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# Evaluation of platelet function using multiple electrode platelet aggregometry in dogs with septic peritonitis

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***Abstract***

**Objective** – To assess platelet function via multiple electrode platelet aggregometry (MEPA) in dogs with septic peritonitis and in healthy dogs. The secondary aim was to determine if there is prognostic significance to changes in platelet function observed in septic dogs.
**Design** – Prospective, observational cohort study conducted from January 2012 to March 2014.
**Setting** – University teaching hospital.
**Animals** – Twenty dogs with septic peritonitis and 23 healthy dogs.
**Interventions** – None.
**Measurements and Main Results** – MEPA using arachidonic acid, adenosine diphosphate, and collagen (COL) as agonists was measured within 24 hours of diagnosis of sepsis. Compared to healthy dogs, platelet aggregation was reduced in dogs with septic peritonitis for all agonists ($P < 0.01$). Overall mortality rate was 40%. MEPA in response to COL was significantly reduced in nonsurvivors compared to survivors ($P = 0.019$). Using receiver-operating characteristic curve statistics, a COL-activated MEPA less than 43.5 aggregation units had a sensitivity and specificity of 85.7% and 90.9%, respectively, for predicting nonsurvival in dogs with septic peritonitis.
**Conclusions** – Circulating platelets from dogs with septic peritonitis have diminished aggregation in response to multiple platelet agonists. MEPA may serve as an assessment tool for illness severity in this patient population.

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***Keywords:*** canine, hemostasis, multiple organ dysfunction syndrome, platelet dysfunction, sepsis

**Abbreviations**

| | |
|---|---|
| AA | arachidonic acid |
| ADP | adenosine diphosphate |
| COL | collagen |
| DIC | disseminated intravascular coagulation |
| HES | hydroxyethyl starch |
| MEPA | multiple electrode platelet aggregometry |
| MODS | multiple organ dysfunction syndrome |
| NSAIDs | nonsteroidal anti-inflammatory drugs |
| ROC | receiver operating characteristic curve |

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## *Introduction*

Despite recent advances in veterinary critical care, mortality rates in dogs with septic peritonitis remain high.[1] Death occurs most commonly in the immediate postoperative period and is associated with the presence of comorbidities, multiple organ dysfunction syndrome (MODS), and alterations in hemostasis.[2–5]

In addition to being the primary effector cell of hemostasis, platelets play an important role in innate immunity and in the augmentation of the inflammatory response.[6,7] Murine and human platelets express functional toll-like receptor-4, which permits recognition of lipopolysaccharides and interaction with neutrophils.[8,9] Previous studies support the notion that sepsis-related changes in platelet function occur, and that the activation of platelets may promote microvascular thrombosis, disseminated intravascular coagulation (DIC) and, subsequently, MODS.[10,11] However, the exact nature of

these derangements is unclear, as experimental and clinical studies often have conflicting results. One study reported that plasma taken from septic human patients stimulated platelets from healthy subjects to aggregate and adhere to endothelial cell cultures in vitro.[12] A report in rabbits demonstrated enhanced platelet rolling and adherence to pulmonary capillaries following experimental endotoxemia, which resulted in pulmonary platelet sequestration.[13] Human clinical studies, however, have found platelet aggregation to be significantly reduced in patients with severe sepsis and septic shock.[14]

Changes in platelet function have been documented in dogs with inflammatory disorders, immune-mediated hemolytic anemia, uremia, and liver disease.[15–17] However, sepsis-related platelet dysfunction has not been extensively studied. Using the Platelet Function Analyzer-100 in a canine endotoxic shock model, Yilmaz et al observed a transient decrease in platelet closure times despite the presence of thrombocytopenia.[18] Dogs with naturally occurring sepsis and systemic inflammatory response syndrome were also found to have increased formation of platelet-neutrophil aggregates.[19]

The Multiplate analyzer, which uses multiple electrodes to evaluate impedance platelet aggregometry (MEPA), can be used for point-of-care assessment of platelet function. Kalbantner et al and Marschner et al have validated the use of the Multiplate analyzer in dogs and have shown that the device is capable of assessing canine platelet function with good precision.[20–22] Because of its ease of use, MEPA has been used to evaluate platelet function in a number of human clinical settings such as intensive care units and in patients undergoing surgery involving cardiopulmonary bypass.[14,23] Decreased platelet aggregation as detected by MEPA was associated with disease severity and predicted early DIC in human patients with severe sepsis and septic shock.[14,24,25] MEPA using collagen (COL) as an agonist was an independent predictor of the prognosis of human patients with severe sepsis.[14] MEPA has not been evaluated in dogs with sepsis.

