Evaluation of peripheral blood and abdominal fluid variables as predictors of intestinal surgical site failure in dogs with septic peritonitis following celiotomy and the placement of closed-suction abdominal drains

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OBJECTIVE
To evaluate peripheral blood and abdominal fluid variables as predictors of intestinal surgical site failure in dogs with septic peritonitis following celiotomy and closed-suction abdominal drain (CSAD) placement.

DESIGN
Prospective study.

ANIMALS
26 dogs with septic peritonitis that underwent celiotomy and CSAD placement.

PROCEDURES
Abdominal fluid and blood samples were collected prior to surgery and daily thereafter until CSAD removal. Abdominal fluid was collected through the CSAD. Analysis of all samples included pH, P_{CO_2}, P_{O_2}, PCV, WBC count, and total solids, glucose, lactate, and electrolyte concentrations. Abdominal fluid samples also underwent cytologic evaluation and bacterial culture, and the volume of fluid removed through the drain was recorded daily. The blood-to-fluid glucose and lactate differences, fluid-to-blood lactate ratio and blood-to-fluid WBC and neutrophil ratios were determined daily. Dogs were categorized into 2 groups on the basis of whether they had an uneventful recovery (UR) or developed postoperative septic peritonitis (POSP).

RESULTS
23 dogs had a UR and 3 developed POSP. On the third day after surgery, the abdominal fluid WBC count was significantly lower and the blood-to-fluid WBC and neutrophil ratios were significantly higher for dogs in the POSP group, compared with those for dogs in the UR group. None of the other blood and abdominal fluid variables assessed differed significantly between the 2 groups.

CONCLUSIONS AND CLINICAL RELEVANCE
Results failed to identify any objective predictive indicators for POSP in dogs with CSADs. Use of blood-to-fluid WBC and neutrophil ratios as predictive indicators for POSP requires further investigation. (*J Am Vet Med Assoc* 2016;249:515–525)

In dogs, the incidence of septic complications after gastrointestinal surgery ranges from 6% to 35%,[1–4] and the mortality rate associated with those complications ranges from 9% to 85%.[1,2] Rapid recognition and appropriate medical and surgical treatment are critical for the management of septic patients to optimize their chance of survival. Identification of surgical site dehiscence requiring additional surgical correction remains a challenge in both human and veterinary medicine. Currently, diagnosis of surgical site failure and the decision regarding the need for additional surgery are made on the basis of the clinical progression of the patient and results of serial blood work and abdominal fluid analyses.[5] Although several diagnostic tests are used to diagnose septic peritonitis, the accuracy of those tests has not been assessed in patients that require repeated surgery, in patients with preoperative septic peritonitis with residual abdominal contamination, or in patients receiving antimicrobial therapy.

Various diagnostic tests have been evaluated for use in the diagnosis of septic peritonitis in dogs that have not undergone celiotomy.[6–8] Bacterial culture of abdominal fluid remains the gold standard for diagnosis of septic peritonitis[6,9]; however, the inherent delay from sample collection to result availability (24 to 72

ABBREVIATIONS

BFG	Blood-to-fluid glucose
BFL	Blood-to-fluid lactate
CSAD	Closed-suction abdominal drain
GIT	Gastrointestinal tract
POSP	Postoperative septic peritonitis
UR	Uneventful recovery

hours) precludes relying on culture results for critical decision-making. Observation of degenerate neutrophils and intracellular bacteria during cytologic examination of abdominal fluid can be used to identify 57% to 100% of dogs with septic peritonitis prior to surgery.[6–8,10] Abdominal fluid glucose and lactate concentrations and BFG and BFL differences have also been evaluated in dogs with septic peritonitis.[6,7] In dogs with septic peritonitis, an abdominal fluid glucose concentration < 50 mg/dL has a diagnostic sensitivity and specificity of 57% and 100%, respectively,[6] whereas an abdominal fluid lactate concentration > 2.5 mmol/L has a diagnostic sensitivity and specificity that are both 100%.[7] The accuracy of abdominal fluid glucose and lactate concentrations for the diagnosis of septic peritonitis is improved by calculation of the BFG and BFL differences. A BFG difference > 20 mg/dL has a 100% diagnostic sensitivity and specificity, and a BFL difference < –2 mmol/L has a diagnostic sensitivity that ranges from 63% to 100% and a diagnostic specificity that ranges from 82% to 100%.[6,7] However, those variables have not been assessed in dogs with septic peritonitis that have undergone celiotomy and have an indwelling CSAD, 2 factors that might affect the results for those variables.

Closed-suction abdominal drains are commonly used for the management of patients with septic peritonitis.[8,11,12] However, evidence suggests that the presence of a CSAD may alter the characteristics of abdominal fluid during the postoperative period. In a study[9] in which healthy dogs had a CSAD placed during a celiotomy without any type of GIT incision, bacterial culture, cytologic examination, and biochemical analysis (including calculation of BFG and BFL differences) of postoperative abdominal fluid frequently yielded results suggestive of septic peritonitis. Thus, the presence of a CSAD can alter the abdominal fluid characteristics resulting in misdiagnosis of septic peritonitis, and previously established criteria for the diagnosis of septic peritonitis might be inappropriate for the diagnosis of POSP in dogs with a CSAD. Furthermore, any type of GIT surgery can alter the composition of abdominal fluid in the postoperative period.[13]

The purpose of the study reported here was to evaluate peripheral blood and abdominal fluid variables as predictors of intestinal surgical site failure in dogs with septic peritonitis following celiotomy and the placement of a CSAD. To do this we compared various peripheral blood and abdominal fluid variables between dogs that had an uneventful recovery from GIT surgery and those that developed complications and required additional surgery.

Materials and Methods

Animals

Client-owned dogs evaluated by the emergency service at the Ontario Veterinary College Health Sciences Centre between January 2012 and June 2014 that underwent surgery of the intestinal tract and had a CSAD[a] placed were prospectively assessed. Only dogs with septic peritonitis prior to surgery were enrolled in the study. Septic peritonitis was diagnosed on the basis of the observation of overt intestinal leakage or necrotic bowel during surgery or the presence of at least 1 of the following criteria: positive results on bacterial culture of abdominal fluid, identification of intracellular bacteria during cytologic examination of abdominal fluid, a BFG difference > 20 mg/dL, and a BFL difference < –2 mmol/L. Intestinal tract surgery was defined as any surgery that involved incision into the small or large intestine. Placement of a CSAD was at the surgeon's discretion and was typically reserved for dogs with a preexisting intestinal perforation with or without abdominal effusion or that had evidence of localized or generalized peritonitis during surgery. Dogs with concurrent diseases that might affect glucose metabolism (eg, diabetes mellitus or insulinoma) were excluded from the study. Dogs that required dextrose supplementation for temporary correction of hypoglycemia or as a carrier fluid for vasopressor administration during the postoperative period were not excluded from the study.

