Case-control study of plasma mean platelet component concentration and survival analysis for dogs with immune-mediated hemolytic anemia

Andrea Zoia DVM
Magda Gerou-Ferriani DVM
Michele Drigo DVM, PhD
Marco Caldin DVM, PhD

From the San Marco Veterinary Clinic, via Sorio 114c, 35141 Padua, Italy (Zoia); the Clinica Veterinaria Malpensa, Via G Marconi 27, 21017 Samarate, Italy (Gerou-Ferriani); the Department of Animal Medicine, Production and Health, Padua University, Agripolis, 35020 Legnaro, Padua, Italy (Drigo); and the Laboratorio d’Analisi Veterinarie San Marco, via Sorio 114c, 35141 Padua, Italy (Caldin).
Address correspondence to Dr. Zoia (zoia.andrea06@gmail.com).

OBJECTIVE
To determine whether dogs with immune-mediated hemolytic anemia (IMHA) had a low plasma mean platelet component (MPC) concentration and whether MPC was associated with outcome.

DESIGN
Retrospective case-control study and survival analysis.

ANIMALS
95 dogs with IMHA (cases) as well as 95 healthy dogs and 95 sick dogs without IMHA (controls) matched to cases by age, reproductive status, and breed.

PROCEDURES
Plasma MPC concentration at initial examination was compared among groups. For dogs with IMHA only, sex, age, serum urea and bilirubin concentrations, Hct, platelet count, and plasma fibrinogen, D-dimer, and MPC concentrations were evaluated for associations with survival to 42 days after initial examination.

RESULTS
Plasma MPC concentration was significantly lower in dogs with IMHA than in the other 2 dog groups. In dogs with IMHA, plasma MPC concentration was the only factor significantly associated with outcome. The optimal plasma MPC concentration cutoff value for predicting nonsurvival of dogs with IMHA was 19.1 g/dL; values ≤ 19.1 g/dL were associated with nonsurvival. Likewise, the survival curve for dogs with plasma MPC concentrations < 19.1 g/dL differed significantly from that for dogs with values > 19.1 g/dL. The mean estimated risk of death for dogs with IMHA decreased by 16% for every unit increase in plasma MPC concentration.

CONCLUSIONS AND CLINICAL RELEVANCE
In dogs with IMHA, platelets appeared to have been activated to a greater degree, as determined by lower plasma MPC concentrations, than in healthy dogs or sick dogs without IMHA. Plasma MPC concentration at initial examination may be useful for predicting prognosis in dogs with IMHA. (J Am Vet Med Assoc 2018;252:1384–1392)

Platelets play an important role in hemostasis, representing the first line of defense against bleeding at sites of vascular injury. Adhesion to damaged endothelium through specific cell surface receptors and interaction with coagulation factors lead to platelet activation, aggregation, and formation of the hemostatic plug at the site of vascular injury. When circulating platelets become activated, they secrete the contents of their alpha, dense, lysosomal granules through their canalicular systems to the exterior.1

Plasma MPC concentration is a measurement derived from the platelet refractive index (which is linearly related to platelet density) and can be determined only by use of a particular automated hematology analyzer system via 2-angle light scatter. This analyte can be used to assess platelet activation.2 When activated platelets degranulate, their density decreases, and the plasma MPC concentration then also decreases.3

Flow cytometry has been used in veterinary medicine for direct or indirect measurement of platelet activation through quantification of P-selectin expression by platelets, detection of platelet-bound fibrinogen, or measurement of platelet-leukocyte aggregates.1–6 P-selectin is a protein of the interior surface of alpha-granule membranes and is exposed on cell surfaces only after platelets degranulation.7–10 Degree of platelet P-selectin expression is correlated with plasma MPC concentration, suggesting that mea-
Measurement of both variables may be useful in the detection of activated platelets in dogs. Quantification of P-selectin expression by platelets is clinically applicable but requires a flow cytometer and approximately 60 minutes of sample preparation time. On the other hand, plasma MPC measurement can be included in a routine CBC and therefore be performed inexpensively and quickly.

Immune-mediated hemolytic anemia is a common hematologic disorder in dogs and can be primary or secondary in nature. Most dogs with IMHA have primary or idiopathic disease, and associated mortality rates can be high. Hemostatic disorders such as thrombosis and DIC can increase the risk of death associated with IMHA.

