

**STANDARD ARTICLE**

# Effects of clopidogrel and prednisone on platelet function in healthy dogs

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**Abstract**

**Background:** Glucocorticoids cause hypercoagulability, but it is unknown if they counteract clopidogrel's antiplatelet effects.

**Hypothesis/Objectives:** Determine the effects of clopidogrel and prednisone on platelet function.

**Animals:** Twenty-four healthy dogs.

**Methods:** Double-blinded, placebo-controlled randomized trial. Platelet function was evaluated using a platelet function analyzer and impedance aggregometry (days 0, 14, and 28) for dogs treated with placebo, clopidogrel (2-3 mg/kg/d), prednisone (2 mg/kg/d), or prednisone with clopidogrel PO for 28 days. Results were categorized as nonresponder versus responder (platelet function analyzer), and inadequate, ideal, or excessive response (aggregometry). Results were compared using mixed model, split-plot repeated measures analysis of variance and generalized estimating equation proportional odds models.  $P < .05$  was considered significant.

**Results:** Closure times differed by treatment ( $F [3, 20] = 10.5$ ;  $P < .001$ ), time ( $F [2, 40] = 14.3$ ;  $P < .001$ ), and treatment-by-time ( $F [6, 40] = 3.4$ ;  $P = .01$ ). Area under the curve (AUC) differed by treatment ( $F [3, 20] = 19.6$ ;  $P < .001$ ), time ( $F [2, 40] = 35.4$ ;  $P < .001$ ), and treatment-by-time ( $F [6, 40] = 13.5$ ;  $P < .001$ ). Based on closure times, 5/6 dogs each in the clopidogrel and prednisone/clopidogrel groups were responders. All dogs in the prednisone/clopidogrel group were overcontrolled based on AUC (days 14 and 28), whereas 5/6 (day 14) and 2/6 (day 28) dogs treated with clopidogrel were overcontrolled. Compared to clopidogrel, dogs receiving prednisone/clopidogrel were 11 times ( $P = .03$ ) more likely to have an excessive response.

**Conclusions and Clinical Importance:** Administration of clopidogrel/prednisone increases platelet dysfunction in healthy dogs.

**KEYWORDS**

antiplatelet, corticosteroid, glucocorticoid, immune-mediated hemolytic anemia, thromboprophylaxis

**Abbreviations:** AUC, area under the curve; IMHA, immune-mediated hemolytic anemia; RI, reference intervals.

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## 1 | INTRODUCTION

Mortality rates in dogs with primary immune-mediated hemolytic anemia (IMHA) historically have been high (50%-70%),<sup>1,2</sup> primarily as a consequence of fatal thromboembolism.<sup>1-3</sup> Standard treatment for dogs with IMHA includes immunosuppression, thromboprophylaxis, and supportive care.<sup>1,4,5</sup> Glucocorticoids are the most commonly used immunosuppressive agent for the treatment of IMHA in dogs.<sup>5</sup> Unfortunately, glucocorticoid administration can cause hypercoagulability in healthy dogs,<sup>6-8</sup> and it has been identified as a risk factor for thromboembolism in clinical patients.<sup>7,9,10</sup> Although commonly administered to dogs with IMHA, many thromboprophylactic medications are either cost prohibitive, administered by injection or both, leaving PO antiplatelet medications a more affordable and practical option for long-term prophylactic treatment.

Clopidogrel has become a popular thromboprophylactic agent for use in dogs with IMHA.<sup>5</sup> In a prospective trial,<sup>11</sup> no difference was found in short-term survival between dogs with IMHA receiving low-dose aspirin or clopidogrel. However, it is unknown if clopidogrel counteracts glucocorticoid-induced hypercoagulability or, alternatively, if glucocorticoid-induced hypercoagulability offsets the antiplatelet effects of clopidogrel.

The objective of this randomized-controlled double-blinded study was to determine the platelet function of healthy dogs receiving placebo, clopidogrel (2-3 mg/kg/d), prednisone (2 mg/kg/d), or prednisone with clopidogrel. Our hypothesis was that sustained administration of clopidogrel would consistently inhibit platelet function when administered singly or concurrently with prednisone.

## 2 | MATERIALS AND METHODS

### 2.1 | Study population

Blood was collected from 24 healthy dogs from the University of Tennessee, College of Veterinary Medicine teaching and research colony during a related study assessing gastrointestinal effects of clopidogrel and prednisone treatment.<sup>12</sup> Sample size calculation was performed using data from a previous study that measured platelet dysfunction in healthy dogs treated with clopidogrel.<sup>13</sup> Based on these results, and assuming a SD of 13.5 and 7.5 for pretreatment and posttreatment samples, respectively, 6 dogs per treatment group would be needed to have 95% power to find a difference of 25% in platelet aggregation significant with an alpha of .05. Animal use was approved by the University of Tennessee, College of Veterinary Medicine Institutional Animal Care and Use Committee (protocol number 2335) and was in compliance with the requirements of a facility accredited by the American Association for Accreditation of Laboratory Animal Care.

