

# Evaluation of platelet count and its association with plateletcrit, mean platelet volume, and platelet size distribution width in a canine model of endotoxemia

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## Key Words

Dog, endotoxin, MPV, PCT, PDW, platelet, sepsis

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**Background:** Platelets are of great importance in the pathogenesis of endotoxemia. Although thrombocytopenia is used as a diagnostic sign of endotoxemia, changes in values for platelet indices (plateletcrit [PCT], mean platelet volume [MPV], and platelet size distribution width [PDW]) in response to endotoxin are still unknown.

**Objective:** The aim of this study was to evaluate platelet count and its relations with platelet indices in a canine model of endotoxemia.

**Methods:** Twenty dogs were divided into 2 groups of 10 each, and treated intravenously with *Escherichia coli* endotoxin (1 mg/kg) or vehicle. Venous blood samples were collected before treatment (0 hour) and 0.5, 1, 2, 4, 6, 8, 12, and 24 hours after treatment. Platelet counts and indices were determined on a CELL-DYN hematology analyzer.

**Results:** The platelet count and PCT decreased by a mean of 73% and 93%, respectively ( $P < .001$ ), at 0.5 hour, and remained 70% and 85% lower than baseline values ( $P < .001$ ) for 24 hours after endotoxin injection. MPV and PDW increased by a mean of 28% and 45%, respectively ( $P < .01$ ), at 0.5 hour, and remained increased by 7% and 16% over baseline values for 24 hours ( $P < .01$ – $.001$ ). Platelet count correlated positively with PCT ( $P < .001$ ), but correlated negatively with MPV ( $P < .001$ ) and PDW ( $P < .01$ ).

**Conclusions:** Changes in platelet count and its association with platelet indices may reflect changes in platelet production and reactivity. Platelet indices have potential value in the diagnosis and monitoring of dogs and humans with endotoxemia.

## Introduction

Platelets are involved in nonspecific inflammatory defense, and activation of the platelets is of great importance in the pathogenesis of endotoxemia. Endotoxin in the outer membranes of gram-negative bacteria is responsible for many of the pathophysiologic events during sepsis and stimulates the release of a broad range of endogenous toxic mediators from macrophages, leukocytes, and platelets, giving rise to systemic inflammatory response syndrome, sepsis, multi-organ failure, and disseminated intravascular coagulation (DIC).<sup>1,2</sup> Thrombocytopenia is one of the markers of endotoxin-induced DIC in humans<sup>3,4</sup> and dogs.<sup>5,6</sup>

Recent advances in automated blood cell analyzers have made it possible to obtain new information

about platelets through the measurement of platelet indices, including mean platelet volume (MPV), platelet size distribution width (PDW), and plateletcrit (PCT).<sup>7,8</sup> It is well known that endotoxin causes marked thrombocytopenia in dogs<sup>3,5,9</sup>; however, changes in platelet indices in response to endotoxin are still unknown. Platelet indices may provide clinical information about the underlying causes of thrombocytopenia.<sup>7,10–13</sup> In particular, MPV has been reported to be an indirect sign of disturbances in platelet production and activity and of bone marrow response in human with sepsis<sup>11–13</sup> and dogs with inflammation.<sup>7</sup> Although MPV in healthy dogs and humans has an inverse correlation with platelet count,<sup>7,14</sup> its clinical meaning as well as inter-relationship with changes in platelet count in endotoxemia is unclear.

Thus, this study was designed to (1) determine changes in platelet count and (2) assess the potential relationship with changes in platelet indices, in response to endotoxin.

## Materials and Methods

### Experimental animals and study design

Twenty adult mongrel dogs (10 males and 10 females), housed in the Animal Husbandry and Diseases Research and Application Centre of Uludag University, were used in the study. The dogs weighed 13–24 kg (mean  $\pm$  SD,  $18.5 \pm 4.8$  kg) and ranged from 2 to 4 years ( $3.2 \pm 0.9$  years) of age. The dogs were clinically healthy based on normal clinical examinations (body temperature, mucous membranes, skin turgor, heart and respiratory rates, capillary refill time, external lymph node palpation, and peripheral pulse quality) and the results of a CBC being within reference limits. Water was provided ad libitum, and the dogs were fed a standardized, pelleted diet twice daily before the experiment. The experimental protocol was approved by the Animal Care and Use Committee of the University of Uludag.