The primary aim of this study was to assess platelet function using MEPA in dogs with septic peritonitis, and to compare this platelet function to that of healthy dogs. The secondary aim of the study was to determine if there is any prognostic significance to changes in platelet function in dogs with septic peritonitis.

## *Materials and Methods*

### Study population

The study protocol was approved by the institutional ethics and welfare committee of the Royal Veterinary College. Client-owned dogs admitted to the Queen Mother Hospital for Animals of the Royal Veterinary College and diagnosed with septic peritonitis were eligible for enrollment. Diagnosis, from peritoneal fluid analysis, was based on cytologic identification of intracellular bacteria or fungi, or positive bacterial culture. Cytologic diagnosis was made by the attending clinician and confirmed by a board-certified clinical pathologist. Patients suspected of having septic peritonitis that did not have cytologic identification of intracellular organisms were temporarily enrolled into the study until further confirmation by positive bacterial cultures. Informed owner consent was required for study inclusion. Dogs were excluded if they weighed <5 kg, or had received anticoagulant or antiplatelet therapy during the preceding 30 days. In the context of this study, drugs such as aspirin and thienopyridines were considered to be antiplatelet drugs. Dogs that had received nonsteroidal anti-inflammatory drugs (NSAIDs) such as meloxicam, carprofen, and firocoxib were included. Dogs were excluded if their platelet count was $<100 \times 10^9$/L [$100 \times 10^3/\mu$L]. Clinical management of patients was directed by their attending clinicians. Signalment, previous medical history, clinical examination findings, and clinicopathological data were recorded on admission to the hospital. Patients were discharged to owners when this was deemed to be clinically appropriate. Healthy dogs undergoing screening for use in the hospital's blood donor program served as the control population.

### Patient sampling

Following patient assessment, diagnostic testing was performed at the discretion of the attending clinician to establish a diagnosis of septic peritonitis. Emergency point-of-care tests including venous blood-gas and electrolyte analyses[a], packed cell volume, and refractometric total plasma protein were performed on all patients at the time of admission.

Once a diagnosis was confirmed and client consent for study enrolment was obtained, blood was collected within 24 hours by 1 of 2 collection methods. In patients with central venous catheters, 5 mL of blood was first removed from the catheter by syringe aspiration before attaching a new syringe to obtain blood samples from the catheter. In patients without central venous catheters, blood samples were collected by direct jugular venipuncture using a 21-Ga blood collection set.[b] The first blood tubes filled were used for CBC[c] and serum biochemistry[d] analysis. Samples for CBC were collected into evacuated potassium EDTA tubes. Samples for serum biochemistry analysis were collected into gel separator tubes. Samples for MEPA were collected into a 3.0 mL evacuated tube containing hirudin[e] as the anticoagulant. Finally, blood was collected into an evacuated tube containing 3.2% sodium citrate for the measurement of

prothrombin time and activated partial thromboplastin time on whole blood using a point-of-care analyzer.[f]

**Multiple electrode platelet aggregometry**

Platelet function was assessed by MEPA[e] according to the manufacturer's recommendations. All samples were analyzed following a 30-minute rest period at room temperature, and within 90 minutes of collection. In brief, 300 μL of hirudin-anticoagulated whole blood and 300 μL of 37°C 0.9% saline were added to each test cell, also warmed to 37°C. After 3 minutes of incubation and stirring, 20 μL of the stock agonist solution was added to each test cell. Platelets were activated with a final concentration of 15 mM arachidonic acid (AA),[g] 0.2 mM adenosine diphosphate (ADP),[h] or 100 mg/mL COL.[i] Each test cell incorporates a duplicate sensor for 2 simultaneous measurements, serving as an internal control. As platelet aggregation occurred on the surface of silver-coated electrodes within the test cell, changes in electrical impedance were reported as mean arbitrary aggregation units and area under the curve over a 6-minute interval. Measurements were repeated if the Pearson's correlation coefficient and difference between the 2 area under the curve were less than 0.98 or greater than 20%, respectively.