### 2.2 | Study design

Dogs were randomized to 1 of 4 treatment groups: (1) placebo (2 placebo capsules), (2) clopidogrel (2-3 mg/kg PO q24h) and placebo,

(3) prednisone (2 mg/kg PO q24h) and placebo, and (4) clopidogrel (2-3 mg/kg PO q24h) and prednisone (2 mg/kg PO q24h). Commercially available clopidogrel (Mylan Pharmaceuticals, Morgantown, West Virginia) and prednisone (West-Ward Pharmaceuticals Corp., Eatontown, New Jersey) tablets were used. The placebo gelatin capsules (LetCo Medical, Decatur, Alabama) contained lactose and were assembled by the College's pharmacy. All treatments were administered in small meatballs before feeding.

The study involved 3 periods: acclimation (days -13 to -7), baseline (days -6 to 0), and treatment (days 1-28). During the acclimation period, dogs received fenbendazole (50 mg/kg/d PO, days -13 to -9) and ivermectin (200 µg/kg SQ once, day -13). As routine colony prophylaxis, dogs also received imidacloprid and moxidectin (Advantage Multi for dogs, Bayer HealthCare, LLC, Shawnee Mission, Kansas), according to each manufacturer's instructions.

Blood was collected at the conclusion of baseline and on days 14 and 28. Blood was collected by jugular venipuncture using a 20-gauge needle directly into Vacutainer tubes containing EDTA (hematocrit and manual platelet count), 3.2% sodium citrate (whole blood platelet analyzer), and hirudin (impedance aggregometry). For both platelet function analyses, blood was kept at room temperature without disturbance until analysis, and all samples were analyzed within 4 hours of collection.

### 2.3 | Hematologic testing

At each time point, hematocrit (Methodology Automated Blood Analyzer, Antech Diagnostics, Fountain Valley, California) and manual platelet count were performed. The manual platelet estimate was performed by calculating the average number of platelets from 10 high power (oil immersion 1000× magnification) microscopic fields representing a typical red blood cell monolayer, and multiplying this number by 16 to obtain a final platelet count ( $\times 10^9/L$ ).

A whole blood platelet function analyzer (PFA-100, Siemens Healthcare Diagnostics, Deerfield, Illinois) previously evaluated for use in dogs<sup>14,15</sup> was used to analyze platelet aggregation. The instrument was used according to manufacturer's instructions. Briefly, the whole blood platelet function analyzer assesses platelet aggregation under high shear forces after activation by agonists; it measures the time, in seconds, needed to form a platelet plug and inhibit blood flow (closure time). The cutoff time for the instrument is >300 seconds. For analysis, the blood samples were gently inverted 3 to 5 times by hand and 800 µL of citrated whole blood was placed into a cartridge and analyzed. The collagen/ADP cartridge (PFA Collagen/ADP Test Cartridge, Siemens Healthcare Diagnostics, Duluth, Georgia) was used before administration of clopidogrel to ensure normal platelet function and during drug administration to assess drug-associated platelet dysfunction. Two cartridges were analyzed concurrently, and closure times were averaged. Platelet response to clopidogrel was categorized as "responder" if closure time increased  $\geq 30\%$  compared to baseline, and "nonresponder" if closure time increased by  $\leq 29\%$  compared to baseline.<sup>16</sup>

A multiple electrode impedance aggregometer (Multiplate Analyzer, Verum Diagnostica GmbH, Munich, Germany) was used according to the manufacturer's instructions (Multiplate Analyzer Manual, Verum Diagnostica GmbH, Munich, Germany) and previously has been validated for use in dogs.<sup>17-19</sup> Briefly, blood was transferred into a single-use test cell that contained warmed 0.9% sodium chloride, a dual sensor unit, and a Teflon-coated magnetic stir bar (1200 revolutions/min). The electrical resistance between the wires within the sensor unit was recorded. Aggregation was assessed using ADP (6.5  $\mu$ M; ADPtest, Roche Diagnostics GmbH, Mannheim, Germany) at a temperature of 37°C for 6 minutes. Platelet aggregation was recorded as area under the curve (AUC). The dual sensor unit generated 2 separate results, which were averaged to yield a single AUC value for each sample. Additionally, deviation from the mean was calculated for the 2 measurements that were used to create the final AUC value. Platelet response to clopidogrel was categorized as inadequate (AUC > 46 U) versus adequate (AUC  $\leq$ 46 U), with positive response also subdivided into ideal (19-46 U) versus excessive control (<19 U).<sup>20</sup> Based on these classification criteria, dogs were classified as poorly controlled, adequately controlled, or overcontrolled.

## 2.4 | Statistical and data analysis

Descriptive statistics were generated for relevant clinical and clinicopathologic variables. Hematocrit, platelet count, closure time, AUC, and AUC deviation from the mean were compared using mixed model, split-plot repeated measures analysis of variance (ANOVA) that included fixed effects of treatment group, sampling time, and treatment-by-time interaction.<sup>21</sup> The repeated measure of time was accounted for in a repeated statement. Dog within treatment group was included as a random effect. Fisher's least significant difference was used to perform posthoc analyses. Box-and-whisker plots and studentized residual diagnostics were performed to evaluate each mixed model for the presence of outliers. The Shapiro-Wilk test of normality and QQ plots of the residuals were evaluated for each marker to confirm that the assumption of normally distributed residuals had been met. Model assumptions regarding equality of variances were verified using Levene's Test for Equality of Variances. Differences in marginal means were determined for markers with significant main effect or interaction terms. Non-normally distributed data were log or rank-transformed, as necessary, to meet underlying statistical assumptions. A generalized estimating equation proportional odds model was used to evaluate level of response over time and between treatments.<sup>22</sup> The multinomial distribution and cumulative logit link function was included to evaluate the odds of each dog moving between the states of poor, adequate, and overcontrolled. Fisher's exact test was performed to assess the relationship between treatment and response (closure time and AUC) individually on days 14 and 28. Statistical computer programs (MedCalc 15.8 MedCalc Software, Ostend, Belgium; SAS 9.4 release TS1M5, SAS Institute Inc., Cary, North Carolina) were used for all analyses and  $P < .05$  was considered significant.