Study design

The study protocol was approved by the Institutional Animal Care and Use Committee of the University of Guelph. Written consent was obtained from the owners of all dogs prior to study enrollment. The duration of the observation period varied among dogs and extended from hospital admission to CSAD removal. For each study dog, all decisions regarding medical and surgical interventions, including CSAD placement and removal, selection of diagnostic tests, and the need for additional surgery were made by the attending surgeon, who remained unaware of (ie, was blinded to) the daily study data.

Dogs were categorized into 1 of 2 groups (UR or POSP group) on the basis of the outcome for each dog at the end of the observation period. The UR group consisted of dogs that were discharged from the hospital without the development of complications that required additional surgery. The POSP group consisted of dogs that required additional surgery to control infection because of failure of the intestinal surgical site. Postoperative septic peritonitis was confirmed by the observation of an abscess or leakage at the original intestinal surgical site during the subsequent surgery or necropsy.[14]

Data collection

For each dog, data extracted from the medical record included signalment, clinical and laboratory findings at hospital admission, all medical treatments initiated for resuscitation, surgical diagnosis, type of surgical intervention performed, duration of anesthesia and surgery, all medical treatments administered before and after surgery, duration between CSAD placement and removal, duration of hospitalization, and outcome.

Sample collection

Samples of blood and abdominal fluid were collected concurrently from each dog at hospital admission prior to surgery (day 0) and at 8 AM each day after surgery until CSAD removal. Because the duration of CSAD retention varied among dogs, the number of samples collected for the study population decreased as the number of days after surgery increased.

A venous blood sample (5 mL) was collected during placement of an intravenous catheter on day 0 and by saphenous or jugular venipuncture on each day thereafter. Immediately after collection, the blood sample was transferred to a heparinized syringe and a blood collection tube that contained EDTA as an anticoagulant (EDTA tube). Within 10 minutes after collection, the blood in the heparinized syringe was analyzed with a point-of-care blood gas analyzer,[b] which measured pH, P_{CO_2}, P_{O_2}, and sodium, potassium, chloride, ionized calcium, bicarbonate, glucose, and lactate concentrations. The PCV and total solids concentration were also measured from the blood in the heparinized syringe. Blood in the EDTA tubes was submitted for CBC and a morphological examination of a blood smear. The extent of neutrophil toxicosis was subjectively assessed on a 4-point scale where 0 = no toxic neutrophils observed, 1 = presence of Döhle bodies and mild basophilia, 2 = moderate basophilia with Döhle bodies or vacuolation, and 3 = marked basophilia and foamy cytoplasm with toxic granules with or without Döhle bodies.

A sample of abdominal fluid was collected by ultrasound-guided abdominocentesis prior to surgery on day 0 when identified and was collected via the port on the collection device[c] of the CSAD daily thereafter until the drain was removed. Prior to collection of abdominal fluid samples from the CSAD, the collection device was emptied by use of a clean technique. Ten minutes later, the device port was swabbed with alcohol and freshly accumulated abdominal fluid was collected into a 10-mL syringe. For patients with multiple CSADs, equal volumes of fluid were collected from each device, and the samples were pooled. The abdominal fluid sample was divided and transferred into a heparinized syringe, EDTA tube, and blood collection tube without any additives (red-top tube). The fluid in the heparinized syringe was analyzed within 10 minutes after collection with the same point-of-care blood gas analyzer that was used to analyze blood samples, and the same variables measured for the peripheral blood samples were measured for the abdominal fluid samples. The fluid in the EDTA tube was analyzed with an automated hematologic analyzer[d] for determination of the RBC count and total nucleated cell count, which included a WBC differential. The concentration of total solids in the sample was measured by a refractometer. Additionally, smears of sedimented and cytocentrifuged abdominal fluid samples were prepared and stained[e] for cytologic examination within 4 hours after sample collection. The slides were reviewed by a clinical pathologist (MAA) who was blinded to the other study findings for each patient. For each slide, a differential count on 400 cells was performed, and the degree of neutrophil degeneration and presence of bacteria were reported. The degree of neutrophil degeneration was subjectively evaluated on a 4-point scale (0 = none, 1 = mild, 2 = moderate, or 3 = severe) on the basis of nuclear swelling and cell lysis. Slides prepared from cytocentrifuged samples were examined at 100X magnification to determine the number of bacteria on the slide. The number of bacteria was determined by use of a semi-quantitative technique where 0 = no bacteria, 1 = < 5 bacteria, 2 = 5 to 10 bacteria, 3 = 11 to 20 bacteria, and 4 = > 20 bacteria. Fluid samples in the red-top tubes were submitted for aerobic and anaerobic bacterial culture. The samples were directly inoculated onto Columbia blood agar[f] within 24 hours after sample collection.

When the CSAD was removed, an aseptic technique was used, and the most distal 10 cm of the drain tubing was placed in a sterile container. The container with the tubing was frozen within 1 hour after CSAD removal and stored at −80°C until analysis. For analysis, the containers were thawed and filled with culture medium[g] until the drain tubing was fully immersed. The containers were aerobically incubated at 35°C for 48 hours. Then, an aliquot of the culture medium from each container was inoculated onto Columbia blood agar and incubated under aerobic and anaerobic conditions for an additional 48 hours. All bacterial colonies cultured were subsequently identified.

Calculations

The BFG and BFL differences were calculated by subtracting the abdominal fluid glucose and lactate concentrations from the corresponding blood glucose and lactate concentrations. Blood-to-fluid ratios were calculated for glucose and lactate concentrations and WBC and neutrophil counts by dividing the value for peripheral blood by the corresponding value for abdominal fluid (eg, $\text{WBC count}_{\text{blood}}/\text{WBC count}_{\text{abdominal fluid}}$). The post hoc abdominal fluid–to–blood ratio for lactate concentration was also calculated.

For each patient, abdominal fluid production was recorded as the number of milliliters per kilogram of patient weight per hour (mL/kg/h) each day. The collection device was emptied as frequently as necessary to maintain negative suction (typically q 1 to 6 hours). To account for changes in the volume of abdominal fluid drained via the CSAD, the daily total nucleated cell count was calculated for abdominal fluid by multiplying the WBC count at 8 AM by the volume of fluid removed from the CSAD during the preceding 24 hours.

