis*. Exp Lung Res 2009;35:605–620.

21. Volakli E, Spies C, Michalopoulos A, et al. Infections of respiratory or abdominal origin in ICU patients: What are the differences? Crit Care 2010;14:R32.

22. Sheu CC, Gong MN, Zhai R, et al. The influence of infection sites on development and mortality of ARDS. Intensive Care Med 2010;36:963–970.

23. Cavaillon JM, Annane D. Compartmentalization of the inflammatory response in sepsis and SIRS. J Endotoxin Res 2006; 12:151–170.

24. Brun-Buisson C, Doyon F, Carlet J. Bacteremia and severe sepsis in adults: A multicenter prospective survey in ICUs and wards of 24 hospitals. French Bacteremia-Sepsis Study Group. Am J Respir Crit Care Med 1996;154:617–624.

25. Matute-Bello G, Frevert CW, Kajikawa O, et al. Septic shock and acute lung injury in rabbits with peritonitis: Failure of the neutrophil response to localized infection. Am J Respir Crit Care Med 2001;163:234–243.

26. Chinnaiyan AM, Huber-Lang M, Kumar-Sinha C, et al. Molecular signatures of sepsis: Multiorgan gene expression profiles of systemic inflammation. Am J Pathol 2001;159: 1199–1209.

27. Schein M, Wittmann DH, Holzheimer R, et al. Hypothesis: Compartmentalization of cytokines in intraabdominal infection. Surgery 1996;119:694–700.

28. Martineau L, Shek PN. Peritoneal cytokine concentrations and survival outcome in an experimental bacterial infusion model of peritonitis. Crit Care Med 2000;28:788–794.

29. Scheingraber S, Bauerfeind F, Bohme J, et al. Limits of peritoneal cytokine measurements during abdominal lavage treatment for intraabdominal sepsis. Am J Surg 2001;181:301–308.

30. Whitehead Z, Sharp CR, DeClue AE. Plasma nitric oxide concentrations in dogs with sepsis and NSIRS. International Veterinary Emergency and Critical Care Symposium, Phoenix, AZ, 2008.

31. Sharp CR, Chang CH, DeClue AE. Inflammatory biomarkers for canine sepsis. The American College of Veterinary Internal Medicine Forum, Anaheim, CA, 2010.

32. Harmon MW, DeClue AE, Chang CH, Sharp CR. Inflammatory profiles of dogs with sepsis and NSIRS at hospital presentation. International Veterinary Emergency and Critical Care Symposium, San Antonio, TX, 2010.

33. Dickinson AE, Shaw SP, de Laforcade AM, Kjelgaard-Hansen M. Use of an endotoxin activity assay to identify infection and evaluate outcome in canine ICU patients. International Veterinary Emergency and Critical Care Symposium, Chicago, IL, 2009.

34. de Laforcade A, Freeman L, Shaw S, et al. Hemostatic changes in dogs with naturally occurring sepsis. J Vet Intern Med 2003;17:674–679.

35. Kenney EM, Rozanski EA, Rush JE, et al. Association between outcome and organ system dysfunction in dogs with sepsis: 114 cases (2003–2007). J Am Vet Med Assoc 2010;236:83–87.

36. Otto CM, Rieser TM, Brooks MB, et al. Evidence of hypercoagulability in dogs with parvoviral enteritis. J Am Vet Med Assoc 2000;217:1500–1504.

37. Kuzi S, Aroch I, Peleg K, et al. Canine procalcitonin messenger RNA expression. J Vet Diagn Invest 2008;20:629–633.

38. Jacobson L, Lobetti R, Becker P, et al. Nitric oxide metabolites in naturally occurring canine babesiosis. Vet Parasitol 2002; 104:27–41.

39. Kjelgaard-Hansen M, Wiinberg B, Aalbaek B, et al. Endotoxin activity in whole blood measured by neutrophil chemiluminescence is applicable to canine whole blood. Comp Immunol Microbiol Infect Dis 2008;31:477–485.

40. Dabrowski R, Kostro K, Lisiecka U, et al. Usefulness of C-reactive protein, serum amyloid A component, and haptoglobin determinations in bitches with pyometra for monitoring early post-ovariohysterectomy complications. Theriogenology 2009;72: 471–476.

41. Gebhardt C, Hirschberger J, Rau S, et al. Use of C-reactive protein to predict outcome in dogs with systemic inflammatory response syndrome or sepsis. J Vet Emerg Crit Care (San Antonio) 2009;19:450–458.

42. Rogers CL, Rozanski EA. Von willebrand factor antigen concentration in dogs with sepsis. J Vet Intern Med 2010;24:228–230.

43. Gardner IA, Greiner M. Receiver-operating characteristic curves and likelihood ratios: Improvements over traditional methods for the evaluation and application of veterinary clinical pathology tests. Vet Clin Pathol 2006;35:8–17.

44. Torab FC, Abu-Zidan FM, Al-Salam S, et al. Peritoneal resorption capacity for the inflammatory mediators in acute experimental *Staphylococcus aureus* peritonitis. Ulus Travma Acil Cerrahi Derg 2009;15:330–336.

45. Humm KR, Boag AK, House AK. TNF-alpha levels in the serum and abdominal fluid of dogs with septic peritonitis. International Veterinary Emergency and Critical Care Symposium, Phoenix, AZ, 2008.

46. Ruaux CG, Pennington HL, Worrall S, et al. Tumor necrosis factor-alpha at presentation in 60 cases of spontaneous canine acute pancreatitis. Vet Immunol Immunopathol 1999;72:369–376.

47. Rivas AL, Tintle L, Kimball ES, et al. A canine febrile disorder associated with elevated interleukin-6. Clin Immunol Immunopathol 1992;64:36–45.

48. Itoh H, Horiuchi Y, Nagasaki T, et al. Evaluation of immunological status in tumor-bearing dogs. Vet Immunol Immunopathol 2009;132:85–90.

49. Mohamed A, Matsumoto Y, Yoshihara K, et al. Establishment of a sandwich enzyme linked immunosorbent assay for canine interleukin-8. J Vet Med Sci 1997;59:39–41.

50. Hegemann N, Wondimu A, Kohn B, et al. Cytokine profile in canine immune-mediated polyarthritis and osteoarthritis. Vet Comp Orthop Traumatol 2005;18:67–72.