## 3 | RESULTS

### 3.1 | Study population

Details of the study population have been reported elsewhere.<sup>12</sup> Briefly, there were 9 intact females, 8 intact males, and 7 neutered males. There were 15 beagles and 9 hounds, which were evenly distributed among the treatment groups. Median age was 3 years (range, 2-7 years), and median body weight was 13 kg (range, 8.1-30.4 kg).

### 3.2 | Hematocrit and platelet count

The hematocrit results were within reference intervals (RI) at all time points for all dogs (Table 1). Hematocrit did not differ significantly by treatment group, sampling time, or treatment-by-time. Platelet count differed significant by treatment-by-time ( $F [6, 40] = 3.25; P = .01$ ) but not treatment group or time (Table 1). The platelet counts were within RI at all time-points for all but 2 dogs. At baseline, 1 dog in the placebo group had a platelet count of 167 000/ $\mu$ L (RI, 170 000-400 000/ $\mu$ L), but platelet clumping was present. Both automated and manual evaluation performed the next day were within RI. On day 28, 1 dog in the prednisone/clopidogrel group had a platelet count of 136 000/ $\mu$ L without evidence of platelet clumping.

### 3.3 | Whole blood platelet function analyzer

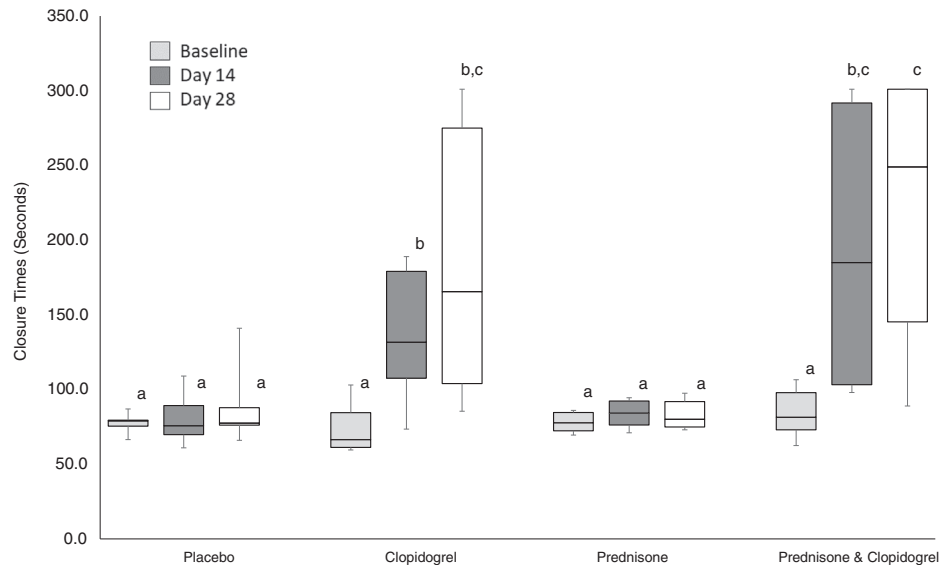
Closure times for the whole blood platelet function analyzer are presented in Figure 1. Results differed significantly by treatment group ( $F [3, 20] = 10.5; P < .001$ ), sampling time ( $F [2, 40] = 14.3; P < .001$ ), and treatment-by-time ( $F [6, 40] = 3.4; P = .01$ ). Platelet response status based on platelet analyzer closure times is summarized in Table 2.

**TABLE 1** The hematocrits and platelet counts (mean  $\pm$  SD) for 24 healthy dogs administered placebo, clopidogrel with placebo, prednisone with placebo, or combination prednisone and clopidogrel for 28 days

	Baseline	Day 14	Day 28
Hematocrit (%)			
Placebo	51.3 $\pm$ 5.6 <sup>a</sup>	52.3 $\pm$ 3.6 <sup>a</sup>	51.0 $\pm$ 3.7 <sup>a</sup>
Clopidogrel	49.3 $\pm$ 4.1 <sup>a</sup>	50.7 $\pm$ 4.7 <sup>a</sup>	50.3 $\pm$ 5.5 <sup>a</sup>
Prednisone	51.2 $\pm$ 4.1 <sup>a</sup>	49.3 $\pm$ 4.6 <sup>a</sup>	49.0 $\pm$ 4.3 <sup>a</sup>
Prednisone and clopidogrel	50.2 $\pm$ 2.4 <sup>a</sup>	50.8 $\pm$ 2.9 <sup>a</sup>	50.2 $\pm$ 2.9 <sup>a</sup>
Platelet count ( $\times 10^3$ / $\mu$ L)			
Placebo	261 $\pm$ 73 <sup>a</sup>	286 $\pm$ 72 <sup>a</sup>	322 $\pm$ 93 <sup>b</sup>
Clopidogrel	311 $\pm$ 56 <sup>a</sup>	364 $\pm$ 36 <sup>b</sup>	294 $\pm$ 35 <sup>a</sup>
Prednisone	324 $\pm$ 61 <sup>b</sup>	280 $\pm$ 40 <sup>a</sup>	294 $\pm$ 35 <sup>b</sup>
Prednisone and clopidogrel	256 $\pm$ 44 <sup>a</sup>	281 $\pm$ 64 <sup>a</sup>	259 $\pm$ 78 <sup>a</sup>