**Review of medical records**

Medical records for each patient were reviewed to determine signalment, the cause of septic peritonitis, hospitalization time, and the results of the bacterial culture of the peritoneal effusion. In addition, the use of oxygen therapy, synthetic colloids, human serum albumin, and vasopressor therapy was recorded. The presence of MODS was recorded, as defined by previously published criteria.[4] Dogs that were discharged from the hospital were considered survivors. Dogs that were euthanized due to financial constraints were censored and not considered as nonsurvivors. Only dogs that died or were euthanized due to moribund state were considered nonsurvivors.

**Statistical analysis**

Normality of the data sets was tested by use of the Shapiro–Wilk test. To compare categorical variables between groups, a 2 by 2 contingency table was constructed and Fisher's exact test performed. Continuous variables were compared using a Mann–Whitney test or *t*-test as appropriate. Correlations between continuous variables were assessed by Pearson correlation coefficient ($r^2$). Receiver operating characteristic (ROC) curves were constructed to determine optimal cut-offs to predict nonsurvival if significant differences between groups were found. The areas under the ROC curves were compared using the method described by Hanley and McNeil.[26] Statistical analysis was performed using commercially available software.[j] An a priori alpha of $P < 0.05$ was considered statistically significant.

## Results

**Patient population and characteristics**

Twenty-three dogs (15 males, 8 females) undergoing screening for the hospital's blood donor program served as the healthy control population. Twenty dogs (10 males, 10 females) with septic peritonitis were prospectively enrolled between January 2013 and March 2014. The median age of the control group was 7 years (range: 5–8). The median age of the septic peritonitis group was 7 years (range: 2.75–16.5). Age was not significantly different between the 2 groups ($P = 0.51$).

Peritoneal fluid was submitted for aerobic culture and antimicrobial susceptibility testing in all 20 dogs with septic peritonitis. The microorganisms isolated were *Escherichia coli* ($n = 14$), *Enterococcus spp.* ($n = 3$), *Streptococcus canis* ($n = 2$), *Clostridium perfringens* ($n = 2$), *Candida albicans* ($n = 2$), and *Pseudomonas aeruginosa* ($n = 1$). Two or more microorganisms were isolated from 8 (40%) dogs. Only *C. albicans* was cultured from 1 dog (5%) with concurrent pyloric perforation and diabetic ketoacidosis. Nineteen dogs had positive identification of intracellular organisms prior to surgery. One dog was temporarily enrolled into the study due to failure to cytologically identify intracellular organisms but was later confirmed by positive bacterial cultures of *P. aeruginosa* and *Enterococcus spp.* in peritoneal fluid. Bacterial culture did not yield any growth in 5 cases. The most common source of septic peritonitis was the gastrointestinal tract followed by urogenital and hepatobiliary systems. The etiology of septic peritonitis is summarized in Table 1. All dogs underwent exploratory laparotomy and had primary closure of the peritoneal cavity except for 1 dog that was euthanized intraoperatively. MODS was identified in 6 (30%) dogs. Five of 6 dogs (83%) with MODS died. Thirteen (65%) dogs survived to discharge, and 7 dogs (35%) died or were euthanized. Only 5 dogs had preoperative measurements of prothrombin time (reference interval: 11–17 s) and activated partial thromboplastin time (reference interval: 72–102 s) with medians of 17 (range: 10–19) and 148 (range: 116–168) seconds, respectively. None of the dogs had any clinical evidence of hemorrhage.