Statistical analysis

For each group (UR and POSP), descriptive statistics were generated for data acquired prior to surgery (baseline). The frequency (percentage) was reported

for categorical variables. The respective distributions for continuous variables were analyzed for normality by means of the Shapiro-Wilk and Kolmogorov-Smirnov tests. The mean ± SD was reported for variables that were normally distributed, and the median (range) was reported for variables that were not normally distributed. Continuous variables (age, body weight, temperature, and duration of hospitalization) were compared between the 2 groups by use of Mann-Whitney *U* tests. Categorical variables (sex and presence of signs of abdominal pain) were compared between the 2 groups by use of Fisher exact tests. All analyses of baseline data were performed with statistical software.[h]

Initially, the respective associations between the results obtained for abdominal fluid and peripheral blood variables from admission to CSAD removal and POSP diagnosis were assessed with mixed logistic regression to identify factors that were significantly associated with POSP. Because of the small number of dogs in the POSP group, only univariate models were assessed. Each model included a random variable for dog to account for repeated measures within individual dogs. For each variable, models were fitted for each of several correlation matrix structures and compared by use of the Akaike information criterion; the correlation matrix that resulted in the lowest Akaike information criterion value was used for all subsequent modeling. Residual plots were visually assessed to ensure that the data met all the required model assumptions.

A mixed linear regression model was used to compare temporal changes for all variables evaluated between the 2 groups of dogs. Dog was included as a random effect in each model to account for repeated measures. Residual and quantile plots were visually assessed to ensure that the required model assumptions of normality and homoscedasticity were not violated. When those assumptions were not met, a logarithmic transformation was applied to the outcome variable, and the assumptions were rechecked. Fixed effects were initially assessed with a type III ANOVA, and a Tukey test was used for post-hoc testing when necessary. Missed or mishandled samples were removed from the analysis. Logistic and linear regression modeling were performed with statistical software,[i] and graphs were generated by use of another software program.[j] For all comparisons and analyses, values of $P \leq 0.05$ were considered significant.

Results

Dogs

Twenty-nine dogs were initially enrolled in the study. Three dogs were subsequently removed from the study; the CSAD was removed 1 day before suspicion of POSP in 1 dog, 1 dog died before the CSAD was removed and a necropsy was not performed, and the remaining dog was euthanized before the outcome could be determined. Thus, 26 dogs were evaluated; 23 dogs were classified in the UR group and 3 dogs were classified in the POSP group. None of the baseline variables differed significantly between the 2 groups except the total solids concentration; the total solids concentration for the UR group was significantly (P = 0.012) greater than that for the POSP group **(Table 1)**.

All 3 dogs in the POSP group died (n = 1) or were euthanized (2). By definition, all dogs in the UR group survived to be discharged from the hospital. The median duration of hospitalization for dogs in the UR group (5 days; range, 3 to 13 days) did not differ significantly (P = 0.44) from that for dogs in the POSP group (6 days; range, 3.5 to 9 days; **Table 2**). For the dogs of the POSP group, the need for additional surgery was suspected at a median of 3 days (range, 2 to 3 days) after the initial surgery and was confirmed during the subsequent surgery for all 3 dogs. Overall, failure of the intestinal surgical site occurred in 3 of the 26 (11.5%) study dogs during the observation period.

Table 1—Descriptive statistics for data acquired prior to surgery (baseline) for 26 dogs with septic peritonitis evaluated at a veterinary teaching hospital between January 2012 and June 2014 that underwent celiotomy and CSAD placement (day 0) and subsequently did (POSP group; n = 3) or did not (UR group; 23) develop POSP, which required additional surgery.

Variable	POSP group	UR group	*P* value
Age (y)	6 (0.5–9)	4.5 (0.5–11)	0.94
Sex*	—	—	0.12
Spayed female	0 (0)	10 (43)	—
Sexually intact female	1 (33)	1 (4)	—
Castrated male	2 (67)	10 (43)	—
Sexually intact male	0 (0)	2 (9)	—
Body weight (kg)	21 (7.3–23.0)	25.0 (4.9–55.2)	0.23
Heart rate (beats/min)	189.6 ± 68.5	123.3 ± 31.6	0.10
Mean arterial pressure (mm Hg)	91.6 ± 27.4	110.0 ± 26.1	0.32
Temperature (°C)	40.2 ± 2.0	38.7 ± 0.8	0.19
Signs of abdominal pain	3 (100)	15 (65)	0.53
Lactate (mmol/L)	4.3 ± 3.0	2.0 ± 1.1	0.26
Total solids (g/dL)	4.4 (4.2–4.4)	5.6 (4.4–9.7)	0.01

Values represent the number (%) of dogs, mean ± SD, or median (range). Values of $P \leq 0.05$ were considered significant.
*Percentages may not sum to 100 because of rounding.
— = Not calculated.

Table 2—Descriptive data for various preoperative, surgical, and postoperative variables for the dogs of Table 1.

Variable	POSP group	UR group
Preoperative treatments		
Crystalloid	3 (100)	23 (100)
Synthetic colloid	2 (67)	2 (23)
Vasopressor (norephinephrine)*	1 (33)	1 (4)
Fresh frozen plasma	1 (33)	0 (0)
Antimicrobial administration		
Ongoing (were receiving antimicrobials before hospital admission)	3 (100)	17 (74)
Prior to surgery	3 (100)	23 (100)
Surgical diagnosis		
Enterotomy or resection and anastomosis site leakage	2 (66)	14 (61)
NSAID-induced gastroduodenal perforation	1 (33)	1 (4)
Perforating intestinal foreign body	0 (0)	2 (8)
Ulcerated intestinal mass	0 (0)	2 (8)
Intestinal abscess	0 (0)	1 (4)
Intestinal ischemia	0 (0)	1 (4)
Acute necrotizing cholecystitis	0 (0)	1 (4)
Mesenteric volvulus	0 (0)	1 (4)
Type of surgery performed		
Primary closure of gastroduodenal defect	1 (33)	1 (4)
Enterotomy	1 (33)	5 (22)
Cholecystectomy and duodenotomy (biliary stenting)	0 (0)	1 (4)
Colotomy	0 (0)	1 (4)
Resection and anastomosis	2 (67)	16 (70)
Surgical site		
Stomach	2 (67)	1 (4)
Duodenum	3 (100)	7 (30)
Jejunum	1 (33)	17 (74)
Ileum	0 (0)	3 (13)
Colon	0 (0)	3 (13)
Bile duct	0 (0)	1 (4)
No. of surgical sites	2 (1–2)	1 (1–5)
Volume of lavage fluid used during surgery (L)	5 (4–5)	4 (1–10)
Duration of anesthesia (h)	3.0 (2.0–3.0)	2.5 (1.8–4.0)
Duration of surgery (h)	2.0 (1.5–2.6)	1.5 (1.0–3.0)
Intraoperative therapy		
Crystalloid	3 (100)	23 (100)
Synthetic colloid	3 (100)	8 (35)
Vasopressors*		
Dopamine	1 (33)	4 (17)
Norepinephrine	1 (33)	8 (35)
Blood products		
RBCs	1 (33)	0 (0)
Fresh frozen plasma	1 (33)	1 (4)
Postoperative treatments		
Antimicrobials	3 (100)	23 (100)
Dextrose	1 (33)	2 (9)
Crystalloid	3 (100)	23 (100)
Synthetic colloid	2 (67)	4 (17)
Vasopressor (norepinephrine)*	2 (67)	1 (4)
Blood products		
RBCs	1 (33)	1 (4)
Fresh frozen plasma	3 (100)	2 (9)
No. of CSADs placed		
1	1 (33)	19 (82)
2	2 (67)	4 (17)
Interval between CSAD placement and removal (d)	3 (2.5–3)	4 (2–6)
Duration of hospitalization (d)	6 (3.5–9)	5 (3–13)