In the past decade, platelet activation has been identified in dogs with septic and nonseptic inflammatory disease, including IMHA, on the basis of high platelet P-selectin expression. Activated platelets may therefore contribute to the development of micro- or macrothrombi, causing illness and death in dogs with IMHA. Treatment with combinations of corticosteroid drugs, azathioprine, and ultralow-dose aspirin reportedly results in significantly greater short- and long-term survival rates in dogs with pIMHA, and dogs with pIMHA receiving standard immunosuppressive treatment with clopidogrel, alone or in combination with ultralow-dose aspirin, have a short-term survival rate similar to that of dogs receiving ultralow-dose aspirin alone. These findings further suggest that platelet activation may be involved in the prothrombotic state of dogs with IMHA. Platelet hyperreactivity has also been identified via thromboelastography in dogs with IMHA, and for some of these dogs, this hyperreactivity may be the primary cause of hypercoagulability.

The primary aim of the retrospective study reported here was to determine whether dogs with IMHA had detectable evidence of activated platelets as inferred by a low plasma MPC concentration at initial examination. Given that platelet activation may lead to a prothrombotic state and IMHA may be complicated by thrombotic conditions such as thromboembolism and DIC (which are both reported causes of death for dogs with IMHA), a secondary aim was to assess the prognostic value of plasma MPC concentration at initial examination for dogs with IMHA. In addition, we sought to identify whether signalment factors, clinicopathologic findings previously associated with death in dogs with IMHA (ie, serum urea and bilirubin concentrations, and Hct) or with risk of thrombosis (ie, plasma fibrinogen and D-dimer concentrations and platelet count), and plasma MPC concentration were associated with outcome in dogs with IMHA.

Materials and Methods

Animals

The electronic medical records database of the San Marco Veterinary Clinic, Padua, Italy, was searched to identify dogs evaluated at the clinic between August 1, 2005, and February 28, 2011, for inclusion in 3 groups. Dogs were included in the IMHA (case) group if they had anemia (Hct < 30%; reference interval, 38.6% to 54.5%) and anti-erythrocyte membrane antibody detected by flow cytometry. Dogs with IMHA and evidence of a concurrent condition such as neoplasia or infectious disease as well as dogs that received medications such as potentiated sulfonamides or cephalosporins or that were vaccinated within 30 days before anemia was detected were classified as having sIMHA. The remaining dogs were classified as having pIMHA. Dogs that had received prednisone treatment for < 14 days prior to initial examination were not excluded from the study; however, they were excluded if they had received prednisone for > 14 days. Dogs with IMHA were also excluded from the study if they had received in the 28 days prior to initial examination any immunsuppressant drugs other than prednisone (eg, azathioprine, cyclophosphamide, or cyclosporine), antiplatelet treatment (eg, aspirin or clopidogrel), or a blood transfusion. Dogs in the IMHA group were also required to have a minimum recorded follow-up period of 42 days after initial examination.

Two control groups were formed. Dogs were included in the healthy control group if the reason for initial examination was routine annual examination, elective surgery, blood donor health screening, or prebreeding examination. Dogs were included in the sick control group if they were examined for any health concern other than IMHA. All control dogs were individually matched to dogs with IMHA by age (within 6 months), reproductive status (ie, sex and neuter status), and breed. When no breed-matched dog of the same age and sex as a given case dog could be identified in the database, a dog of similar body weight (within 5 kg [11 lb]) was selected instead. Dogs were selected as closely as possible to the admission date of the corresponding case dog. When ≥ 2 dogs fulfilled these criteria, the records system was used to randomly select 1 dog.

To be included in any group, dogs were required to have a complete medical record, including medical history and results of physical examination, CBC (including blood smear examination), serum biochemical analysis, coagulation profile analysis, and urinalysis. All dogs in the IMHA group and some dogs in the sick control group had also undergone thoracic radiography and abdominal ultrasonography. Dogs in these 2 groups also had other diagnostic tests performed when judged necessary by the attending clinician. To be included in the study, dogs in the IMHA group and sick control group were also required to have received a specific diagnosis relevant to the initial reason for examination. Dogs eligible for either control group were excluded if they had received during the 28 days prior to initial examination any antiplatelet treatment, such as aspirin or clopidogrel, or a blood transfusion.
Data collection

Data recorded at initial examination were retrieved from the electronic medical records of each dog regarding signalment (reproductive status, age, and breed) and plasma MPC concentration. For dogs with IMHA only, data were also retrieved from the electronic medical records regarding serum urea and bilirubin concentrations, Hct, plasma fibrinogen and D-dimer concentrations, platelet count, and prednisone treatment duration, when applicable. For dogs in the IMHA group that failed to survive for at least 42 days after initial examination, the time from initial examination to death was retrieved.