Two dogs were excluded from the nonsurvivor group; 1 dog was euthanized intraoperatively due to the presence of metastatic neoplasia and the other was euthanized in the postoperative period due to identification of gastrointestinal lymphoma and persistent vomiting. In the nonsurvivor group, 3 dogs experienced

**Table 1:** Characterization of etiology of septic peritonitis in dogs

| Etiology | Patients with septic peritonitis ($n = 20$) | Survivors ($n = 13$) | Non-survivors ($n = 5$) |
|---|---|---|---|
| Gastrointestinal | | | |
| Postoperative dehiscence | 5 | 3 | 2 |
| Neoplasia | 5 | 3 | 0 |
| Use of NSAIDs | 2 | 0 | 2 |
| Strangulation and incarceration | 1 | 1 | 0 |
| Perforation (idiopathic) | 1 | 1 | 0 |
| Foreign body | 1 | 1 | 0 |
| Urogenital | | | |
| Prostatic abscess | 2 | 2 | 0 |
| Pyometra | 1 | 1 | 0 |
| Hepatobiliary | | | |
| Bacterial cholecystitis | 2 | 1 | 1 |

NSAIDs, nonsteroidal anti-inflammatory drugs.

**Table 2:** Comparison of demographic variables and interventions in dogs with septic peritonitis

| | Survivors ($n = 13$) | Nonsurvivors ($n = 5$) | *P* |
|---|---|---|---|
| Age (years) | 8 (3.4, 10.5) | 6 (5.6, 9.8) | 0.777 |
| Sex (male/female) | (7/6) | (2/3) | 1.000 |
| Vasopressor therapy | 0 | 3 | 0.0245* |
| Oxygen therapy or need of mechanical ventilation | 0 | 4 | 0.0049* |
| Use of synthetic colloids† | 3 | 3 | 0.344 |
| Use of HSA‡ | 0 | 3 | 0.0245* |
| MODS | 1 | 5 | 0.0039* |
| Length of hospitalization (days) | 6.0 (4.25, 9.0) | 1.5 (0.56, 4.0) | 0.006* |

Continuous variables are expressed as median (25th and 75th percentiles).
HSA, human serum albumin; MODS, multiple organ dysfunction syndrome.
*Significant differences.
†6% hydroxyethyl starch 130/0.4 in 0.9% sodium chloride solution.[k]
‡20% human serum albumin.

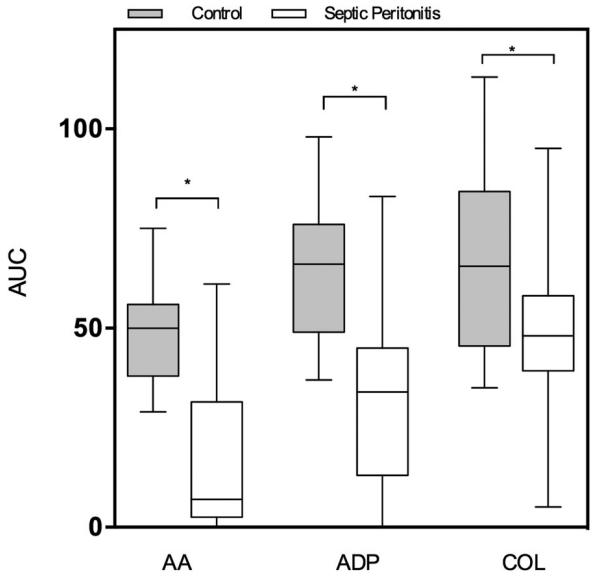


**Figure 1:** Box and whisker plots of platelet aggregation, as determined by multiple electrode impedance platelet aggregometry, in healthy dogs and dogs with septic peritonitis. Aggregometry findings are shown as the area under the curve (AUC) with activators arachidonic acid (AA), adenosine diphosphate (ADP), and collagen (COL). The first and third quartiles are represented by the lower and upper boundaries of the box, respectively, and the line within the box represents the median. The whiskers represent the complete range of data and outliers are shown. A significant difference (*) was detected between groups when AA ($P < 0.001$), ADP ($P < 0.001$), and COL ($P = 0.010$) were used as agonists.

cardiopulmonary arrest in the postoperative period. The other 2 dogs were euthanized in the postoperative period. One of these dogs was euthanized because of anuria secondary to acute kidney injury, for whom the owners declined to pursue renal replacement therapy. The other was euthanized due to acute respiratory distress syndrome. Summary statistics of both groups are presented in Table 2.