Values represent number (%) of dogs or median (range).
*All vasopressors were administered in a solution of 5% dextrose in water.
See Table 1 for remainder of key.

Blood and abdominal fluid

The daily postoperative abdominal fluid variables **(Table 3)** and BFG and BFL differences **(Figure 1)** were summarized. None of the peripheral blood or abdominal fluid variables differed significantly between the 2 groups during the postoperative period. Likewise, the BFG and BFL differences did not differ significantly between the 2 groups at any time during the postoperative period. The percentage of dogs in the UR group with a BFG difference > 20 mg/dL

Table 3—Descriptive data for various abdominal fluid variables for the dogs of Table 1 on a daily basis before (day 0) and after surgery.

Variable	Group	Day 0	1	2	3	4	5	6
Glucose (mg/dL)	POSP	66.0 ± 30.4 (3)	86.4 ± 14.4 (2)	48.6 ± 11.2 (3)	27.9 ± 24.3 (2)	—	—	—
	UR	45.3 ± 6.5 (19)	79.6 ± 6.5 (23)	60.5 ± 7.0 (23)	47.4 ± 7.9 (18)	46.3 ±11.3 (12)	10.2 ± 5.7 (3)	72 (1)
No. (%) with glucose < 50 mg/dL*	POSP	1 (33)	0 (0)	2 (67)	1 (50)	—	—	—
	UR	9 (47)	3 (13)	9 (39)	9 (50)	7 (58)	3 (100)	0 (0)
Lactate (mmol/L)	POSP	9.1 ± 3.7 (3)	4.3 ± 0.0 (2)	7.5 ± 1.1 (3)	10.1 ± 0.5 (2)	—	—	—
	UR	7.5 ± 0.8 (19)	5.6 ± 0.6 (23)	7.3 ± 0.8 (23)	8.5 ± 1.0 (18)	8.7 ± 1.1 (12)	12.2 ± 2.9 (3)	3.8 (1)
No. (%) with lactate > 2.5 mmol/L*	POSP	2 (67)	2 (100)	3 (100)	2 (100)	—	—	—
	UR	18 (95)	21 (91)	23 (100)	18 (100)	12 (100)	3 (100)	1 (100)
WBC (X 10^3 cells/µL)	POSP	87.7 ± 57.3 (3)	8.6 ± 1.9 (3)	20.6 ± 10.5 (3)	0.4 ± 0.0 (2)	—	—	—
	UR	73.8 ± 17.6 (17)	15.1 ± 2.4 (23)	19.5 ± 5.8 (22)	20.9 ± 4.2 (19)	17.1 ± 6.1 (13)	42.3 ±32.5 (3)	87.3 (1)
Neutrophils (X 10^3 cells/µL)	POSP	57.4 ± 45.2 (3)	6.4 ± 2.8 (3)	8.24 ± 4.8 (3)	0.08 ± 0.06 (2)	—	—	—
	UR	58.96 ± 15.2 (17)	10.8 ± 2.1 (23)	15.1 ± 5.5 (22)	16.6 ± 3.9 (19)	13.3 ± 5.8 (13)	36.8 ± 28.3 (3)	77.7 (1)
Total nucleated cells (X 10^3 cells/µL)†	POSP	—	6.1 ± 2.5 (3)	13.6 ± 7.0 (3)	0.4 ± 0.4 (2)	—	—	—
	UR	—	6.2 ± 1.7 (23)	10.9 ± 4.3 (22)	8.1 ± 2.1 (19)	5.2 ± 2.0 (13)	13.0 ± 11.8 (3)	25.4 (1)
Fluid production (mL/kg/h)	POSP	2.4 ± 0.6 (3)	1.3 ± 0.3 (3)	1.6 ± 1.1 (3)	2.6 (1)	—	—	—
	UR	1.6 ± 0.2 (23)	0.9 ± 0.1 (23)	0.7 ± 0.1 (21)	0.6 ± 0.1 (16)	0.7 ± 0.3 (6)	0.3 ± 0.2 (2)	—

Values represent the mean ± SD unless otherwise indicated. The number in parentheses following a mean ± SD value represents the number of dogs that contributed to that mean. The duration of the observation period varied among dogs and extended from hospital admission (day 0) to CSAD removal; therefore, the number of dogs within each group generally decreased as the number of days after surgery increased. Also, missed or mishandled samples were removed from the analysis; thus, within a group, the number of dogs evaluated on a given day may have varied among variables.

*The denominator used for calculating the percentage was the number of dogs that contributed to the corresponding mean for this variable. †The daily total nucleated cell count was calculated by multiplying the WBC count at 8 AM by the volume of fluid removed from the CSAD during the preceding 24 hours.

— = Not evaluated.

See Table 1 for remainder of key.

progressively increased from day 1 (16/23 [70%]) through day 5 (20/23 [87%]), when all 3 remaining dogs had a BFG difference > 20 mg/dL. The percentage of dogs in the UR group with a BFL difference < –2 mmol/L varied from 82% to 100% during the postoperative period; on day 5, all 3 remaining dogs had a BFL difference < –2 mmol/L. All 3 dogs in the POSP group, had a BFG > 20 mg/dL and a BFL difference < –2 mmol/L throughout the postoperative period.