All clinicopathologic tests had been performed at the Laboratorio d’Analisi San Marco for all dogs at initial examination. A blood sample was collected from each dog via cephalic (for medium-sized or large dogs) or jugular (for small dogs) venipuncture into sterile 10-mL plastic syringes. Two milliliters of blood was immediately transferred to plastic tubes containing K$_2$-EDTA for a CBC and anti-erythrocyte membrane antibody testing via flow cytometry. The plasma MPC concentration was measured as part of the routine CBC, which was performed by use of a hematology analyzer$^b$ ≤ 60 minutes after sample collection.

Percentages of erythrocytes with IgG or IgM membrane binding for dogs with IMHA were measured ≤ 60 minutes after sample collection by use of a flow cytometer$^c$ as described elsewhere.$^{30}$ A sample was considered positive for antibody binding if a positive peak was observed in the fluorescence histogram in addition to > 1.47% of erythrocytes with membrane-binding IgG or 1.38% of erythrocytes with membrane-binding IgM.$^{30}$

Statistical analysis

Continuous data were assessed for normality of distribution with the Shapiro-Wilk test. Normally distributed data were reported as mean ± SD, and non-normally distributed data were reported as median (range). Differences among the 3 groups in mean age were evaluated by ANOVA. The plasma MPC concentration was compared among groups via 1-way ANOVA. The plasma MPC concentration at the time of initial examination was also calculated. For all statistical analyses, values of $P < 0.05$ were considered significant.

Results

Animals

Breed classifications and reproductive statuses of dogs in the IMHA group and the 2 control groups (healthy or sick without IMHA) were summarized (Table 1). During the study period, 191 dogs received a diagnosis of IMHA at the San Marco Veterinary Clinic, but only 95 (50%) fulfilled all inclusion criteria and were therefore included in the IMHA group. All of these dogs were anemic (mean ± SD Hct, 14.63 ± 5.89%) and had positive results of flow cytometric testing for erythrocyte membrane-binding IgG (n = 49), IgM (4), or both (42). Spherocytes were detected on blood smear examination for 66 (69%) dogs; 25 dogs had mild spherocytosis (ie, 1 to 10 spherocytes/hpf [1,000×]), 13 had moderate spherocytosis (ie, 11 to 50 spherocytes/hpf), 13 had marked spherocytosis (ie, 51 to 150 spherocytes/hpf), and 15 had severe spherocytosis (ie, > 150 spherocytes/hpf). Thirty-six (38%) dogs in the IMHA group received prednisone treatment (mean ± SD dose, 1.90 ± 1.07 mg/kg/d [0.86 ± 0.49 mg/lb/d]; range,
before initial examination, for a median duration of 3 days (range, 1 to 14 days). On the basis of whether dogs had concurrent disease in addition to IMHA, 64 (67%) dogs were classified as having pIMHA and 31 (33%) as having sIMHA. Underlying causes of sIMHA included bacterial infection (n = 11), neoplasia (8), ehrlichiosis (2), and other causes (10).

Dogs in the healthy control group (n = 95) were 100% matched to dogs in the IMHA group with respect to reproductive status and 77% with respect to breed (Table 1). Dogs in the sick control group (n = 95) were 100% matched to dogs in the IMHA group with respect to reproductive status and 98% with respect to breed. Causes of sickness for dogs in the sick control group included urogenital disease (n = 16), neoplasia (14), gastrointestinal disorder (11), brain and spinal cord disease (10), endocrinopathy (7), road traffic accident or other traumatic disease (7), respiratory problems (6), sepsis and infectious disease (5), inflammatory or immune-mediated disease (3), cardiac problems (3), liver disease (3), and other causes (10).

Comparisons among groups
No significant (P = 0.99) difference in mean age was identified among dogs with IMHA (mean ± SD, 7.32 ± 3.87 years; range, 8 weeks to 16.5 years), healthy dogs (7.29 ± 3.81 years; range, 16 weeks to 15.3 years), and sick dogs without IMHA (7.29 ± 3.80 years; range, 12 weeks to 15.4 years). Mean plasma MPC concentration (reference interval, 19.1 to 24.4 g/dL) at initial examination was significantly (P < 0.001) lower in dogs with IMHA (20.21 ± 2.58 g/dL) than in healthy dogs (22.01 ± 2.07 g/dL) or sick dogs without IMHA (21.31 ± 2.01 g/dL). Mean plasma MPC concentration in healthy dogs was significantly (P = 0.03) greater than in sick dogs without IMHA (Figure 1).

