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Serial Evaluation of Abdominal Fluid and Serum Amino-terminal pro-C-type Natriuretic Peptide in Dogs with Septic Peritonitis

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Background: Serum N-terminal pro-C-natriuretic peptide (NT-proCNP) has shown promise as a diagnostic biomarker for sepsis. Its sensitivity to detect dogs with septic peritonitis (SP) is reportedly low, perhaps attributable to the compartmentalization of NT-proCNP in the abdominal cavity.

Objectives: To evaluate the use of an ELISA for the measurement of NT-proCNP in canine abdominal fluid and to describe the peri-operative pattern of abdominal fluid and serum NT-proCNP concentrations in dogs with SP.

Animals: Five client-owned dogs with nonseptic abdominal effusion of varying etiologies and 12 client-owned dogs with SP undergoing abdominal surgery and placement of a closed-suction abdominal drain (CSAD). Six dogs were included upon hospital admission; 6 were included the day after surgery.

Methods: Prospective pilot study. A commercially available ELISA kit was analytically validated for use on canine abdominal fluid. The NT-proCNP concentrations were measured in the abdominal fluid of control dogs, and in serum and abdominal fluid of dogs with SP from admission for CSAD removal.

Results: In dogs with SP, admission abdominal fluid NT-proCNP concentrations were lower than the concurrent serum concentrations ($P = 0.031$), and lower than control canine abdominal fluid concentrations ($P = 0.015$). Postoperatively, abdominal fluid NT-proCNP concentrations remained lower than serum concentrations ($P < 0.050$), except on day 4.

Conclusions and Clinical Importance: The ELISA kit was able to measure NT-proCNP in canine abdominal fluid. In dogs with SP, low serum NT-proCNP concentrations cannot be explained by abdominal compartmentalization.

Key words: Biomarkers; Canine; ELISA; Monitoring; Surgery.

Diagnostic biomarkers are measurable and quantifiable substances that can indicate the likely presence of a disease or a physiologic abnormality.[1] The use of molecular biomarkers is receiving increasing attention because of the advances in technology and the difficulties encountered in diagnosing many conditions using standard test methods. Better understanding of disease pathogenesis has allowed the identification of specific biomarkers that are being used to screen patients at risk for a variety of diseases, as well as to evaluate response to therapy and predict outcome.[1] Among others, amino-terminal pro-C-type natriuretic peptide (NT-proCNP), a stable product of cleavage of C-type natriuretic peptide (CNP), is a novel biomarker with potential diagnostic utility in sepsis.[2–4]

Amino-terminal pro-C-type natriuretic peptide participates in vascular homeostasis and modulates inflammation in the vascular wall.[5] *In vitro* studies using cultures of canine aortic endothelial cells,[6] human circulating macrophages,[7] and mouse peritoneal macrophages[7] reveal that NT-proCNP secretion is enhanced by lipopolysaccharide (LPS) and pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), and interleukin- (IL-)1β, suggesting a role for NT-proCNP in the cross talk between inflammatory cells, microbial markers, and the endothelium.

Individual and serial serum NT-proCNP measurements predict sepsis in heterogeneous populations of critically ill and trauma patients.[2,3] There has been limited NT-proCNP research in veterinary medicine, with 1 study suggesting a potential use of serum NT-proCNP for the identification of sepsis in dogs.[4] That study proposed a serum NT-proCNP cut-off of 10.1 pmol/L to differentiate dogs with heterogeneous sources of sepsis from dogs with noninfectious systemic inflammatory

Abbreviations:

CNP	C-type natriuretic peptide
CSAD	closed-suction abdominal drain
CV	coefficient of variation
IL	interleukin
IV	intravenous
LPS	lipopolysaccharide
NSIRS	noninfectious systemic inflammatory response syndrome
NT-proCNP	amino-terminal pro-C-type natriuretic peptide
O/E	observed-to-expected
SIRS	systemic inflammatory response syndrome
SP	septic peritonitis
TNCC	total nucleated cell count
TNF	tumor necrosis factor
TS	total solids
WBC	white blood cell