Differential cell counts for blood and abdominal fluid samples

During the postoperative period, none of the differential cell counts for blood and abdominal fluid differed significantly between the 2 groups or were predictive of intestinal surgical site failure, except for the WBC count in abdominal fluid, which was significantly (P = 0.002) lower for the POSP group, compared with that for the UR group on day 3 (Table 3). In the UR group, the inflammatory cell count reported during the postoperative period remained lower than that at admission in all dogs except one. In the POSP group, 1 of the 3 dogs had an increase in abdominal fluid WBC count, whereas the 2 remaining dogs had unexpectedly low abdominal fluid WBC counts (< 500 cells/µL) on the day of intestinal surgical site failure. In the POSP group, a significant (P = 0.002) and drastic drop in abdominal fluid WBC count from baseline to day 3 was observed. The daily mean total nucleated cell count in abdominal fluid did not differ significantly between the 2 groups at any time during the postoperative period when adjusted for the daily fluid production.

The blood-to-fluid WBC ratios for each group during the postoperative period were summarized (Figure 1). The blood-to-fluid WBC (P = 0.003) and neutrophil (P = 0.003; data not shown) ratios for the POSP group were significantly greater than those for the UR group on day 3.

Abdominal fluid cytology and bacterial culture

The abdominal fluid cytologic findings including neutrophil count, degree of neutrophil degeneration, and presence or absence of intracellular bacteria were summarized **(Table 4)**. None of the variables evaluated differed significantly between the 2 groups or were predictive of POSP. Neutrophils were the predominant cell type identified in abdominal fluid samples during the postoperative period for both groups. The number of dogs in the UR group with intracellular bacteria observed in the abdominal fluid gradually decreased during the first 4 days after surgery. For 2 dogs in the UR group, the attending clinician removed the CSAD on the basis of clinical improvement despite the observation of intracellular bacteria in abdominal fluid samples. Intracellular bacteria were not observed in the abdominal fluid obtained from any of the 3 dogs in the POSP group on the day (day 2 [n = 1 dog] or 3 [2]) that the intestinal surgical site failed.

The number of dogs in the UR group that had abdominal fluid samples with positive bacterial culture