Among dogs with IMHA, no significant (P = 0.73) difference in mean plasma MPC concentration at initial examination was identified between those with pIMHA (20.27 ± 2.46 g/dL) and those with sIMHA (20.09 ± 2.23 g/dL). There was also no significant (P = 0.63) difference in mean plasma MPC concentrations between dogs that received (20.03 ± 2.36 g/dL) or did not receive (20.06 ± 2.36 g/dL) prednisone in the 2 weeks prior to initial examination.

Table 1 —Number (%) of dogs with various signalment characteristics, grouped by whether dogs had IMHA (n = 95; case group), were healthy (95; healthy control group), or had health concerns other than IMHA (95; sick control group) at initial examination.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>IMHA</th>
<th>Healthy</th>
<th>Sick without IMHA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breed or type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed-breed dog</td>
<td>31 (33)</td>
<td>40 (42)</td>
<td>32 (34)</td>
</tr>
<tr>
<td>English Cocker Spaniel</td>
<td>5 (5)</td>
<td>6 (6)</td>
<td>5 (5)</td>
</tr>
<tr>
<td>Shih Tzu</td>
<td>5 (5)</td>
<td>4 (4)</td>
<td>5 (5)</td>
</tr>
<tr>
<td>Boxer</td>
<td>3 (3)</td>
<td>3 (3)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Toy Poodle</td>
<td>3 (3)</td>
<td>0 (0)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Yorkshire Terrier</td>
<td>3 (3)</td>
<td>3 (3)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Other</td>
<td>45 (47)</td>
<td>39 (41)</td>
<td>44 (46)</td>
</tr>
<tr>
<td>Reproductive status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sexually intact male</td>
<td>30 (32)</td>
<td>30 (32)</td>
<td>30 (32)</td>
</tr>
<tr>
<td>Castrated male</td>
<td>8 (8)</td>
<td>8 (8)</td>
<td>8 (8)</td>
</tr>
<tr>
<td>Sexually intact female</td>
<td>29 (31)</td>
<td>29 (31)</td>
<td>29 (31)</td>
</tr>
<tr>
<td>Spayed female</td>
<td>28 (29)</td>
<td>28 (29)</td>
<td>28 (29)</td>
</tr>
</tbody>
</table>

Figure 1 —Box-and-whisker plots of plasma MPC concentrations at initial examination for dogs with IMHA (n = 95), healthy dogs (95), and sick dogs without IMHA (95). For each box, the central horizontal line represents the median, and the lower and upper boundaries represent the 25th and 75th percentiles, respectively. Whiskers represent the most extreme observations that were not outliers. Circles represent outliers (ie, values that were less than or greater than the 25th or 75th percentile values by > 1.5 times the interquartile range). The shaded region represents the reference interval for plasma MPC concentration. a–c Mean values of the represented data with different superscript letters differ significantly (P ≤ 0.03).

Figure 2 —Box-and-whisker plots of plasma MPC concentrations at initial examination for dogs with IMHA that survived (n = 56) or did not survive (39) to 42 days after initial examination. a,b Mean values of the represented data with different superscript letters differ significantly (P = 0.004). See Figure 1 for remainder of key.
By the end of the 42-day follow-up period, 39 of the 95 (41%) dogs with IMHA had died (n = 25) or were euthanized (14) because of worsening of their clinical condition. No difference in mortality rates was identified by IMHA type (pIMHA, 24/64 [38%]; sIMHA, 15/31 [48%]; P = 0.43) or whether prednisone had been received prior to diagnosis (yes, 16/36 [44%]; no, 23/59 [39%]; P = 0.76). Twenty-seven of the 39 (69%) nonsurvivors had died within 1 week after initial examination (overall mortality rate at this point, 27%), and 30 (77%) died within 2 weeks after initial examination (overall mortality rate at this point, 52%). At initial examination, mean plasma MPC concentration was significantly (P = 0.004) lower in nonsurvivors (19.37 ± 2.64 g/dL) than in survivors (20.79 ± 2.00 g/dL; Figure 2). Differences between survivors and nonsurvivors regarding sex and age, serum urea and bilirubin concentrations, Hct, plasma fibrinogen concentration, platelet count, and plasma D-dimer concentration at initial examination were summarized (Table 2).