Note: Results that do not share a superscript letter differed significantly ( $P < .05$ ) on posthoc analysis. Reference intervals: Platelet count = 170-400  $\times 10^3$ / $\mu$ L, hematocrit = 36%-60%.

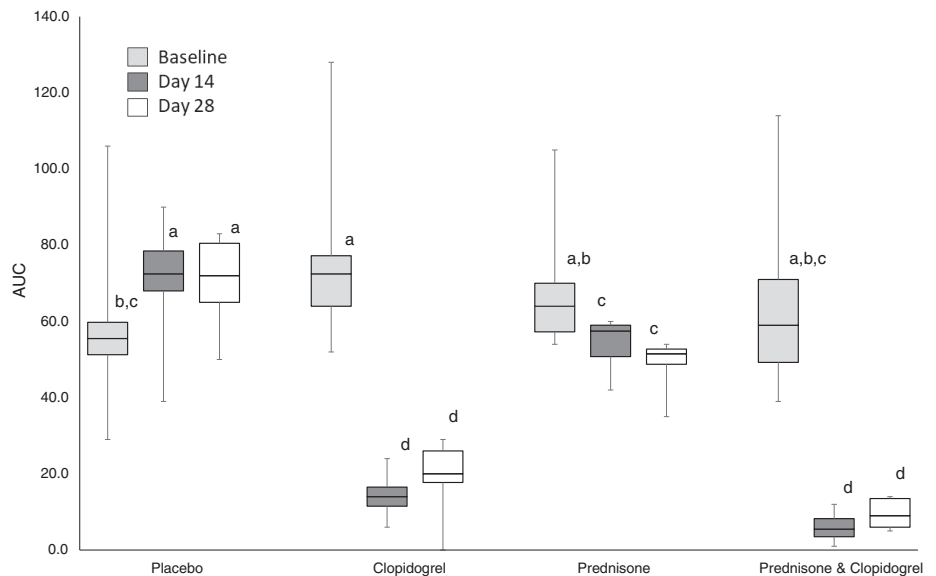
**FIGURE 1** Closure times for 24 healthy dogs administered placebo, clopidogrel with placebo, prednisone with placebo, or combination prednisone and clopidogrel for 28 days. The box and whiskers plot demonstrate the median (line), interquartile range (box), and total range (whiskers). Results that do not share a letter differed significantly ( $P < .05$ ) on posthoc analysis



**TABLE 2** Platelet response status based on the whole blood platelet analyzer closure times for 24 healthy dogs administered placebo, clopidogrel with placebo, prednisone with placebo, or combination prednisone and clopidogrel for 28 days

	Day 14		Day 28	
	Nonresponder (<30%)	Responder (≥30%)	Nonresponder (<30%)	Responder (≥30%)
Placebo	5/6	1/6	5/6	1/6
Prednisone	6/6	0/6	6/6	0/6
Clopidogrel	1/6	5/6	1/6	5/6
Prednisone and clopidogrel	1/6	5/6	1/6	5/6

**FIGURE 2** AUC for 24 healthy dogs administered placebo, clopidogrel with placebo, prednisone with placebo, or combination prednisone and clopidogrel for 28 days. The box and whiskers plot demonstrate the median (line), interquartile range (box), and total range (whiskers). Results that do not share a letter differed significantly ( $P < .05$ ) on posthoc analysis



Platelet responder status differed significantly by treatment group for both days 14 and 28 ( $P = .002$ , for each).

### 3.4 | Impedance aggregometry

The AUCs for whole-blood aggregometry are presented in Figure 2. Results differed significantly by treatment group ( $F [3, 20] = 19.6$ ;

$P < .001$ ), sampling time ( $F [2, 40] = 35.4$ ;  $P < .001$ ), and treatment-by-time ( $F [6, 40] = 13.5$ ;  $P < .001$ ). Platelet response status based on AUC results is summarized in Table 3. Platelet response status differed significantly by treatment group for both days 14 and 28 ( $P < .001$ , for each).

When considering whether a dog would be classified as poorly controlled, ideally controlled, or overcontrolled, both treatment group ( $\chi^2 [3] = 14.12$ ;  $P = .003$ ) and time point ( $\chi^2 [2] = 7.06$ ;  $P = .03$ ) were

	Day 14			Day 28		
	Poor >46 U	Adequate 19-46 U	Over ≤19 U	Poor >46 U	Adequate 19-46 U	Over ≤19 U
Placebo	5/6	1/6	0/6	6/6	0/6	0/6
Prednisone	5/6	1/6	0/6	5/6	1/6	0/6
Clopidogrel	0/6	1/6	5/6	0/6	4/6	2/6
Prednisone and clopidogrel	0/6	0/6	6/6	0/6	0/6	6/6

**TABLE 3** Platelet response status based on AUC results for 24 healthy dogs administered placebo, clopidogrel with placebo, prednisone with placebo, or combination prednisone and clopidogrel for 28 days

determined to be significant predictors. Administration of clopidogrel alone was associated with a 74% chance of being at least adequately controlled with a 14% chance of overcontrol. Conversely, coadministration of prednisone/clopidogrel was associated with a 97% chance of being at least adequately controlled with a 65% chance of overcontrol. Compared to the clopidogrel treatment group, dogs receiving prednisone/clopidogrel were 11.1 times ( $P = .03$ ) more likely to be classified as overcontrolled than ideally or poorly controlled.