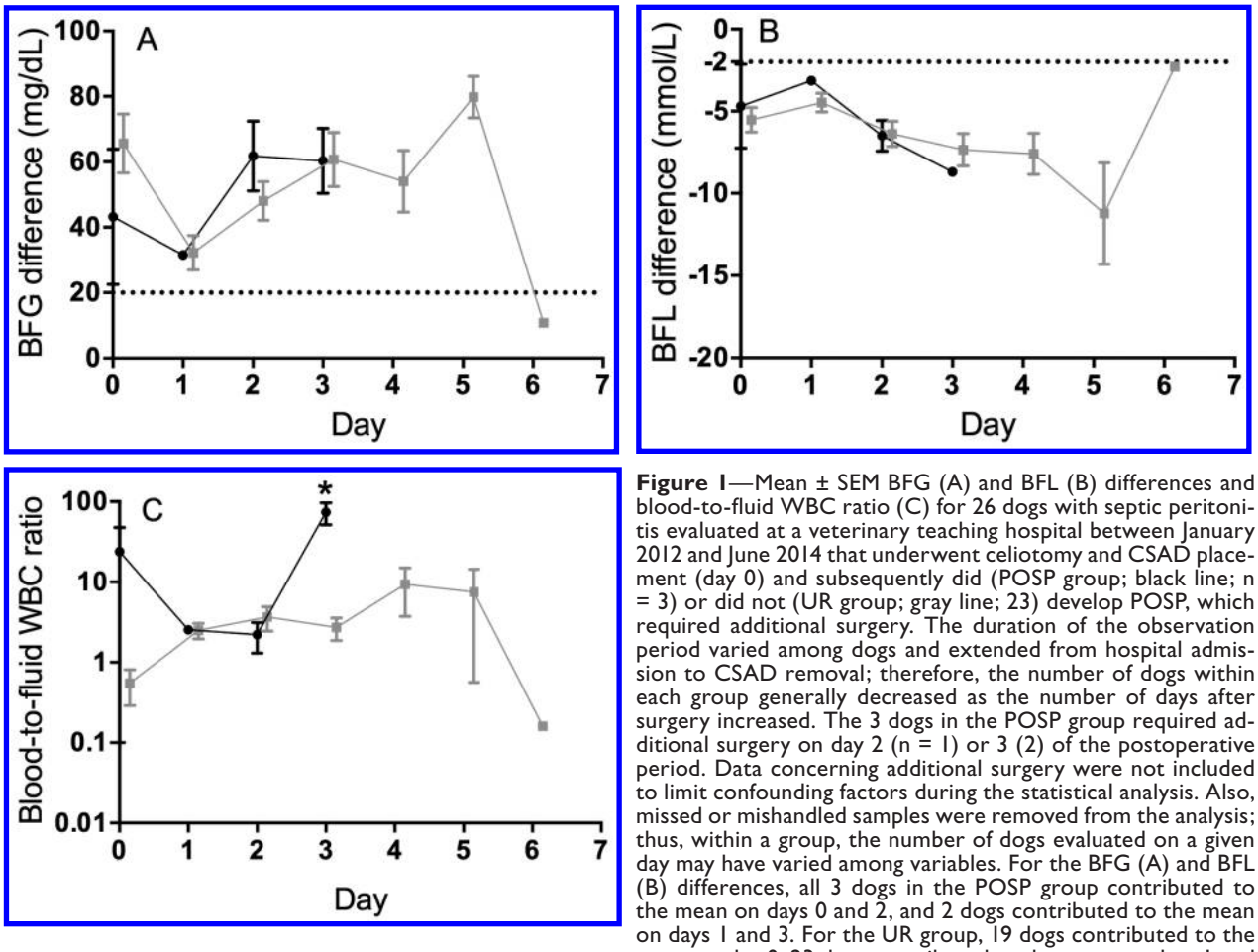

Figure 1—Mean ± SEM BFG (A) and BFL (B) differences and blood-to-fluid WBC ratio (C) for 26 dogs with septic peritonitis evaluated at a veterinary teaching hospital between January 2012 and June 2014 that underwent celiotomy and CSAD placement (day 0) and subsequently did (POSP group; black line; n = 3) or did not (UR group; gray line; 23) develop POSP, which required additional surgery. The duration of the observation period varied among dogs and extended from hospital admission to CSAD removal; therefore, the number of dogs within each group generally decreased as the number of days after surgery increased. The 3 dogs in the POSP group required additional surgery on day 2 (n = 1) or 3 (2) of the postoperative period. Data concerning additional surgery were not included to limit confounding factors during the statistical analysis. Also, missed or mishandled samples were removed from the analysis; thus, within a group, the number of dogs evaluated on a given day may have varied among variables. For the BFG (A) and BFL (B) differences, all 3 dogs in the POSP group contributed to the mean on days 0 and 2, and 2 dogs contributed to the mean on days 1 and 3. For the UR group, 19 dogs contributed to the mean on day 0, 23 dogs contributed to the mean on days 1 and 2, 18 dogs contributed to the mean on day 3, 11 dogs contributed to the mean on day 4, 3 dogs contributed to the mean on day 5, and only 1 dog was evaluated on day 6. For the blood-to-fluid WBC ratio, all 3 dogs in the POSP group contributed to the mean on days 0, 1, and 2, and 2 dogs contributed to the mean on day 3. For the UR group, 16 dogs contributed to the mean on day 0, 23 dogs contributed to the mean on day 1, 21 dogs contributed to the mean on day 2, 19 dogs contributed to the mean on day 3, 13 dogs contributed to the mean on day 4, 3 dogs contributed to the mean on day 5, and only 1 dog was evaluated on day 6. The dotted line in panels A and B represents the BFG (20 mg/dL) and BFL (–2 mmol/L) differences recommended for diagnosis of septic peritonitis in dogs without a CSAD. *Within a day, the means for the 2 groups differ significantly ($P \leq 0.05$).

results decreased during the first 48 hours after surgery, and none of the dogs had positive culture results on days 2 and 3. However, *Enterococcus faecium* was isolated from the abdominal fluid of 2 dogs on day 4 and from 1 of those dogs again on day 5. Those 2 dogs were the only dogs in the UR group that had culture positive abdominal fluid samples at CSAD removal. Abdominal fluid samples remained culture positive from admission until CSAD removal (day of second surgery) for 2 of the 3 dogs in the POSP group; for the remaining dog in that group, the abdominal fluid sample collected prior to CSAD removal yielded negative results.

Abdominal fluid production

The volume of abdominal fluid produced on a daily basis after surgery did not differ significantly between the 2 groups of dogs and was not predictive of POSP (Table 3). The volume of abdominal fluid produced gradually decreased after the initial surgery for 22 of the 23 dogs in the UR group and 2 of the 3 dogs in the POSP group. For the other dog in the POSP group, the volume of abdominal fluid produced the day before the second surgery was greater than that produced the previous day.

Bacterial culture of CSADs

Following CSAD removal, the distal portion (tip) of the drain was unavailable for bacterial culture for 5 dogs in the UR group and 1 dog in the POSP group. Consequently, 18 and 2 drain tips were cultured from dogs in the UR and POSP groups, respectively. Bacteria were isolated from all tips cultured. Of the 18 drain tips retrieved from dogs in the UR group, 11 had 1 organism isolated and the remaining 7 had multiple organisms isolated. Bacteria isolated from the UR group included *E faecium* (n = 9), *Enterobacter cloacae* (5), *Micrococcus luteus* (3), *Clostridium perfringens* (2), an unidentified coccus (2), and *Escherichia coli*, *Moraxella canis*, *Clostridium tertium*, *Staphylococcus* sp, *Enterobacter aerogenes*,

Table 4—Descriptive data for various cytologic and bacterial culture results for abdominal fluid obtained from the dogs of Table 1 on a daily basis before (day 0) and after surgery.

Group	Day	No. of dogs evaluated	No. (%) with intracellular bacteria	Median (range) percentage neutrophils	Median (range) neutrophil degeneration*	No. (%) culture positive‡
POSP	0	3	1 (33)	87 (85–89)	2 (1–3)	3 (100)
	1	3	1 (33)†	92 (90–93)	2 (1–2)	3 (100)
	2	3	0 (0)	92 (90–95)	2 (2)	2 (67)
	3	2	0 (0)	—	1 (0–2)	2 (100)
UR	0	20	14 (70)	90 (60–97)	2 (0–3)	17 (74)
	1	21	9 (43)	95 (67–97)	2 (1–3)	4 (17)
	2	22	5 (23)†	93 (82–97)	1 (0–3)	0 (0)
	3	18	2 (11)	92 (82–97)	1 (0–2)	0 (0)
	4	12	1 (8)	92 (75–98)	1 (0–2)	2 (15)
	5	3	1 (33)	96 (95–97)	1 (0–2)	1 (33)
	6	1	0 (0)	96	0 (0)	0 (0)

*Neutrophil degeneration was evaluated on the basis of the extent of nuclear swelling and cell lysis and was scored on a 4-point scale where 0 = none, 1 = mild, 2 = moderate, and 3 = severe. †1 dog had intracellular bacteria that did not have intracellular bacteria the previous day. ‡For the UR group, the denominator used for calculating this percentage was 23 on days 0, 1, and 2; 19 on day 3; 13 on day 4; 3 on day 5; and 1 on day 6.
— = No cells observed during this evaluation.
See Tables1 and 3 for remainder of key.

Staphylococcus xylosus, *Lactobacillus marinus*, and an unidentified gram-positive bacillus (1 each). *Candida* spp were isolated from 3 drains. Multiple bacterial organisms were isolated from both drains cultured from the POSP group and included *E cloacae*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Enterobacter asburiae*, *C perfringens* (1 each). *Candida* spp were not isolated from either drain cultured from the POSP group.

Discussion

Results of the present study indicated that previously defined cutoff values for BFG (20 mg/dL) and BFL (–2 mmol/L) differences for the diagnosis of septic peritonitis by investigators of other studies[6,7] did not accurately predict POSP in dogs when abdominal fluid was collected via an indwelling CSAD. Had those cutoffs been applied to the dogs of the present study, a high percentage of the dogs in the UR group would have been misclassified as having POSP. Many dogs in the present study that underwent celiotomy with CSAD placement had altered abdominal fluid biochemical variables yet recovered uneventfully, a finding that was consistent with the results of 2 other studies.[9,12] There was substantial overlap between the UR and POSP groups in terms of the respective ranges for abdominal fluid glucose and lactate concentrations, which might preclude identification of cutoffs for those variables that would accurately predict POSP in dogs. The use of glucose concentration as a predictor of POSP might have been hindered in the present study because a 5% dextrose solution was used as the carrier fluid for the administration of vasopressors in a small subset of dogs in both the UR and POSP groups. We did not exclude those dogs from the analysis of glucose variables because that is a common protocol for the treatment of similar patients in a clinical or hospital setting.