### Platelet aggregation in dogs with septic peritonitis

In comparison to healthy dogs, platelet aggregation was significantly reduced in dogs with septic peritonitis when AA ($P < 0.001$), ADP ($P < 0.001$), and COL ($P < 0.05$) were used as agonists (Figure 1). Platelet aggregation using any of the 3 agonists was not significantly different among dogs with or without MODS (Table 3). Among dogs with septic peritonitis, COL-induced platelet aggregation was significantly lower in

**Table 3:** Comparison of MEPA findings, packed cell volume, platelet, and neutrophil counts in study groups

| | Groups | | |
|---|---|---|---|
| | **Control** | **Septic peritonitis** | ***P*** |
| AA | 50.0 (38, 56) | 7 (2.5, 31.5) | <0.001* |
| ADP | 66.0 (49, 76) | 34 (13, 45) | <0.001* |
| COL† | 65.5 (22.4) | 48.65 (21.6) | 0.010* |
| Platelet ($\times 10^9$/L) | 290 (212, 395) | 177 (150, 238) | 0.002* |
| PCV (%)† | 42 (9.0) | 46 (11.0) | 0.291 |
| Neutrophils ($\times 10^9$/L) | 6.04 (4.97, 6.49) | 15.54 (8.42, 24.63) | <0.001* |
| | **Survivors** | **Nonsurvivors** | |
| AA | 13.0 (3.0, 43.0) | 7.0 (1.5, 12.0) | 0.335 |
| ADP | 35.5 (16.5, 51.0) | 20.0 (4.9, 50.0) | 0.206 |
| COL | 55.0 (45, 63.5) | 39.5 (15.5, 44.5) | 0.019* |
| Platelet ($\times 10^9$/L)† | 173.55 (55.69) | 213.50 (126.10) | 0.488 |
| PCV (%) | 45 (40, 52) | 43 (30, 53) | 0.820 |
| Neutrophils ($\times 10^9$/L) | 11.79 (6.8, 18.7) | 15.2 (10.2, 25.0) | 0.922 |
| | **No MODS** | **MODS** | |
| AA | 9.0 (3.0, 41.0) | 7.0 (1.5, 11.8) | 0.355 |
| ADP | 36.0 (14.0, 49.0) | 22.0 (14.5, 56.5) | 0.657 |
| COL | 48.0 (35.0, 63.0) | 41.5 (30.5, 51.3) | 0.219 |
| Platelet ($\times 10^9$/L) | 204.5 (150.8, 242.3) | 144 (80.0, 287.0) | 0.272 |
| PCV (%) | 47.5 (40.5, 54.5) | 43.0 (37.5, 51.8) | 0.480 |
| Neutrophils ($\times 10^9$/L) | 14.5 (7.9, 24.6) | 15.2 (5.6, 25.0) | 0.735 |

Continuous variables and median (25th, 75th percentile) are reported.
*Statistical significance.
†Continuous variables and mean (standard deviations) are reported.
PCV, packed cell volume.

**Table 4:** Receiver-operating curve statistics for multiple electrode platelet aggregometry for the prognosis of septic peritonitis in dogs

| Agonists | AUC | Optimal cut-off | Sensitivity | Specificity | 95% Confidence interval | *P* |
|---|---|---|---|---|---|---|
| AA | 0.675 | 27 | 100 | 41.7 | 0.418–0.932 | 0.268 |
| ADP | 0.700 | 28 | 80.0 | 66.7 | 0.404–0.996 | 0.206 |
| COL | 0.817 | 43.5 | 83.3 | 83.3 | 0.608–1.000 | 0.045 |

Area under the curve (AUC) with 95% confidence interval and asymptotic *P*-values are provided using activators arachidonic acid (AA), adenosine diphosphate (ADP), and collagen (COL).

nonsurvivors ($P < 0.05$; Figure 2). Platelet count did not correlate with platelet aggregation induced by AA ($r^2 = 0.020$, $P = 0.50$), ADP ($r^2 = 0.069$, $P = 0.29$), or COL ($r^2 = 0.017$, $P = 0.60$). Neutrophil count also did not correlate with platelet aggregation induced by AA ($r^2 = 0.022$, $P = 0.54$), ADP ($r^2 = 0.11$, $P = 0.18$), or COL ($r^2 = 0.000025$, $P = 0.98$).