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response syndrome (NSIRS), with an overall sensitivity and specificity of 65 and 89%, respectively. Subcategorization of dogs based on the origin of the septic focus revealed that the sensitivity of this biomarker increased to 94% when dogs with septic peritonitis (SP) were removed from analysis. Indeed, dogs with SP had low serum NT-proCNP concentrations that were comparable to those of healthy or NSIRS dogs.[4] One potential explanation for these findings is that NT-proCNP could be concentrated within the abdominal cavity of dogs with SP rather than in the blood stream, a concept known as "compartmentalization." To date, abdominal fluid NT-proCNP concentrations have not been measured in experimental or clinical studies; however, the principle of compartmentalization for a variety of cytokines including TNF-α, IL-1, IL-6, and IL-10 has been previously described in human patients with SP.[8,9] Similarly, in a population of client-owned dogs with SP, TNF-α concentrations were higher in abdominal fluid than serum, suggesting the occurrence of compartmentalization in dogs.[10]

The objectives of this pilot study were to validate the use of a commercially available ELISA[a] for NT-proCNP measurement in abdominal fluid and to measure abdominal fluid and serum NT-proCNP concentrations at admission and during the postoperative period in client-owned dogs with pre-operative SP that underwent abdominal surgery and had a closed-suction abdominal drain[b] (CSAD) placed. We hypothesized that the ELISA would be able to measure NT-proCNP concentrations in canine abdominal fluid, and that in dogs with SP, abdominal fluid NT-proCNP concentration would be significantly greater than the concurrently measured serum NT-proCNP concentration.

Materials and Methods

This prospective observational pilot study included client-owned dogs presented to the Ontario Veterinary College Health Sciences Centre between May 2013 and July 2014. This study was approved by the University of Guelph Animal Care Committee.

Dogs with Septic Peritonitis

With owner consent, all dogs undergoing celiotomy for source control of SP, in which a CSAD was placed intra-operatively, were eligible for inclusion. Diagnosis of pre-operative SP was based on the presence of 1 or more of the following criteria: positive abdominal fluid culture, cytologic evidence of intracellular bacteria, a blood-to-abdominal fluid glucose difference >20 mg/dL, and a blood-to-abdominal fluid lactate difference <−2 mmol/L,[11] overt intestinal leakage or necrotic bowel at the time of surgery. Postoperatively, all dogs were housed in the intensive care unit. Dogs had blood and abdominal fluid collected daily throughout the study period, until CSAD removal. Patient care, including the time of CSAD removal, was at the discretion of the attending clinician.

The following data were recorded for each enrolled dog: age, sex, breed, physical examination findings, presence or absence of SIRS, antimicrobial therapy, cause of SP, surgical procedure, abdominal fluid bacterial culture results, duration of hospitalization, and outcome defined as an uneventful postoperative recovery or recurrence of SP. Each day, abdominal fluid cytology [total nucleated cell count (TNCC) and total solids (TS)], as well as total fluid production (24-hour volume, mL/kg/h) were also recorded.

Dogs met criteria for SIRS if they had at least 2 of the 4 following: hypo/hyperthermia (<100.6 or >102.6°F); tachycardia (>120 beats/min); tachypnea (>20 breaths/min); leukocytosis or leukopenia [white blood cell (WBC) count <6 or >16 × 10^3/μL]; and left shift (>3% bands).[12]

Sample Collection and Storage

Blood and abdominal fluid were collected concurrently at admission or within 24 hours of diagnosis of SP and postoperatively each day at 8 AM until CSAD removal.

At admission, blood was collected via an intravenous catheter immediately at the time of its placement. Postoperatively, a 5-mL blood sample was drawn via venipuncture from a saphenous or jugular vein. Blood was placed in a plastic Vacutainer™ tube with no additive[c] and allowed to clot for 15 minutes before being centrifuged (1,500 × *g*, 7 minutes). The serum was collected and stored at −80°C within 1 hour of collection, for later batch analysis.

At admission, abdominal fluid was collected by ultrasound-guided abdominocentesis. Postoperatively, abdominal fluid was collected via the port on the collection reservoir[d] of the CSAD. The drain port was swabbed with alcohol, then the drain was emptied, and subsequently 5 mL of freshly accumulated abdominal fluid was collected into a syringe. Abdominal fluid was placed into a plastic Vacutainer™ tube with no additive and an EDTA tube.[e] The tube with no additive was centrifuged (1,500 × *g*, 7 minutes) within 15 minutes of collection. The supernatant was collected and stored at −80°C within 1 hour of collection, for batch analysis at a later date. The EDTA tube was submitted within 4 hours of collection for abdominal fluid cytologic examination. For each fluid sample, TS were measured and an automated TNCC was performed using a Coulter counter.[f]