### ROC curve analysis and mortality rate

The optimal cutoff value identified through ROC curve analysis for plasma MPC concentration (ie, the value that maximized the sum of sensitivity and specificity [Youden index] in discriminating survivors from nonsurvivors) was 19.1 g/dL. Sensitivity at this cutoff value was 51%, and specificity was 84% (AUC, 0.67; 95% CI for the AUC, 0.56 to 0.79; P = 0.004; Figure 3).

The frequency of dogs with pIMHA having a plasma MPC concentration ≤ 19.1 g/dL (the optimum cutoff value shown in Figure 3; solid line; n = 29) or > 19.1 g/dL (dotted line; 66).

### Table 2—Comparison of various characteristics at initial examination for dogs with IMHA that survived (n = 56) or did not survive (39) to 42 days after that examination.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Survivors</th>
<th>Nonsurvivors</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>7.0 ± 3.91</td>
<td>7.8 ± 3.83</td>
<td>0.33</td>
</tr>
<tr>
<td>Male sex</td>
<td>21 (38)</td>
<td>17 (44)</td>
<td>0.55</td>
</tr>
<tr>
<td>Serum urea concentration (mg/dL)</td>
<td>55 (12–366)</td>
<td>61 (15–444)</td>
<td>0.89</td>
</tr>
<tr>
<td>Serum bilirubin concentration (mg/dL)</td>
<td>0.45 (0.04–39.14)</td>
<td>0.65 (0.06–33.94)</td>
<td>0.22</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>14.5 ± 6.5</td>
<td>14.8 ± 4.9</td>
<td>0.78</td>
</tr>
<tr>
<td>Plasma fibrinogen concentration (mg/dL)</td>
<td>647 ± 329</td>
<td>577 ± 291</td>
<td>0.29</td>
</tr>
<tr>
<td>Platelet count (× 10^3/µL)</td>
<td>286 (25–1,629)</td>
<td>266 (28–880)</td>
<td>0.19</td>
</tr>
<tr>
<td>Plasma D-dimer concentration (µg/dL)</td>
<td>0.21 (0.01–5.43)</td>
<td>0.35 (0.01–7.53)</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Values for age, Hct, and plasma fibrinogen concentration are mean ± SD. Values for sex are number (%) of dogs in each group. Values for all other variables are median (range). Characteristics with P values < 0.25 on univariate analysis were included in a multivariate model.

Figure 3—Receiver operating characteristic curve for the use of plasma MPC concentration at initial examination for discriminating dogs with IMHA that survived (n = 56) from those that did not survive (39) to 42 days after that examination. The optimum cutoff value (19.1 g/dL; circle) was identified as the value that provided the greatest sum of sensitivity and specificity (Youden index). At that value, sensitivity was 51% and specificity was 84% (AUC, 0.67; 95% CI for the AUC, 0.56 to 0.79; P = 0.004).

Figure 4—Kaplan-Meier survival curves for the 42-day period after initial examination for dogs with IMHA with an initial plasma MPC concentration ≤ 19.1 g/dL (the optimum cutoff value shown in Figure 3; solid line; n = 29) or > 19.1 g/dL (dotted line; 66).
ference in mortality rates was identified between dogs with pIMHA versus sIMHA regarding proportions with a plasma MPC concentration ≤ 19.1 g/dL (pIMHA, 13/19; sIMHA, 7/10; P = 0.73) or > 19.1 g/dL (pIMHA, 11/45 [24%]; sIMHA, 8/21 [38%]; P = 0.35).

A significant (P < 0.001) association was identified between plasma MPC concentration categorized by the cutoff value (≤ 19.1 g/dL or > 19.1 g/dL) and the probability of survival to 42 days after initial examination (OR, 5.50; 95% CI, 1.94 to 15.98). Kaplan-Meier analysis involving dogs with IMHA revealed a significant (P < 0.001) association between categorized plasma MPC concentration and the probability of survival to 42 days after initial examination (Figure 4). Dogs with values ≤ 19.1 g/dL were significantly less likely to survive for the 42-day period than dogs with values > 19.1 g/dL.

Results of multivariate Cox proportional hazards regression revealed that plasma MPC concentration at initial examination was the only factor significantly associated with death after controlling for other factors (Table 3). The mean estimated risk of death for dogs with IMHA decreased by 16% (95% CI, 4% to 27%; P = 0.01) for every 1-unit increase in plasma MPC concentration. The estimated risk of death per incremental unit of plasma MPC concentration from 18 to 23 g/dL and associated survival rates were summarized (Table 4).