### Prognostic significance of platelet aggregation in dogs with septic peritonitis

ROC curves were constructed to determine separate cut-offs for platelet aggregation to predict nonsurvival using AA, ADP, and COL as agonists (Figure 3). Notably, the area under the ROC curve of COL-induced aggregation was 0.82 (95% confidence interval: 0.61–1.00). A cut-off of 43.50 for COL-induced aggregation resulted in a sensitivity and specificity of 83.3% for predicting nonsurvival. Summary data of ROC-curve statistics are detailed in Table 4.

## *Discussion*

Platelet aggregation was significantly reduced in dogs with septic peritonitis as measured by MEPA, using multiple agonists. The decreased AA-induced platelet aggregation may be an exacerbation of the already variable and weak response to AA in most canine platelets.[27] In addition, sepsis may result in alterations of the cyclooxygenase pathway that is responsible for converting

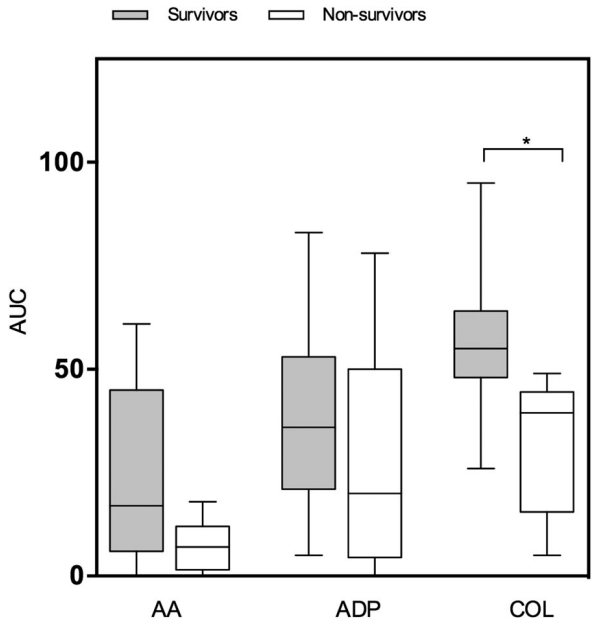


**Figure 2:** Box and whisker plots of platelet aggregation, as determined by multiple electrode impedance platelet aggregometry, in survivors and nonsurvivors, after exclusion of euthanized dogs, with septic peritonitis. Aggregometry findings are shown as area under the curve (AUC) with activators arachidonic acid (AA), adenosine diphosphate (ADP), and collagen (COL). The first and third quartiles are represented by the lower and upper boundaries of the box, respectively, and the line within the box represents the median. The whiskers represent the complete range of data and outliers are shown. A significant difference (*) was detected between groups when COL was used as an agonist ($P = 0.019$).

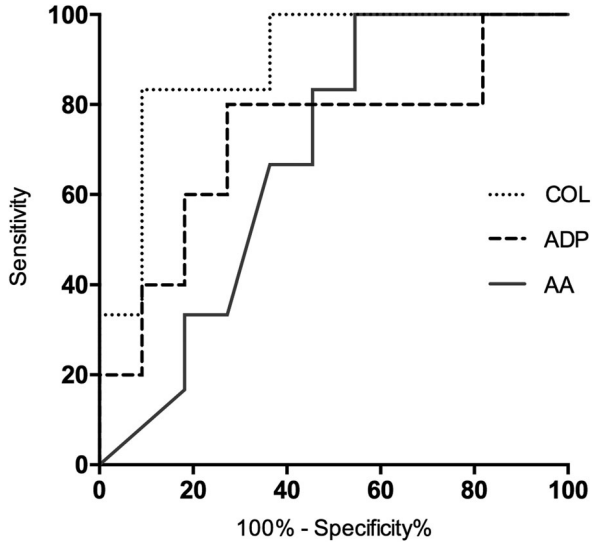


**Figure 3:** Receiver operating curve for the prediction of mortality by multiple electrode impedance platelet aggregometry in dogs with septic peritonitis with activators arachidonic acid (AA), adenosine diphosphate (ADP), and collagen (COL).