Control Dogs

Five client-owned dogs undergoing abdominocentesis for diagnostic or therapeutic purposes unrelated to sepsis or SP were included as controls. The following data were recorded for control dogs: age, sex, breed, antibiotic therapy, and etiology of the abdominal effusion. In these dogs, sepsis was excluded based on at least 2 of the following criteria: SIRS criteria not fulfilled,[12] recovery of effusate from patients with confirmed diagnosis consistent with abdominal effusion of a nonseptic origin, absence of organisms or degenerate neutrophils on abdominal fluid cytologic examination, or patient follow up with no signs of sepsis or SP. Abdominal fluid samples were centrifuged and stored as described above.

Amino-terminal pro-C-type Natriuretic Peptide ELISA

Amino-terminal pro-C-type natriuretic peptide concentrations in serum and abdominal fluid were measured using a commercially available NT-proCNP ELISA[a] according to the manufacturer's instructions. In the absence of previous validation of this ELISA on canine abdominal fluid, spiking-and-recovery, dilutional parallelism, detection limit, and intra- and inter-assay variability were performed. Spiking-and-recovery and dilutional parallelism were determined using 3 abdominal fluid samples of varying NT-proCNP concentrations. Spiking-and-recovery was determined by adding 10 and 20 pmol/L of standard NT-proCNP provided in the kit. For dilutional parallelism, abdominal fluid samples were serially diluted with assay buffer at 1:2, 1:4, 1:8. Observed-to-expected ratios (O/E) were calculated. Dilutional parallelism and spiking-

and-recovery are reported as mean ± SD. Intra- and inter-assay coefficients of variation (CV) were each determined using abdominal fluid with 2 different NT-proCNP concentrations. Abdominal fluid samples were each assayed 5 times using the same ELISA kit to determine the intra-assay CV and using 5 different ELISA kits to determine the inter-assay CV. For inter-assay measurements, 1 of the abdominal fluid samples used was thawed once, at which time 5 aliquots were made and refrozen at −80°C for interassay measurements. Intra-assay and inter-assay CVs were calculated by dividing the standard deviation (SD) by the mean of the 5 results obtained for each sample. Those data are reported as mean ± SD.

Statistical Analysis

Data were analyzed for normality using the following tests: Shapiro–Wilk, Kolmogorov–Smirnov, Cramer–von Mises, and Anderson–Darling. Logarithmic transformations were performed to meet assumptions of normality when necessary. A Fischer's exact test was used to compare NT-proCNP concentrations in abdominal fluid from dogs with SP to control dogs, and a Wilcoxon sign rank test was used to compare NT-proCNP concentrations from serum and abdominal fluid from dogs with SP at admission. ANOVA for repeated measures was used to test for significant differences between abdominal fluid and serum NT-proCNP concentrations in dogs with SP from admission to CSAD removal, as well as to test significant changes over time in abdominal fluid and serum NT-proCNP concentrations during the same period. The assumptions of the ANOVA were assessed by comprehensive residual analysis. A Dunnett's adjustment or a multivariate *t*-test between treatments was applied when appropriate. When sample mishandling occurred, the paired samples were removed from statistical analysis. In samples with a reported NT-proCNP concentration below the detection limit, half of the lower detection limit was used for statistical analysis to allow for logarithmic transformation.[13] A mixed linear model that accounted for repeated measures was used to determine if a significant association existed between abdominal fluid NT-proCNP concentrations and concurrently measured TNCC, TS, and abdominal fluid production (24-hour volume).

Statistical significance was set at a P value <0.05 for all comparisons. Statistical analysis was performed using commercial software.[g] Graphs were generated using commercial software.[h]

Results

Study Population Characteristics

Seventeen client-owned dogs were enrolled in the study, including 5 control dogs and 12 dogs with SP monitored until CSAD removal. At admission, paired abdominal fluid and serum samples were collected from 6 dogs with SP. Subsequently, 6 dogs were added within 24 hours of diagnosis of SP, after surgical intervention. Because of sample mishandling, NT-proCNP analysis had 1 serum sample removed from day 1, and 2 serum samples removed from day 2 during the postoperative period.