**Discussion**

In the case-control study reported here, the primary aim was to investigate whether dogs with IMHA had lower plasma MPC concentrations, consistent with high numbers of activated platelets, than both healthy dogs and sick dogs without IMHA. The use of healthy dogs as the sole control group would have introduced some bias because they can be generally expected to have different management characteristics than the general population (eg, routine vaccination and regular endo- and ectoparasite prophylaxis). For this reason, we also included a group of sick control dogs without IMHA that was unaffected by the healthy control bias. Findings indicated that the mean plasma MPC concentration at initial examination was significantly lower in dogs with IMHA than in healthy dogs or sick dogs without IMHA, and it was also significantly lower in sick dogs without IMHA than in healthy dogs. In accordance with findings of other studies, these data provided support for the hypothesis that platelets circulate in a more activated state in dogs with IMHA than in healthy dogs. Findings of the present study also suggested that in dogs with IMHA, platelets circulated with a low plasma MPC concentration, a finding consistent with high platelet activation, compared with the situation in sick dogs without IMHA.

In contrast, no significant decrease in plasma MPC concentration was identified in another study for dogs with IMHA versus healthy dogs or dogs with other types of inflammatory or noninflammatory diseases. A few factors may explain this discrepancy. First, each of the 3 groups in the present study contained 95 dogs, whereas dogs in the other study (n = 228) were separated into 8 groups containing between 9 and 47 dogs each (median, 30 dogs), limiting statistical power to detect small differences among the groups. Second, the previous study involved no strategy for matching dogs by signalment during enrollment; therefore, the influence of confounding factors such as age, sex (including neuter status), and breed was not controlled for. Third, some dogs with IMHA in the present study may have also had a concurrent immune-mediated thrombocytopenia owing to the occasional association of these 2 diseases with...

**Table 3**—Results of multivariate Cox proportional hazards analysis of characteristics associated with failure to survive to 42 days after initial examination for 95 dogs with IMHA.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Hazard ratio</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma MPC concentration (g/dL)</td>
<td>0.84</td>
<td>0.73–0.96</td>
<td>0.01</td>
</tr>
<tr>
<td>Serum bilirubin concentration (mg/dL)</td>
<td>1.02</td>
<td>0.99–1.06</td>
<td>0.18</td>
</tr>
<tr>
<td>Platelet count (X 10^9/µL)</td>
<td>0.999</td>
<td>0.97–1.001</td>
<td>0.22</td>
</tr>
<tr>
<td>Plasma D-dimer concentration (µg/dL)</td>
<td>1.0</td>
<td>0.81–1.24</td>
<td>0.99</td>
</tr>
</tbody>
</table>

Only variables with P values < 0.25 on univariate analysis were included in this model.

**Table 4**—Survival rate (proportion [%]), cumulative survival rate (proportion [%]), and OR for death during the 42-day period after initial examination per incremental unit of plasma MPC concentration for 95 dogs with IMHA.

<table>
<thead>
<tr>
<th>MPC (g/dL)</th>
<th>Survival (%)</th>
<th>Cumulative survival (%)</th>
<th>OR for death (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 18.0</td>
<td>4/14 (29)</td>
<td>4/14 (29)</td>
<td>4.48 (1.29–15.57)</td>
<td>0.01</td>
</tr>
<tr>
<td>18.0–18.9</td>
<td>4/11 (36)</td>
<td>8/25 (32)</td>
<td>4.63 (1.74–12.36)</td>
<td>0.001</td>
</tr>
<tr>
<td>19.0–19.9</td>
<td>6/11 (55)</td>
<td>14/36 (39)</td>
<td>3.88 (1.62–9.32)</td>
<td>0.002</td>
</tr>
<tr>
<td>20.0–20.9</td>
<td>16/21 (76)</td>
<td>30/57 (53)</td>
<td>1.95 (0.83–4.60)</td>
<td>0.12</td>
</tr>
<tr>
<td>21.0–21.9</td>
<td>11/18 (61)</td>
<td>41/75 (55)</td>
<td>2.49 (0.82–7.54)</td>
<td>0.10</td>
</tr>
<tr>
<td>22.0–22.9</td>
<td>8/10 (80)</td>
<td>49/85 (58)</td>
<td>1.71 (0.41–7.09)</td>
<td>0.45</td>
</tr>
<tr>
<td>≥ 23.0</td>
<td>7/10 (70)</td>
<td>56/95 (59)</td>
<td>0.58 (0.11–2.76)</td>
<td>0.68</td>
</tr>
</tbody>
</table>
most dogs with IMHA in the present study had the idiopathic form or primary form of the disease. Interestingly, no difference in plasma MPC concentrations was identified between dogs with pIMHA and dogs with sIMHA. This finding suggested that, in dogs with IMHA, platelet activation may not be dependent on the underlying cause of IMHA but may instead stem from the IMHA itself. Similarly, no difference was identified in platelet activation, as measured by plasma MPC concentration, between dogs with IMHA that received prednisone before initial examination (mean dosage, 1.90 mg/kg/d for a median duration of 3 days) and dogs with IMHA that received no such treatment. This latter result is in agreement with reported findings that treatment of healthy dogs with prednisone at 2 mg/kg/d (0.9 mg/lb/d) for 15 days causes no alteration of platelet function, as measured by turbidimetric aggregometry. Likewise, despite the extensive use of corticosteroid drugs in the treatment of humans with cardiovascular and immune disorders, these drugs reportedly have minimal to no effect on platelet function in humans.