AA to thromboxane $A_2$, an agonist that induces conformational changes of integrin $\alpha_{IIb}\beta_3$, and mediating further ligand binding and platelet aggregation.[10,28] Reduced platelet aggregation in response to COL reflects the presence of hyporeactive circulating platelets in dogs with septic peritonitis. Although the exact causes of this phenomenon are unknown, several studies suggested that the decrease in platelet aggregation in patients with sepsis may be secondary to a shift of platelet function from hemostasis toward vascular healing and innate immunity.[10,29] In human and other animal models, endotoxemia promotes platelet cytokine release, platelet-neutrophil interaction, and the formation of neutrophil extracellular traps, which have both antimicrobial and prothrombotic properties. [9–11,30–33] Another possibility for the decreased aggregation seen in the circulating platelet population is the sequestration of reactive platelets in the microvasculature. This was demonstrated by Clark et al, who induced platelet-neutrophil interaction in the liver sinusoids of mice treated with lipopolysaccharide.[8]

The present study further characterizes the hemostatic imbalance found in canine sepsis. Although elements of sepsis have been considered to promote a hypercoagulable state, this study supports the notion that the presence of a hypocoagulable state is associated with poor outcome and nonsurvival in dogs with sepsis.[5,34] In Bentley et al's study, nonsurvivors not only had increased D-dimers but had hypocoagulability that failed to resolve in the postoperative period, suggesting that decompensated and excessive thrombosis resulting in hypocoagulability and hyperfibrinolysis may be associated with nonsurvival.[5] Enhanced platelet-neutrophil interaction and sequestration of reactive platelets in the microvasculature can lead to thrombosis, impairment of organ perfusion, and ultimately MODS.[11,12,35,36]

In agreement with human clinical studies, MEPA using COL was significantly associated with nonsurvival in our study.[11,14] Adamzik et al showed that decreased COL-induced MEPA was more sensitive and specific than procalcitonin and C-reactive protein for predicting outcome in people with severe sepsis.[14] Out of the 3 agonists used in this study, only COL-induced aggregation was significantly different between survivors and nonsurvivors. One possible explanation is that platelets can become refractory to in vitro ADP stimulation following in vivo or ex vivo exposure to ADP.[37] Because erythrocytes can release ADP, increased hemolysis or prolonged incubation of whole blood prior to MEPA analysis may have resulted in refractoriness to ADP. Further studies are needed to evaluate serial changes in platelet aggregation in dogs with septic peritonitis, as it may carry greater prognostic significance than measurement of aggregation responses at a single time point.

Kenney et al reported a mortality rate of 70% for dogs with MODS, which is similar to the current study, where nearly all dogs (83%) with MODS failed to survive. However, no differences in platelet function were detected between dogs with or without MODS. Given the small number of dogs in this study, the failure to detect any differences among these 2 groups of dogs may have been caused by type II error. Also, the present study only classified organ dysfunction as either present or absent, based on criteria established by a previous study.[4] Serial assessment of organ dysfunction or employment of other scoring systems such as the acute patient physiologic and laboratory evaluation (APPLE) score or survivor prediction index 2 (SPI2) that evaluate severity of organ dysfunction may have resulted in a more sensitive detection of MODS.[38,39] In addition, the lack of standardized management and diagnostics performed on the dogs of this report represents a limitation of this study and prevented the authors from using published disease severity scoring systems.

Thrombocytopenia is a common hematologic finding in critically ill people, and may be a result of bone marrow suppression, splenic sequestration, hemodilution, or consumption related to microvascular thrombosis or DIC.[40] Similarly, in the present study, dogs with septic peritonitis had a significantly lower median platelet count compared to healthy dogs. This finding, however, did not influence platelet aggregation, as platelet count did not correlate with the MEPA results. Because the results obtained with MEPA are influenced by both platelet function and platelet count, we strictly excluded thrombocytopenic dogs in the present study, which may have prevented a true representation of the overall septic population.[41] The platelet count of $100 \times 10^9$/L was chosen based on an experimental study in human platelets which showed a platelet count of $100 \times 10^9$/L or less significantly decreased aggregation as measured by MEPA by 18%, irrespective of the agonist. The study also showed a large interindividual variation and no changes in MEPA once platelet count was above $150 \times 10^9$/L.[42] Since platelet dysfunction was noted in nonsurvivors without moderate to severe thrombocytopenia, the detection of platelet hyporesponsiveness may indicate the presence of early DIC, before the onset of thrombocytopenia and prolonged coagulation times.[5,24] Further investigations on the influence of canine platelet count on MEPA are required.