Dogs with SP included 6 neutered males, 4 spayed females, and 2 intact females, with a median age of 7 years (range: 6 months to 11 years). At the time of enrollment, all dogs with SP met the criteria for SIRS and 10 (83%) dogs were receiving antibiotics, median duration 3 days (range 2 hours to 10 days). At surgery, 8 dogs were diagnosed with failure of previous enterotomy (n = 5), colotomy (n = 1), or resection and anastomosis sites (n = 2). Surgical correction included revision of the enterotomy (n = 1) and colotomy (n = 1) sites or intestinal resection-and-anastomosis (n = 6). The previously failed surgeries were performed 1–8 days (median = 4 days) before presentation to our institution. Three dogs underwent intestinal resection-and-anastomosis for surgical correction of extensive necrotic bowel of unknown origin (n = 1), mesenteric torsion (n = 1), and jejunal perforation secondary to a foreign body (n = 1). The last dog had an acute necrotizing cholecystitis with cystic duct perforation that was treated with cholecystectomy, duodenotomy, and biliary stenting. Abdominal fluid bacterial cultures were positive for 92% (n = 11/12) of dogs. All dogs recovered uneventfully and survived to hospital discharge with no recurrence of SP. Median duration of hospitalization was 5 days (range 4–13 days).

The control group included dogs with nonseptic abdominal effusion. There were 2 spayed females, 2 neutered males, and 1 intact female, with a median age of 10 years (range: 7.5 months to 10 years). The cause for abdominal effusion diagnosed in these dogs included portosystemic shunt (n = 2), congestive heart failure, liver mass, and fluid overload (each n = 1). Three dogs were receiving prophylactic metronidazole at the time of abdominal fluid collection (2 for medical management of a portosystemic shunt and 1 with right-sided congestive heart failure).

Evaluation of the Commercial ELISA for Measurement of Amino-terminal pro-C-type Natriuretic Peptide in Abdominal Fluid

Observed-to-expected ratios for spike-and-recovery ranged from 82.3% to 102.4% with a mean ± SD of 90.8 ± 6.6%. For dilutional studies, linearity was maintained to a 1:8- dilution, and O/E ratios ranged from 66.6% to 104.4% with a mean ± SD of 80.6 ± 19.4%. The minimum detection limit of the assay was determined to be 0.40 pmol/L. The intra-assay CV was 3.3–5.9%, whereas the inter-assay CV was 14.4–15.1%.

Serum and Abdominal Fluid Amino-terminal pro-C-type Natriuretic Peptide Concentrations

In dogs with SP, admission serum NT-proCNP concentrations ranged from 1.95 to 30.57 pmol/L (median = 5.56 pmol/L; n = 6), and were significantly higher than concurrent abdominal fluid concentrations (P = 0.031) that were below the abdominal fluid detection limit (0.40 pmol/L) in all but 1 dog whose abdominal fluid NT-proCNP concentration was 0.48 pmol/L (Fig 1). The control dogs' abdominal fluid NT-proCNP concentrations ranged from 3.74 to 8.23 pmol/L (median = 4.79 pmol/L; n = 5) and were significantly higher than that of the dogs with SP at admission (P = 0.015) (Fig 1).

From admission to CSAD removal, abdominal fluid NT-proCNP concentrations were significantly lower than serum NT-proCNP concentrations (P < 0.050), except on day 4 (P = 0.114) (Table 1). Abdominal fluid

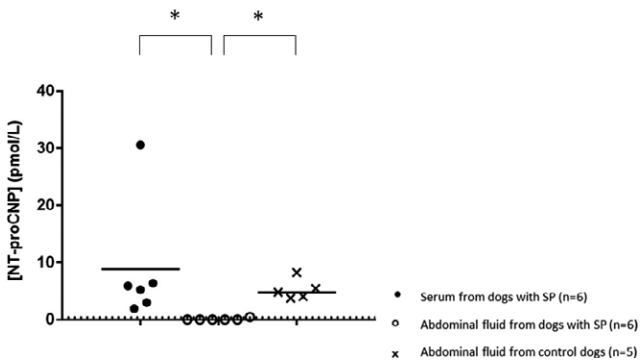

Fig 1. Admission abdominal fluid and serum amino-terminal pro-C-type natriuretic peptide (NT-proCNP) concentrations in dogs with septic peritonitis (SP) and abdominal fluid NT-proCNP concentrations in control dogs. The dotted line (. . .) represents the determined abdominal fluid detection limit (0.40 pmol/L). All but 1 admission abdominal fluid NT-proCNP concentrations in dogs with SP were below the detection limit. Significant differences are indicated with * ($P < 0.05$).