The dogs were divided equally into 2 groups. Dogs in the treatment group received endotoxin (lipopolysaccharide, *Escherichia coli* serotype 055:B5, purity > 97%; Sigma, St. Louis, MO, USA) dissolved in sterile saline (0.9% NaCl solution; Baxter, Istanbul, Turkey) and administered intravenously at a dosage of 1 mg/kg, as described previously.<sup>9</sup> Dogs in the control group received 0.2 mL/kg sterile saline solution intravenously. Dogs were provided water 3 times a day, and food (the pelleted diet) twice a day during the experiments. Dogs were monitored clinically and hematologically at 0.5- to 4-hour intervals for 24 hours.

### Sample collection and measurements

Venous blood samples were collected before treatment (0 hour) and 0.5, 1, 2, 4, 6, 8, 12, and 24 hours after treatment from the brachiocephalic vein into Vacutainer tubes containing K<sub>2</sub>EDTA (BD Vacutainer System, BD Diagnostics, Franklin Lakes, NJ, USA) for a CBC, which included measurement of total WBC count, RBC count, HCT, hemoglobin concentration, MCV, MCH, MCHC, red cell distribution width, platelet count, MPV, PCT, and PDW. The anticoagulant tubes were inverted several times immediately postcollection to allow for adequate mixing. A CBC was performed just after the blood collection using an automatic analyzer with optical scatter and impedance methods (CELL-DYN 3500; Abbott, Wiesbaden, Germany). Peripheral blood smears were not examined.

### Statistical analysis

Results were expressed as mean  $\pm$  SD. Data were analyzed by Kruskal–Wallis 1-way ANOVA on ranks for within-group changes. Percentage change from baseline was analyzed for each parameter in the 2 groups. Mann–Whitney rank sum test was used to determine if differences between groups were significant. Pearson product moment correlation coefficients were determined to evaluate the possible relationships among parameters (SPSS 10.0 Statistical Program, SPSS Inc, Chicago, IL, USA). A *P*-value < .05 was considered significant.