Most reported mortality rates associated with IMHA in dogs have been high. Previously reported mortality rates for dogs with pIMHA range from 20% to 70%, and this wide range could be attributable, at least in part, to the duration of follow-up. In the present study, the overall mortality rate was 41%, with no significant difference identified between dogs with pIMHA (37%) and dogs with sIMHA (48%) or between dogs that briefly received (44%) or did not receive (39%) prednisone treatment before initial examination. In addition, most (77%) dogs that failed to survive in the present study died within the first 2 weeks after initial examination. By 2 weeks after initial examination, the overall mortality rate reached 32%. These results were similar to those in a previous study that included only dogs with pIMHA, for which the estimated mortality rate for a similar period was 22.5%, followed by a marked decrease in risk of death after this time. The period of 42 days after initial examination was chosen for survival-related calculations in the present study to allow inclusion of most of the surviving dogs, for which follow-up information had been obtained at the second reevaluation after initial examination.

Dogs with IMHA are often systemically ill and can have evidence of multiorgan dysfunction. Several factors previously identified as associated with a poor prognosis for dogs with IMHA include a high plasma urca concentration, high band neutrophil count, hyperbilirubinemia, thrombocytopenia, and the presence of petechiae at the time of diagnosis. Investigators who performed a systematic review of factors associated with mortality rate in dogs with IMHA excluded studies that included no adjustment for potential confounding factors via multivariate (vs univariate) analysis. Their aim in doing so was to avoid spurious associations between single biochemical or hematologic variables with survival times. For the same reason, a multivariate approach was used in the present study to control for variables that might confound the association between plasma MPC concentration and outcome. Moreover, dogs enrolled in each of the 3 groups were matched by potential confounders (age, reproductive status, and breed), minimizing the effect of these variables on the results involving plasma MPC concentration. Although plasma D-dimer and MPC concentrations appeared to have had prognostic value on univariate analysis, only plasma MPC concentration had a significant association with outcome on multivariate analysis. To the authors’ knowledge, the present study represents the first in which plasma MPC concentration was identified as a prognostic indicator for dogs with IMHA. Neither prednisone treatment prior to IMHA diagnosis (at the first plasma MPC evaluation) nor type of IMHA (pIMHA vs sIMHA) influenced the observed prognostic value of plasma MPC concentration.

Interestingly, the optimal cutoff value for plasma MPC concentration identified by ROC curve analysis to discriminate survivors from nonsurvivors coincided with the lower reference limit for this analyte at the study laboratory. The accuracy of plasma MPC concentration for predicting death over the 42-day period after initial examination for dogs with IMHA was 0.67. An AUC between 0.50 and 0.70 is generally considered to indicate low accuracy for a diagnostic test. The observed result may have reflected the fact that dogs with IMHA have multiorgan dysfunction and therefore multiple causes of death, which cannot be predicted by a single test.