NT-proCNP concentrations at admission were significantly lower than concentrations obtained each day postoperatively ($P < 0.0001$). Neither abdominal fluid nor serum NT-proCNP concentrations significantly changed over time (all $P > 0.050$) during the postoperative period.

Amino-terminal pro-C-type natriuretic peptide concentrations measured at admission and until CSAD removal were associated with lower TNCC ($P < 0.001$), TS ($P < 0.001$), and abdominal drain fluid production ($P = 0.029$) (Tables 2 and 3).

Discussion

Results of this study show that NT-proCNP can be detected in canine abdominal fluid using a commercially available ELISA previously validated on canine serum.[4] Unexpectedly, dogs with SP had lower pre-operative abdominal fluid NT-proCNP concentrations compared to paired serum NT-proCNP concentrations. In addition, dogs with SP had lower admission abdominal fluid NT-proCNP concentrations than control dogs. For dogs with SP, abdominal fluid NT-proCNP concentrations increased during the recovery period compared to admission, however, NT-proCNP concentrations remained lower in the abdominal fluid compared to the serum.

Based on the results of the validation experiments, the ELISA used in this study can adequately measure abdominal fluid NT-proCNP concentrations in dogs. The spiking-and-recovery and dilutional parallelism results obtained indicate good mean recovery and linearity with this sample matrix. What constitutes acceptable O/E ratios are not clearly described in the literature. Previous veterinary studies have reported clinically validated immunoassays with an O/E ratio ranging from 68.1 to 129.7% for spike-and-recovery and 75 to 148.8% for dilutional parallelism.[14,15] In the present study, the mean O/E ratios for dilutional parallelism ranged from 66.6 to 104.4%; the lowest O/E ratio (66.7%) was obtained from a 1:8 dilution when the expected abdominal NT-proCNP concentration fell near the determined detection limit. These results suggest that the accuracy of the measured NT-proCNP concentration declines in the low end of the detection range. With this value removed the O/E ratios for the linearity study ranged from 78.4 to 104.4%. As such, results of linearity and reproducibility experiments demonstrate that NT-proCNP is appropriately detected within the types of abdominal effusions tested.

The results for both intra- and inter-assay variability were also considered acceptable. In this study, the reported interassay CV (14.4–15.1%) was similar to a previously reported interassay CV for use of this ELISA

Table 1. Daily abdominal fluid and serum amino-terminal pro-C-type natriuretic peptide concentrations from dogs with septic peritonitis.

	Abdominal Fluid [NT-proCNP] (pmol/L)		Serum [NT-proCNP] (pmol/L)		
Day	No. of Dogs	Median (Range)	No. of Dogs	Median (Range)	*P* Value
0	6	ND (ND–0.48)	6	5.56 (1.95–30.57)	0.001
1	12	3.19 (ND–11.21)[a]	11	6.86 (1.41–42)	0.008
2	12	4.75 (0.99–10.86)	10	9.46 (2.65–42)	0.026
3	12	4.02 (2.06–6.59)	12	9.85 (2.04–31.09)	0.032
4	9	2.91 (2.04–8.23)	9	4.71 (2.55–28.76)	0.11
5	2	2.61 (1.38–3.84)	2	18.09 (12.24–23.93)	0.044

ND, not detectable.

Statistical significance was set at *P* value <0.05.

[a]Only 1 dog had a nondetectable abdominal fluid NT-proCNP concentration on day 1.

Table 2. Daily abdominal fluid total nucleated cell count and total solids from dogs with septic peritonitis.

Day	No. of Dogs	TNCC ($\times 10^3/\mu L$)	TS (g/dL)
0	6	83.7 (15.9–285)	3.5 (2.8–4.8)
1	12	12.3 (3.5–34.7)	2.0 (2.0–4.3)
2	12	12.5 (4–141.6)	2.1 (2.0–4.8)
3	12	17.6 (2.5–100.8)	2.3 (2.0–4.4)
4	9	8.7 (1–109)	2.0 (2.0–2.6)
5	2	89.1 (2.1–176)	3.1 (2.7–3.4)

Data are reported as median (range).

Table 3. Daily abdominal drain fluid production from dogs with septic peritonitis.