Deviation in the mean for AUC differed significantly for sampling time ( $F [2, 40] = 4.1; P = .02$ ), but did not differ significantly by treatment group or treatment-by-time. Posthoc analysis indicated that deviation in the mean AUC significantly increased on days 14 ( $P = .02$ ) and 28 ( $P = .02$ ).

## 4 | DISCUSSION

Clopidogrel has become a popular thromboprophylactic agent for use in dogs with primary IMHA.<sup>5</sup> Our results suggest that clopidogrel not only counteracts glucocorticoid-induced platelet reactivity, but the combination of prednisone at the dosage used in our study and clopidogrel enhanced platelet dysfunction in healthy dogs. In people, platelet function testing is used to ensure that thromboprophylactic effects fall within the therapeutic window between inadequate inhibition (non- or partial responders) and excessive loss of platelet reactivity (overresponders).<sup>20</sup> Based on antiplatelet monitoring standards for humans using the multiple electrode impedance aggregometer, all of the dogs in the prednisone/clopidogrel group were classified as overcontrolled on both days 14 and 28, whereas only 5 dogs on day 14 and 2 dogs on day 28 in the clopidogrel group were considered overcontrolled. Overall, dogs were 11.1 times more likely to develop excessive platelet dysfunction when receiving clopidogrel with prednisone compared to receiving clopidogrel alone.

The mechanism underlying synergistic effects of prednisone and clopidogrel on platelet dysfunction is unknown. However, in humans, the *in vitro* addition of prednisolone to whole blood decreases platelet aggregation and thrombus formation by inhibition of the ADP receptors (P2Y<sub>1</sub> and P2Y<sub>12</sub>) on platelets.<sup>23</sup> Additionally, when prednisolone is incubated with known ADP receptor inhibitors, a decrease in the amount of platelet aggregation is observed compared to blood exposed only to ADP receptor inhibitors. Interestingly, other glucocorticoids, such as dexamethasone and triamcinolone, do not have the

same effect on the platelet ADP receptor.<sup>23</sup> In both our study and previously published studies,<sup>24,25</sup> the administration of prednisone as a sole treatment does not inhibit platelet function when ADP is used to stimulate platelet activation. This observation suggests that clopidogrel is the primary inhibitor of platelet function, and prednisone either contributes a small but potentially relevant amount of platelet inhibition or enhances the antiplatelet effects of clopidogrel.

The clinical importance of the synergistic effects of prednisone and clopidogrel on platelet function is unknown. Our study was performed on healthy dogs, not hypercoagulable dogs; the synergistic effect of this drug combination might be beneficial to dogs that are predisposed to thrombus formation. However, in addition to clopidogrel, many hypercoagulable dogs receive additional preventative treatments, such as unfractionated heparin, low-molecular weight heparin, or rivaroxaban. When multiple thromboprophylactic agents are used concurrently, especially with the enhanced effects of clopidogrel, patients might be at risk for excessive hemorrhage. Although no difference was identified in gastrointestinal hemorrhage and ulceration between dogs treated with prednisone alone versus in combination with clopidogrel, actual blood loss was not quantified.<sup>12</sup> Further evaluation regarding the clinical impact of the synergistic effects of prednisone and clopidogrel on platelet function in hypercoagulable dogs is warranted.

Our study also is the first to identify changing clopidogrel requirements over time in dogs, although the mechanism that causes the change in platelet dysfunction is unknown. In people, clopidogrel responsiveness changes over the first week of treatment,<sup>26</sup> after which it stabilizes. In our study, platelet sensitivity to clopidogrel monotherapy decreased over time, with overcontrol resolving by day 28 in 3 of 5 dogs that had initially excessive responses. If platelet sensitivity to clopidogrel continues to decrease after 28 days in dogs, this effect could result in decreased thromboprophylactic benefits and increased thrombotic risk. Interestingly, the antiplatelet effects of clopidogrel appear to be consistent when coadministered with prednisone, making this finding of lesser concern for dogs being treated with prednisone for IMHA. Because premature cessation of thromboprophylaxis can increase the risk thrombosis, recent treatment guidelines for dogs with IMHA recommend thromboprophylaxis be continued until resolution of the hypercoagulable state, which includes the entire duration of glucocorticoid treatment.<sup>5</sup>