To the authors’ knowledge, no other prognostic factors for dogs with IMHA have been investigated through ROC curve analysis, precluding comparisons of the findings for the plasma MPC assay used in our study with those of other diagnostic tests. The clinical importance of the ROC curve–derived cutoff value (19.1 g/dL) for plasma MPC concentration was supported by the significant degree of association between plasma MPC concentrations equal to or below this cutoff and mortality rate, which was an expected result given that both these analyses involved survival status at 42 days after initial examination for dogs with IMHA. However, the Kaplan-Meier curves of the probability of survival over the 42-day period further supported the clinical importance of the cutoff plasma MPC value. The significant mean estimated risk of death for dogs with IMHA with respect to plasma MPC concentration at initial examination allowed calculation of the estimated risk of death for each incremental unit of plasma MPC concentration (Table 4). In the authors’ opinion, these latter estimates would be more clinically informative and useful to attending clinicians and dog owners than a generic population mortality rate range of 20% to 70%.
The overall mortality rate was significantly higher in dogs with a lower plasma MPC concentration than in dogs with a higher concentration in the study reported here. However, it was not possible to establish whether plasma MPC concentration was purely an indicator of platelet activation secondary to the prothrombotic state of dogs with IMHA or whether a low MPC concentration had direct harmful effects that contributed to hemostatic disorders such as thrombosis and DIC, which are potential causes of death in dogs with IMHA.\textsuperscript{3,13–24} Interestingly, 2 studies reported in the past decade showed that antiplatelet treatment with ultralow-dose aspirin could improve short- and long-term survival rates for dogs with IMHA\textsuperscript{25} and that clopidogrel was as effective as ultralow-dose aspirin for the short-term survival of dogs with pIMHA.\textsuperscript{25} These findings might indicate that a high degree of platelet activation, as suggested by a low plasma MPC concentration, has a direct effect on the likelihood of survival, although additional research would be needed to explore this possibility.

The hypercoagulable state in dogs with IMHA is an important cause of illness and death, resulting in an increased risk of thromboembolism and DIC.\textsuperscript{5,13–24,26} Results of the present study suggested that the hypercoagulable state characteristic of dogs with IMHA could be at least in part attributable to a higher degree of platelet activation, as indicated by a lower plasma MPC concentration at initial examination than in healthy dogs and sick dogs without IMHA. The identified prognostic value of plasma MPC concentration for dogs with IMHA regardless of type (primary or secondary) or brief prednisone treatment prior to plasma MPC measurement could theoretically justify the use of antiplatelet drugs such as clopidogrel or aspirin for all dogs with IMHA.

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Footnotes

a. POA System-Plus, version 9.0, Ingegnere Luigi Coppola, Venezia Mestre, Italy.

b. ADVIA 120 and 2120 hematology system, Siemens Healthcare GmbH, Erlangen, Germany.

c. Epics XL-MCL, Beckman Coulter Inc, Brea, Calif.

References


Small Animals & Exotic


From this month’s *AJVR*

Pharmacokinetics of fentanyl after intravenous administration in isoflurane-anesthetized red-tailed hawks (*Buteo jamaicensis*) and Hispaniolan Amazon parrots (*Amazona ventralis*)

Peter J. Pascoe et al

**OBJECTIVE**
To compare the disposition of fentanyl citrate after a single IV injection to isoflurane-anesthetized red-tailed hawks (*Buteo jamaicensis*) and Hispaniolan Amazon parrots (*Amazona ventralis*).

**ANIMALS**
6 adult red-tailed hawks and 6 adult Hispaniolan Amazon parrots.

**PROCEDURES**
Anesthesia was induced and maintained with isoflurane; intermittent positive-pressure ventilation was provided. The minimum alveolar concentration of isoflurane was determined for each bird by use of the bracketing method and a supramaximal electrical stimulus. Fentanyl (20 µg/kg) was administered IV. Arterial (red-tailed hawks) or jugular venous (Hispaniolan Amazon parrots) blood samples were obtained immediately before and 1, 2, 4, 8, 15, 30, 60, 120, 180, 240, and 480 minutes (red-tailed hawks) and 1, 5, 10, 15, 30, 60, 120, and 180 minutes (Hispaniolan Amazon parrots) after fentanyl administration.

**RESULTS**
A 3-compartment and a 2-compartment model best described fentanyl pharmacokinetics in red-tailed hawks and Hispaniolan Amazon parrots, respectively. Median apparent volume of the central compartment and volume of distribution at steady state were 236 mL/kg and 948 mL/kg, respectively, for the red-tailed hawks and 5,108 mL/kg and 13,079 mL/kg, respectively, for the Hispaniolan Amazon parrots. Median clearance and terminal half-life were 10.2 mL/min/kg and 76 minutes, respectively, for the red-tailed hawks and 199 mL/min/kg and 51 minutes, respectively, for the Hispaniolan Amazon parrots.

**CONCLUSIONS AND CLINICAL RELEVANCE**
Pharmacokinetic results for fentanyl in isoflurane-anesthetized red-tailed hawks and Hispaniolan Amazon parrots indicated large differences and should strongly discourage extrapolation of doses between these 2 species. (*Am J Vet Res* 2018;79:606–613)