This is the first study documenting the use of MEPA in a veterinary critical care setting. Platelet aggregometry using MEPA provides a rapid, low-cost, point-of-care assessment of platelet function. Although optical aggregometry has been considered to be a gold standard for assessing platelet aggregation, it is poorly standardized across laboratories and is subjected to numerous preanalytical and analytical variables that may influence results.[43] Unlike optical aggregometry, MEPA does not require the generation of platelet rich plasma. This not only eliminates the requirement of larger volumes of blood and shortens preparation time, but also minimizes the activation of platelets prior to analysis.[44] The utilization of diluted whole blood in MPEA is also considered more physiologic because it preserves the opportunity for erythrocyte- and leukocyte-platelet interactions. Erythrocytes can augment platelet function by increasing platelet AA release and expression of integrin $\alpha_{IIb}\beta_{3,}$ while neutrophils can downregulate platelet reactivity.[45] While neutrophil count did not correlate with MEPA findings in the present study, it is unknown at this time if the significant neutrophilia noted in the septic group may have contributed to platelet hypofunction in these dogs.

The present study has several limitations. The small sample size may have limited the statistical power of the study, specifically in the ability to detect platelet dysfunction in patients with MODS. Since dogs that were euthanized were considered nonsurvivors for the purpose of statistical analysis in this study, this complicates the interpretation of survival as an outcome. Despite a relatively homogeneous population, the differences of underlying etiology of septic peritonitis and lack of standardized patient management may also have had an impact on platelet function and patient survival. For instance, antibiotic therapy with beta-lactams can cause reversible in vivo dysfunction of human platelets.[46] Prior use of NSAIDs in some dogs may also have influenced the results of this study. Brainard et al demonstrated that therapeutic dosages of carprofen, meloxicam, and deracoxib decreased ADP-induced platelet aggregation but had little effect on COL-induced aggregation.[47] In addition, since the use of NSAIDs is commonly associated with gastrointestinal perforation and septic peritonitis in dogs, we did not exclude dogs that had received NSAIDs prior to admission.[3] Because only 2 dogs were treated with NSAIDs prior to enrollment into the study, a comparison of those with or without treatment was not attempted.

Because of the lack of standardized dosing and timing of blood sampling, this study was not designed to search for a causative relationship between platelet dysfunction and the administration of hydroxyethyl starch (HES) 130/0.4 solutions. Previous studies have documented in vitro impairment of canine platelet function by HES 130/0.4 solutions, and a similar process may also occur in vivo and, therefore, influence the results of this study.[48,49] Large clinical trials evaluating the risk of HES administration in dogs with sepsis are needed. Lastly, univariate and multivariate analyses would be required for identification of additional factors such as platelet

count, hematocrit, surgical procedures, drug therapies and preexisting diseases affecting platelet function and prognosis in this study population.

In conclusion, circulating platelets in dogs with septic peritonitis have diminished aggregation in response to AA, ADP, and COL. COL-induced MEPA may be a candidate assay for prediction of nonsurvival in septic dogs. Further studies are needed to evaluate the progression of changes in platelet function during sepsis and to determine the underlying causes of platelet dysfunction. MEPA may also serve as an assessment tool for disease severity in sepsis.

## Footnotes

[a] Critical Care Xpress, NOVA Biomedical Corporation, Waltham, MA.
[b] BD Safety-Lok, Becton Dickinson, UK.
[c] Cell-Dyn 2500, Abbott Diagnostics, Abbott Park, IL.
[d] ILAB 600, Holliston, MA.
[e] Roche Diagnostic International Limited, Rotkreuz, Switzerland.
[f] CoagDx Analyzer, IDEXX Laboratories, Westbrook, ME.
[g] ASPItest, Roche Diagnostic International Limited.
[h] ADPtest, Roche Diagnostic International Limited.
[i] COLtest, Roche Diagnostic International Limited.
[j] Prism 6, GraphPad Software, La Jolla, CA.
[k] Voluven®, Fresenius Kabi Ltd, Bad Homburg vor der Höhe, Germany.

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