Our study used 2 methods to assess drug-induced platelet dysfunction. Platelet aggregometry measures the ability of platelets to aggregate after activation with specific agonists in either platelet-rich

plasma (optical aggregometry) or whole blood (impedance aggregometry). Optical aggregometry has been considered the ideal method to assess platelet function.<sup>27</sup> However, optical aggregometry requires additional sample processing and manipulation, creation of platelet-rich plasma and removal of blood components from the sample, and is not an accurate representation of the natural environment for the platelet. Impedance aggregometry, on the other hand, requires minimal sample preparation and manipulation, and creates a more natural environment in which to assess platelet function. Our study did not use optical aggregometry, and additional studies using this technique could provide additional information about prednisone/clopidogrel-induced platelet dysfunction. Additionally, aggregometry does not evaluate platelet function under shear forces, which is why the whole blood platelet function analyzer was included in our analysis. In humans<sup>28-30</sup> and dogs,<sup>31</sup> the INNOVANCE PFA P2Y cartridge (Siemens Healthcare, Erlangen, Germany) has been reported to provide a better assessment of clopidogrel-associated platelet dysfunction than a standard collagen/ADP cartridge. Unfortunately, this instrument and cartridge were not available in the United States at the time our study was conducted. Because some test-to-test variability occurred in closure times in dogs, 2 samples per dog per time point were analyzed and averaged.

The criteria used to classify dogs based on their clopidogrel response was extrapolated from human medicine<sup>16,20</sup> because species-specific classification criteria have not been established for dogs.<sup>31</sup> Application of canine-specific classification criteria could result in different findings. The classification criteria used for the multiple electrode impedance aggregometer was extrapolated from humans receiving antiplatelet treatment for percutaneous coronary intervention, and dogs with IMHA may not have a similar clinical response to prophylactic treatment. Categorization of response status (responder versus nonresponder) differed slightly for the 2 platelet function analyzers. All dogs in the clopidogrel and prednisone/clopidogrel groups were categorized as ideally controlled or over-controlled based on AUC results, whereas 1 of 6 dogs in each clopidogrel-receiving group was classified as a nonresponder based on closure times. Additionally, closure times of the whole-blood platelet analyzer did not indicate a decrease in platelet inhibition during sustained administration of clopidogrel monotherapy. Prior studies evaluating antiplatelet treatment in dogs have focused primarily on aspirin.<sup>31-33</sup> Additionally, these studies used multiple criteria to define response to treatment. For example, for the whole blood platelet function analyzer, previous studies have defined a response to aspirin treatment as a significant increase in closure time compared to pre-treatment values,<sup>34</sup> a closure time of >300 seconds,<sup>32,33</sup> and a closure time greater than the upper limit of the reference interval.<sup>31</sup> Because of variability among instruments that assess platelet function, and a lack of universally accepted reference ranges, it is recommended that population-based RI not be used to establish response criteria and the use of subject-based RI is more appropriate.<sup>31,35</sup> The classification criteria and benefits of routine assessment of platelet function during thromboprophylactic treatment in dogs with naturally occurring disorders have not been established.

The mechanisms of exogenous glucocorticoid-induced hypercoagulability in dogs are unknown, but they might include increased fibrinogen concentration, decreased antithrombin activity, and decreased fibrinolysis.<sup>6,7</sup> Most studies that have evaluated the coagulation status in dogs receiving exogenous glucocorticoids have focused primarily on measures of secondary hemostasis, such as prothrombin time, activated partial thromboplastin time, activated clotting time, fibrinogen concentration, antithrombin activity, thrombin-antithrombin complex, thromboelastography, and thrombin generation.<sup>6-8</sup> In contrast, fewer studies have been performed in dogs, with variable results, that have assessed the effects of exogenous glucocorticoids on platelet reactivity. One study did not detect a significant difference in platelet function after administration of prednisone (2 mg/kg PO q24h) to healthy dogs.<sup>24</sup> In contrast, another study identified increased platelet aggregation in dogs treated with prednisone at a dosage of 2 mg/kg PO q12h, but not at a prednisone dosage of 1 mg/kg PO q12h.<sup>25</sup> Our study focused on primary hemostasis, and did not assess glucocorticoid-induced hypercoagulability that involved secondary hemostasis. Although clopidogrel and the combination of clopidogrel and prednisone consistently inhibited platelet function, other mechanisms that induce a hypercoagulable state still may be present. Additional studies are required to better understand how glucocorticoids affect platelet function and contribute to hypercoagulability, especially when administered with antiplatelet treatment.

Our study had several limitations. First, we used healthy dogs with no evidence of disease or hypercoagulability. Dogs that are hypercoagulable or have hyperactive platelets as a result of naturally occurring disorders might respond differently to clopidogrel, prednisone, or both. Second, the sample size calculation indicated 6 dogs per group should be adequate to detect significant differences during drug administration. Although our study detected significant differences among some treatment groups, enrollment of a larger sample size could have yielded different results, particularly between the prednisone/clopidogrel and clopidogrel groups. Third, our study only evaluated 2 time points during drug administration. Although additional samples would have provided additional assessment of drug-induced platelet dysfunction, the changes in platelet function during antiplatelet treatment are gradual. In previous studies that used antiplatelet dosages of aspirin, inhibition of platelet function changed gradually over multiple days or weeks.<sup>32,33</sup> Given significant differences in AUC results for days 28 versus 14, a potentially more important limitation is the duration of the study. Further evaluation will be necessary to determine whether platelet sensitivity to clopidogrel stabilizes after 28 days or continues to decrease.