Abdominal fluid lactate concentration might be a better predictor of POSP than abdominal fluid glucose concentration.[15] Interestingly, in a study[15] of human patients who underwent surgery of the GIT and had a CSAD placed, an abdominal fluid–to–serum lactate ratio > 4.5 had a sensitivity and specificity of 91.3% and 81%, respectively, for the diagnosis of POSP, whereas an abdominal fluid lactate concentration > 9.1 mmol/L had a sensitivity and specificity of 81.9% and 82%, respectively, for the same purpose. We chose to evaluate the abdominal fluid–to–blood lactate ratio in the present study on the basis of those findings[15]; however, there was substantial overlap in that value between dogs in the UR and POSP groups. Additional studies with a larger population of dogs with POSP than that of the present study are necessary before a definitive conclusion can be made regarding the usefulness of abdominal fluid lactate concentration for the diagnosis of POSP in dogs.

In the present study, the mean abdominal fluid WBC count on day 3 (ie, 3 days after the initial surgery) for the dogs in the POSP group was significantly lower than that for dogs in the UR group. This was an unexpected finding. Two of the 3 dogs in the POSP group had exceedingly low abdominal fluid WBC counts (< 500 WBCs/µL) on the day that the second surgery was performed. Interestingly, those 2 dogs had a concurrent mild to moderate neutrophilic leukocytosis (peripheral blood WBC count, > 20,000 WBCs/µL). The abdominal fluid total nucleated cell count that was calculated on a daily basis to account for changes in the volume of abdominal fluid produced did not differ significantly from the abdominal fluid WBC count, which suggested that the WBC count was not artificially diluted by an increase in abdominal fluid production. Thus, the reason that the abdominal fluid WBC count for those 2 dogs was so

low on the day of the second surgery is unknown, but that finding highlights the challenges associated with the use of abdominal fluid WBC count to predict POSP. Other explanations for an unexpectedly low abdominal fluid WBC count include factors that result in poor preservation of inflammatory cells in abdominal fluid or exogenous factors such as administration of corticosteroids, which decreases the exiting of neutrophils from the peripheral blood circulation.[16,17] Corticosteroid administration was ruled out as the cause of the low abdominal fluid WBC count in the 2 dogs in the POSP group because neither dog received corticosteroids before hospital admission or during the observation period.

We postulated that a low blood-to-fluid WBC ratio would be indicative of patients with severe intraabdominal inflammation with sequestration of neutrophils in the abdominal cavity. It was hypothesized that an increase in abdominal fluid WBC count with a concurrent peripheral blood leukopenia would be suggestive of a worsening clinical condition. However, 2 of the 3 dogs in the POSP group had extremely low abdominal fluid WBC counts on day 3, which resulted in those dogs having blood-to-fluid WBC and neutrophil ratios that were much higher than expected. This finding should be interpreted with caution because only 2 dogs remained in the POSP group on day 3.

Cytologic evaluation of abdominal fluid from the dogs in the UR group revealed degenerate neutrophils in all dogs throughout the postoperative period, a finding that was consistent with the results of another study[9] that involved clinically normal dogs that underwent celiotomy, abdominal lavage, and CSAD placement. These results were in contrast to those previously published involving experimental intestinal resection-anastomosis and peritonitis induced by mesenteric ligation when a CSAD was not used because only dogs that developed POSP had a predominant population of degenerate neutrophils and intracellular bacteria in the abdominal fluid.[13] Collectively, these results suggest that the presence of an indwelling CSAD and constant suction might promote local inflammation of the peritoneum and alter neutrophil response and provide further support to concerns about the use of cutoffs derived from other populations for patients with CSADs. However, neutrophil degeneration can be difficult to differentiate from poor cell preservation, the latter being an in vitro artifact and can be seen during inappropriate abdominal fluid sample processing (eg, long-standing fluid retrieved from the collection device or drain tubing, samples stored at room temperature, and traumatic slide preparation).[18] As such, additional exogenous factors can affect cell morphology in patients from which abdominal fluid is collected by a CSAD; we attempted to avoid those factors during collection of the abdominal fluid samples from the dogs of the present study.

Declining numbers of intracellular bacteria observed in abdominal fluid samples over successive days in several dogs of the UR group likely reflected progressive decontamination of the abdomen by the local immune system and the effects of antimicrobial administration. This finding highlighted the need to avoid over-reliance on cytology or culture results alone for the diagnosis of POSP or as an indication of prognosis because bacteria can be present in the abdominal fluid of dogs with uneventful recoveries, particularly when identified early in the recovery period. The results of the present study differed from those of another study[9] in which intracellular and extracellular bacteria were observed in abdominal fluid samples collected for up to 7 days after surgery from 4 healthy research dogs that recovered uneventfully from celiotomy without incision into the GIT. The declining frequency of observed bacteria in abdominal fluid samples over time for the dogs of the present study might be the result of routine antimicrobial administration during the perioperative period. Antimicrobials were not administered to the healthy dogs of that other study.[9] Alternatively, differences between the present study and that other study[9] in the method used to collect abdominal fluid from the CSADs could have accounted for the varying bacterial contamination rates of abdominal fluid observed.

None of the dogs in the POSP group had bacteria observed in the abdominal fluid collected on the day that POSP was diagnosed. Although the POSP group consisted of only 3 dogs, this finding was notable because it suggested that the absence of bacteria in abdominal fluid during cytologic evaluation cannot be used to rule out intestinal surgical site failure and the need for additional surgery, especially during the early stages of dehiscence. All 3 of those dogs were receiving antimicrobials, which likely contributed to the lack of bacteria in the abdominal fluid, and 2 of them had low abdominal fluid WBC counts, which limited the number of cells available for assessment. It is also possible that overt failure of the intestinal surgical site had not yet occurred at the time (8 AM) the abdominal fluid sample was collected.