Day	No. of Dogs	Drain Production (mL/kg/h)
0	6	1.83 (0.9–3.16)
1	12	0.99 (0.35–2.39)
2	12	0.74 (0.163–2.99)
3	11	0.44 (0.086–2.26)
4	5	0.58 (0.22–2.1)
5	1	0.49

Data are reported as median (range).

on canine serum,[16] when higher CVs were obtained with the lowest NT-proCNP concentrations.

At the time of admission, 83% (5/6) of dogs with SP had a serum NT-proCNP concentration below 10.1 pmol/L, a value that was previously suggested as a cut-off value for differentiating sepsis from NSIRS in dogs.[4] The findings from the present study confirm the previously stated poor sensitivity and specificity of this biomarker to detect SP. It has been suggested that the low serum NT-proCNP concentrations in dogs with SP may be explained by sequestration of NT-proCNP in the abdominal cavity, such that NT-proCNP concentration of abdominal fluid may provide a potential biomarker for the diagnosis of SP.[4] This concept of compartmentalization has been previously described using other biomarkers in human patients with SP.[8,9] Indeed, human studies report higher cytokine concentrations including IL-1, IL-6, IL-10, and TNF-α in the abdominal fluid compared to plasma during SP.[8,9] It has also been shown in rat models of sepsis-induced peritonitis that accumulation of fibrin, leukocytes, and erythrocytes could obstruct the peritoneal stomata, thereby decreasing the peritoneal permeability and impairing the clearance of various intra-abdominal cytokines.[17]

Results of this study do not support the hypothesis of abdominal compartmentalization of NT-proCNP in dogs with SP, since all dogs had significantly lower abdominal fluid NT-proCNP concentrations compared to paired serum concentrations from admission to CSAD removal. While the reason for this finding is uncertain, some hypotheses can be made. One experimental study[7] reported the secretion of CNP by peritoneal mouse macrophages after stimulation by LPS suggesting a local source for the release of CNP during SP. It is not known whether canine abdominal macrophages secrete CNP; species differences might exist. Furthermore, as abdominal fluid NT-proCNP concentrations were lower in septic effusions compared to nonseptic effusions, and abdominal fluid NT-proCNP concentrations were lower than paired serum concentrations in dogs with SP, these results might suggest that certain components present in septic abdominal effusion could participate in early clearance of NT-proCNP from abdominal fluid of dogs with SP. Further *in vitro* studies would be required to test these hypotheses. Previous surgery might also affect the intra-abdominal concentrations of NT-proCNP. In this study, 8/12 (66%) dogs had abdominal surgery before presentation. While it seems logical to consider that successive abdominal surgeries might increase the degree of trauma to the peritoneum, these effects on NT-proCNP concentration remain unknown. Human studies evaluating abdominal cytokines (TNF-α, IL-6, and IL-10) in the postoperative period have had contradicting results with some reporting increasing concentrations with prolonged surgery[18] and others finding no difference when emergency and elective surgeries were compared.[9] The use of antibiotics might also alter the abdominal environment and its ensuing responses. In this study, 10/12 (83%) dogs with SP were already receiving antibiotics for varying durations at the time of inclusion in the study. Antibiotics can affect the degree of abdominal inflammation and bacterial load during SP; therefore, secondary effects on abdominal fluid NT-proCNP concentrations are possible. However, decontamination of the abdomen, and continued antibiotic administration resulted in increasing abdominal NT-proCNP concentrations throughout the recovery period.

Interestingly, based on lower admission abdominal fluid NT-proCNP concentrations from dogs with SP compared to abdominal effusion from nonseptic dogs, and based on higher abdominal fluid NT-proCNP concentrations during recovery of dogs with SP, it is possible that low or nondetectable NT proCNP concentrations in abdominal effusion could be indicative of SP and be used to differentiate septic from nonseptic effusions. Further studies enrolling a larger population of dogs with septic and nonseptic effusions would be required to further explore this hypothesis.

Investigation of an association between the abdominal fluid characteristics (TNCC and TS) and NT-proCNP concentrations revealed that low NT-proCNP concentrations were found in samples with higher TNCC and TS. While such relationships cannot prove cause and effect, results might indicate a link between NT-proCNP concentration and the type of effusion. Likewise, an inverse relationship was found between abdominal fluid NT-proCNP concentration and drain fluid volume production with an increase in NT-proCNP concentration associated with declining abdominal fluid production. It must be noted that the volume of abdominal effusion was not quantifiable at admission (pre-operatively), at which time the lowest abdominal fluid NT-proCNP concentrations were obtained. The

negative association between fluid production and NT-proCNP concentration must be interpreted in light of a lack of significant change post-admission in abdominal fluid NT-proCNP concentration during the postoperative period. While cause and effect cannot be demonstrated, a dilutional effect cannot be excluded based on these results.