An additional limitation of our study is that recurrent anesthesia, endoscopy performed to evaluate gastrointestinal bleeding for the related study<sup>12</sup> or both could have altered platelet function and confounded the results of the present study. However, blood was collected for analysis of platelet function before administration of any anesthetic medication and performance of endoscopy, and there was at least a 14-day recovery period before collection of the next blood sample. Additionally, the anesthetic protocol used in the related study

(acepromazine, butorphanol, and isoflurane) has been shown to have no significant sustained effect on platelet function.<sup>36-39</sup> However, the instruments used to assess platelet function in these studies were different than the instruments used in our study.<sup>36-39</sup> Finally, our study only used 1 thromboprophylactic agent. According to the American College of Veterinary Internal Medicine consensus statement on the treatment of IMHA in dogs, anticoagulants, such as heparin or rivaroxaban, are considered the preferred anticoagulant treatment in dogs with IMHA.<sup>5</sup> Unfortunately, these medications require multiple injections, can be cost-prohibitive or both, given current recommendations to continue thromboprophylaxis 6 weeks beyond discontinuation of glucocorticoid treatment in dogs with IMHA.<sup>5</sup> Thus, PO clopidogrel remains an affordable, long-term thromboprophylactic treatment.

Dogs with IMHA are predisposed to developing thromboembolism, and administration of glucocorticoids might contribute to a hypercoagulable state. Our study indicates that sustained administration of clopidogrel not only counteracts potential glucocorticoid-induced platelet reactivity, but the combination of prednisone and clopidogrel enhances platelet dysfunction in healthy dogs. Additional studies using a wider array of thromboprophylactic treatments in hypercoagulable dogs need to be performed.

#### CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

#### OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

#### INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

The study protocol was approved by the IACUC of the University of Tennessee, Knoxville (protocol number 2335) and performed in compliance with "The Guide for the Care and Use of Laboratory Animals" in laboratory animal facilities that are AAALAC certified.

#### HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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#### REFERENCES

- Weinkle TK, Center SA, Randolph JF, Warner KL, Barr SC, Erb HN. Evaluation of prognostic factors, survival rates, and treatment protocols for immune-mediated hemolytic anemia in dogs: 151 cases (1993-2002). *J Am Vet Med Assoc.* 2005;226:1869-1880.
- Reimer ME, Troy GC, Warnick LD. Immune-mediated hemolytic anemia: 70 cases (1988-1996). *J Am Anim Hosp Assoc.* 1999;35:384-391.
- Carr AP, Panciera DL, Kidd L. Prognostic factors for mortality and thromboembolism in canine immune-mediated hemolytic anemia: a retrospective study of 72 dogs. *J Vet Intern Med.* 2002;16:504-509.
- Garden OA, Kidd L, Mexas A, et al. ACVIM consensus statement on the diagnosis of immune-mediated hemolytic anemia in dogs and cats. *J Vet Intern Med.* 2019;33:313-334.
- Swann JW, Garden OA, Fellman CL, et al. ACVIM consensus statement on the treatment of immune-mediated hemolytic anemia in dogs. *J Vet Intern Med.* 2019;33:1141-1172.
- O'Kell AL, Grant DC, Panciera DL, et al. Effects of oral prednisone administration with or without ultralow-dose acetylsalicylic acid on coagulation parameters in healthy dogs. *Am J Vet Res.* 2012;73:1569-1576.
- Flint SK, Abrams-Ogg ACG, Kruth SA, Bersenas AM, Wood RD. Independent and combined effects of prednisone and acetylsalicylic acid on thromboelastography variables in healthy dogs. *Am J Vet Res.* 2011;72:1325-1332.
- Rose LJ, Dunn ME, Allegret V, Bédard C. Effect of prednisone administration on coagulation variables in healthy Beagle dogs. *Vet Clin Pathol.* 2011;40:426-434.
- Johnson LR, Lappin MR, Baker DC. Pulmonary thromboembolism. *J Vet Intern Med.* 1999;13:338-345.
- McManus PM, Craig LE. Correlation between leukocytosis and necropsy findings in dogs with immune-mediated hemolytic anemia: 34 cases (1994-1999). *J Am Vet Med Assoc.* 2001;218:1308-1313.
- Mellet AM, Nakamura RK, Bianco D. A prospective study of clopidogrel therapy in dogs with primary immune-mediated hemolytic anemia. *J Vet Intern Med.* 2011;25:71-75.
- Whittemore JC, Mooney AP, Price JM, Thomason J. Clinical, clinicopathologic, and gastrointestinal changes from administration of clopidogrel, prednisone, or combination in healthy dogs: a double-blind randomized trial. *J Vet Intern Med.* 2019;33:2618-2627.
- Thames BE, Lovvorn J, Papich MG, et al. The effects of clopidogrel and omeprazole on platelet function in normal dogs. *J Vet Pharmacol Therap.* 2016;40:130-139.
- Callan MB, Giger U. Assessment of a point-of-care instrument for identification of primary hemostatic disorders in dogs. *Am J Vet Res.* 2001;62:652-658.
- Mischke R, Keidel A. Influence of platelet count, acetylsalicylic acid, vonWillebrand's disease, coagulopathies, and haematocrit on results obtained using a platelet function analyser in dogs. *Vet J.* 2003;165:43-52.
- Linnemann B, Schwonberg J, Rechner AR, Mani H, Lindhoff-Last E. Assessment of clopidogrel non-response by the PFA-100<sup>®</sup> system using the new test cartridge INNOVANCE<sup>®</sup> PFA P2Y. *Ann Hematol.* 2010;89:597-605.
- Defontis M, Cote S, Stirn M, et al. Optimization of Multiplate<sup>®</sup> whole blood platelet aggregometry in the Beagle dog and Wistar rat for ex vivo drug toxicity testing. *Exp Toxicol Pathol.* 2013;65:637-644.
- Marschner CB, Kristensen AT, Spodsborg EH, Wiinberg B. Evaluation of platelet aggregometry in dogs using the multiplate platelet analyzer: impact of anticoagulant choice and assay duration. *J Vet Emerg Crit Care.* 2012;22:107-115.
- Kalbantner K, Baumgarten A, Mischke R. Measurement of platelet function in dogs using a novel impedance aggregometer. *Vet J.* 2010;185:144-151.
- Gross L, Aradi D, Sibbing D. Platelet function testing in patients on antiplatelet medications. *Semin Thromb Hemost.* 2016;42:306-320.
- Littell RC, Milliken GA, Stroup WW, et al. *SAS for Mixed Models.* 2nd ed. Cary, NC: SAS Institute Inc.; 2006.
- Stokes ME, Davis CS, Koch GG. *Categorical Data Analysis Using SAS.* 3rd ed. Cary, NC: SAS Institute Inc.; 2012.
- Liverani E, Banerjee S, Robers W, et al. Prednisolone exerts exquisite inhibitory properties on platelet functions. *Biochem Pharmacol.* 2012;83:1364-1374.
- Thomason J, Archer T, Wills R, et al. Effects of immunosuppressive agents on the hemostatic system in normal dogs. *J Vet Intern Med.* 2018;32:1325-1333.