The volume of abdominal fluid produced decreased over time during the postoperative period for all dogs except 1 in the UR group; unfortunately, the reason for the increase in abdominal fluid production for that dog was not identified. In another study,[11] 2 of 20 dogs managed with a CSAD after GIT surgery had an increase in abdominal fluid production after drain placement; 1 of those dogs recovered uneventfully and the other required additional surgery for the treatment of POSP. In the present study, only 1 of the 3 dogs in the POSP group had an increase in the volume of abdominal fluid produced the day before the second surgery and none of the dogs had an increase in the volume of abdominal fluid produced the day of the second surgery. The lack of an increase in abdominal fluid production in those dogs could include a negative fluid balance or incomplete drainage of abdominal fluid because of fibrinous adhesions or partial obstruction of the drain by omentum. None of

the dogs in the present study underwent an omentectomy to optimize abdominal fluid drainage, and abdominal ultrasonography was not performed daily to assess the efficiency with which the CSADs drained fluid from the abdominal cavity. Consequently, the mean daily abdominal fluid production for both the UR and POSP groups should be interpreted cautiously. Although we routinely monitor the volume of abdominal fluid drained from patients with CSADs at our institution, little information is available regarding the association between the volume of fluid drained from CSADs and various clinical outcomes. Investigators of 2 clinical studies[8,11] reported appropriate abdominal fluid drainage for up to 11 days from indwelling CSADs that were placed in dogs with septic peritonitis following source-control celiotomy. Even though occlusion of the CSAD was not suspected in any of the dogs of those studies,[8,11] abdominal ultrasonography was not performed to confirm complete drainage of abdominal fluid; therefore, CSAD occlusion or dysfunction cannot be ruled out. Results of another study[12] in which dogs managed with a CSAD following intestinal resection and anastomosis were evaluated with abdominal ultrasonography on each of the first 2 days after surgery indicate that the amount of abdominal fluid varied among dogs with some dogs having multiple large pockets of free fluid in the abdominal cavity despite the presence of a CSAD. Thus, placement of a CSAD does not ensure complete drainage of accumulated abdominal fluid, and the volume of abdominal fluid drained from a CSAD on a daily basis should be cautiously interpreted.

If compartmentalization of abdominal fluid occurs, the characteristics of the fluid collected through the CSAD might not be representative of the fluid surrounding the intestinal surgical site. In an experimental study[19] in which sump-Penrose drains were surgically placed in healthy dogs, the amount of saline (0.9% NaCl) solution recovered during a 24-hour period following abdominal infusion (20 mL/kg) varied among dogs. Interestingly, when the dogs of that study[19] were necropsied, the sump-Penrose drain in each dog was encased by omentum, which undoubtedly affected the retrieval of the infused fluid. Although the sump-Penrose drains used in that study[19] are different from the CSADs used in the present study, encasement of a CSAD by omentum could result in incomplete and inhomogeneous abdominal drainage. Additional investigation of the patency of CSADs in patients that develop POSP and require a second surgery is warranted.

Bacteria were isolated from all of the drain tips cultured in the present study, whereas bacteria were isolated from only 4 of 9 drain tips cultured in another study.[9] The discrepancy in the CSAD bacterial colonization rate between the 2 studies was most likely a reflection of the fact that all dogs in the present study had naturally occurring septic peritonitis and underwent celiotomy with invasion of the GIT, whereas the dogs of that other study[9] were healthy and underwent an exploratory celiotomy for drain placement without incision into the GIT. On the basis of these findings, it appears that CSADs represent a nidus for bacterial growth. This is not particularly surprising because any foreign material within the body is predisposed to bacterial colonization. It is unclear whether CSAD placement constitutes a risk for the subsequent development of an infection; however, the fact that bacteria were isolated from all CSADs that were removed from dogs in the UR group, suggested that bacterial colonization of the drain might be clinically irrelevant. Additional studies to evaluate bacterial growth on CSADs similar to those conducted for indwelling urinary catheters are necessary. Current veterinary guidelines[20] do not recommend bacterial culture of the tip of indwelling urinary catheters or urine collected through those catheters as an aid for the definitive diagnosis of catheter-associated urinary tract infections. Similar recommendations regarding bacterial culture of CSADs and abdominal fluid obtained from those drains may be applicable in regard to the definitive diagnosis of POSP. Finally, the fact that bacteria were cultured from all CSADs evaluated in the present study despite all dogs being treated with antimicrobials suggested that the drains developed a biofilm during the postoperative period in a manner analogous to indwelling urinary catheters.[21,22]

The present study had several limitations. The study population was small and only 3 dogs developed POSP, the outcome of interest. It is likely that a prospective multicenter study will be required to enroll a sufficient number of dogs managed with a CSAD to identify criteria for the diagnosis POSP. Unfortunately, even large-scale studies[23,24] involving human patients have been unable to elucidate objective criteria for the identification of patients that subsequently develop POSP. The small study population limited the power of the present study, and although the findings should be interpreted cautiously, they represent potential criteria for further investigation. Another limitation of this study was that blood and abdominal fluid samples were collected only once daily, and even though that approach allowed for determination of the kinetics of the variables assessed over time, it may not have been sufficient for detection of short-term changes in fluid or blood composition or the exact onset time of POSP.

Results of the present study failed to identify any objective criteria for the prediction of POSP and the need for additional surgery in dogs. In dogs with conditions managed with CSADs, abdominal fluid glucose and lactate concentrations and BFG and BFL differences are not reliable prognostic indicators of POSP. Although 2 of the 3 dogs that developed POSP in this study had exceedingly low abdominal fluid WBC counts and high blood-to-fluid WBC and neutrophil ratios, investigation of a larger population of dogs with CSADs that subsequently develop POSP is necessary before those indices can be recommended as prognostic indicators. Additionally, cytologic find-

ings of intracellular bacteria or degenerate neutrophils in abdominal fluid obtained via a CSAD should not be used as the sole indicators of POSP. The characteristics of abdominal fluid collected via a drain are frequently altered and should be interpreted in conjunction with the clinical status of the patient.

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Footnotes

a. Hubless silicone flat drain (7 or 10 mm), CR Bard Inc, Covington, Ga.
b. ABL800 FLEX, Radiometer Canada, London, ON, Canada.
c. 100-cc silicone closed wound suction evacuator, CR Bard Inc, Covington, Ga.
d. Advia 2120, Siemens, Burlington, ON, Canada.
e. Wright stain, Fisher Scientific Company LLC, Kalamazoo, Mich.
f. Columbia blood agar, Oxoid Microbiology Products, Nepean, ON, Canada.
g. Brain heart infusion, Oxoid Microbiology Products, Nepean, ON, Canada.
h. R, version 3.0.1, R Foundation for Statistical Computing, Vienna, Austria.
i. R-package version 3.1–103, R development core team (2012); R foundation for statistical computing, Vienna, Austria.
j. Prism 6, GraphPad Software Inc, La Jolla, Calif.

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