Throughout the postoperative period, abdominal fluid NT-proCNP concentrations were higher than at admission. The cause for the increase in abdominal fluid NT-proCNP concentration from admission is unknown and could be related to elapsed time from surgical manipulation, bacterial decontamination, the effects of an indwelling abdominal drain, or return to normal steady state. It should be emphasized that abdominal fluid was collected from a CSAD during the postoperative period. Previous veterinary studies have shown that abdominal fluid characteristics including glucose and lactate concentration are altered when collected from a CSAD.[19] Effects of the presence of an indwelling drain on NT-proCNP concentration will require further investigation.

In this study, none of the dogs included developed recurrence of SP. As such, no comment can be made regarding potential changes associated with intestinal surgical site failure on serum or abdominal fluid NT-proCNP concentrations. Further investigation is warranted to permit any conclusion regarding diagnostic utility of abdominal fluid NT-proCNP concentration for intestinal dehiscence.

Unexpectedly, dogs with pre-operative SP had lower abdominal fluid NT-proCNP concentrations than abdominal fluid from control dogs. The control patients were selected based on a lack of SP, however, underlying disease processes in the control dogs might have contributed to the higher abdominal fluid NT-proCNP concentrations observed. While it is known that serum NT-proCNP concentrations are higher in human patients with cardiac and liver diseases,[20] it is currently unknown whether these diseases affect the abdominal fluid or serum NT-proCNP concentrations in dogs. The effects of these nonseptic conditions on abdominal NT-proCNP concentrations warrant further investigation.

This pilot study has several important limitations. Validation of the ELISA kit for use on canine abdominal fluid was performed on a limited number of samples. NT-proCNP concentration in abdominal fluid associated with differing etiologies has not been established, and the ELISA requires further validation on abdominal effusion of different causes before it could be considered to provide any clinical utility. In this study, abdominal fluid was centrifuged within an hour of collection and stored at −80°C, as recommended by the ELISA manufacturer for the measurement of NT-proCNP in serum. Such abdominal fluid processing has also been reported in previously published studies evaluating cytokine concentrations and acute phase protein concentrations in the abdominal fluid of human patients for potential diagnostic/screening purposes.[8] Abdominal fluid sample handling and the effects of freezing and storage on NT-proCNP concentrations have not been studied. The study population also included a small number of dogs with SP, with only 6 dogs enrolled at the time of admission, and had limited paired abdominal fluid and serum samples, particularly by Day 5 as all dogs with SP recovered uneventfully; this might limit the clinical and statistical significance of our findings. Finally, because all dogs recovered uneventfully, this precluded comparison of abdominal fluid and serum NT-proCNP concentration between the dogs that recovered uneventfully and the dogs that developed surgical site failure.

In conclusion, NT-proCNP was successfully measured in abdominal fluid from control dogs and dogs with SP during the postoperative period using an ELISA kit previously validated for canine serum. Lack of abdominal compartmentalization was identified based on lower concentrations of NT-proCNP in the abdominal fluid of dogs with SP compared to paired serum samples. Further studies including larger populations of dogs are needed to determine the potential diagnostic utility of abdominal fluid NT-proCNP concentrations for the diagnosis of SP at admission and during the postoperative period.

Footnotes

[a] NT-proCNP ELISA, Biomedica Gruppe, Vienna, Austria
[b] Hubless Silicone Flat Drain drain (7 or 10 mm), CR Bard Inc., Covington, GA
[c] BD Vacutainer plus plastic serum tubes, Becton Dickinson and Company, Franklin Lakes, NJ
[d] 100 cc Silicone Closed Wound Suction Evacuator, CR Bard Inc., Covington, GA
[e] EDTA tube Vacutainer plus plastic plasma tubes, Becton Dickinson and Company, Franklin Lakes, NJ
[f] Z2 Coulter Particle Count and Size Analyzer, Beckman Coulter Inc., Mississaugua, ON, Canada
[g] SAS v.9.2, SAS Institute Inc., Cary, NC
[h] Prism 5, GraphPad Software, La Jolla, CA

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Conflict of Interest Declaration: Authors disclose no conflict of interest.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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