25. Romao FG, Campos EF, Mattoso CRS, et al. Hemostatic profile and thromboembolic risk in healthy dogs treated with prednisone: a randomized controlled trial. *BMC Vet Res.* 2013;9:268.
26. Khanna V, Mikael R, Thayalasamy K, et al. Does the response to aspirin and clopidogrel vary over 6 months in patients with ischemic heart disease? *J Thromb Haemost.* 2015;13:920-930.
27. Lordkipanidze M, Pharand C, Schampaert E, Turgeon J, Palisaitis DA, Diodati JG. A comparison of six major platelet function tests to determine the prevalence of aspirin resistance in patients with stable coronary artery disease. *Eur Heart J.* 2007;28:1702-1708.
28. Edwards A, Jakubowski J, Rechner A, et al. Evaluation of the INNOVANCE PFA P2Y test cartridge: sensitivity to P2Y12 blockade and influence of anticoagulant. *Platelets.* 2012;23:106-115.
29. Tsantes A, Ikonomidis I, Papadakis I, et al. Evaluation of the role of the new INNOVANCE PFA P2Y test cartridge in detection of clopidogrel resistance. *Platelets.* 2012;23:481-489.
30. Lim ST, Coughlan CA, Murphy S, et al. Platelet function testing in transient ischaemic attack and ischaemic stroke: a comprehensive systematic review of the literature. *Platelets.* 2015;26:402-412.
31. Saati S, Abrams-Ogg ACG, Blois SL, Wood RD. Comparison of Multiplate, Platelet Function Analyzer-200, and Plateletworks in healthy dogs treated with aspirin and clopidogrel. *J Vet Intern Med.* 2018;32:111-118.
32. Dudley A, Thomason J, Fritz S, et al. Cyclooxygenase expression and platelet function in healthy dogs receiving low-dose aspirin. *J Vet Intern Med.* 2013;27:141-149.
33. Haines JM, Thomason JM, Seage EC, et al. In vitro and in vivo assessment of platelet function in healthy dogs during administration of a low-dose aspirin regimen. *Am J Vet Res.* 2016;77:174-185.
34. Nielsen LA, Zois NE, Pedersen HD, et al. Platelet function in dogs: breed differences and effect of acetylsalicylic acid administration. *Vet Clin Pathol.* 2007;36:267-273.
35. Blois SL, Lang ST, Wood RD, Monteith G. Biologic variability and correlation of platelet function testing in healthy dogs. *Vet Clin Pathol.* 2015;44:503-510.
36. Elrashidy AA, Abdelrahman RS, Ghali AM, et al. Effects of sevoflurane and isoflurane on coagulation system: a comparative study. *Tanta Med Sci J.* 2007;2:11-22.
37. Connor BJ, Hanel RM, Hansen B, et al. Effects of acepromazine maleate on platelet function assessed by use of adenosine diphosphate activated- and arachidonic acid- active modified thromboelastography in healthy dogs. *Am J Vet Res.* 2012;73:595-601.
38. Lemke KA, Runyon CL, Horney BS. Effects of preoperative administration of ketoprofen on whole blood platelet aggregation, buccal mucosal bleeding time, and hematologic indices in dogs undergoing elective ovariohysterectomy. *J Am Vet Med Assoc.* 2002;220:1818-1822.
39. Bozdogan N, Madenoglu H, Dogru K, et al. Effects of isoflurane, sevoflurane, and desflurane on platelet function: a prospective, randomized, single-blind, in vivo study. *Curr Ther Res Clin Exp.* 2005;66:375-384.

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