### Results

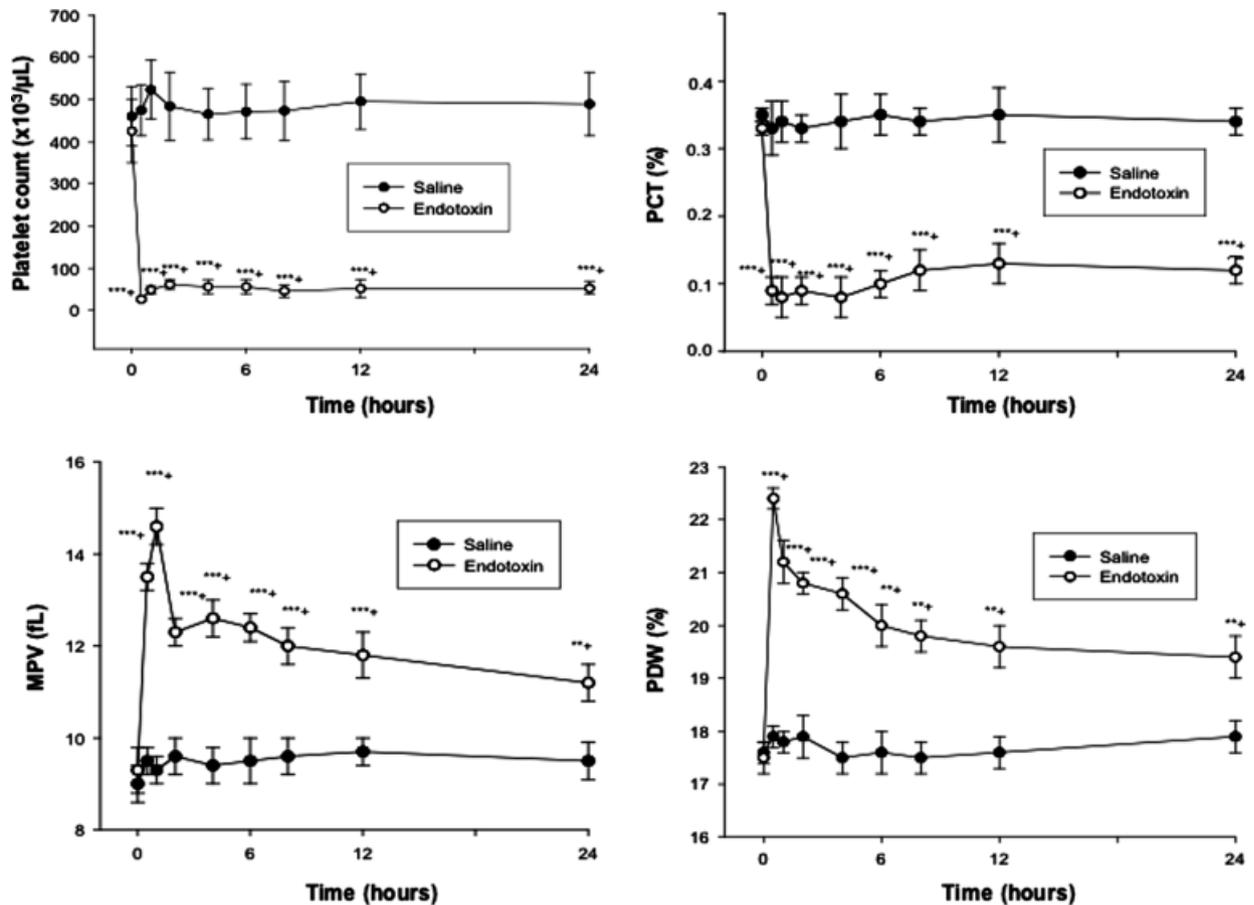
Endotoxin caused fever, tachycardia, tachypnea, prolonged capillary refill time, hypotension, depression, anorexia, vomiting, diarrhea, hemoconcentration (increased RBC count, hemoglobin concentration, and HCT), leukopenia, and neutropenia within 0.5 hour after injection (data not shown). All dogs survived to the end of the study.

Platelet counts and PCT in dogs receiving endotoxin were significantly lower (*P* < .001) than the values observed in dogs receiving saline injection at all time points except 0 hour (Figure 1). Platelet count decreased significantly from a mean of  $425 \pm 75 \times 10^3$  cells/ $\mu$ L at 0 hour to  $27 \pm 5 \times 10^3$  cells/ $\mu$ L at 0.5 hour (by 73%); PCT also decreased significantly, from  $0.33\% \pm 0.01\%$  to  $0.09\% \pm 0.02\%$  (by 93%) (Figure 1). Platelet count and PCT remained significantly lower (by 70% and 85%, respectively) than baseline values for 24 hours. MPV increased from  $9.3 \pm 0.5$  fL at 0 hour to  $13.5 \pm 0.3$  fL at 0.5 hour (by 45%), and remained significantly increased (by 56%) for 24 hours after endotoxin injection. PDW increased from  $17.5\% \pm 0.3\%$  at 0 hour to  $22.5\% \pm 0.5\%$  at 0.5 hour (by 28%). PDW remained significantly increased for 24 hours (by 8%–21%) compared with the baseline value. Platelet count was correlated positively with PCT ( $r = 0.853$ , *P* < .001), but was correlated negatively with MPV ( $r = -0.991$ , *P* < .0014) and PDW ( $r = -0.754$ , *P* < .01).

Mean MCV ranged from 67.0 to 69.8 and was not significantly different between groups or at different time points (data not shown).

### Discussion

In this study, we showed that platelet count and PCT decreased, but MPV and PDW increased, in dogs in response to endotoxin. In addition, the thrombocytopenia was significantly associated with changes in PCT, MPV, and PDW. These findings may reflect changes in



**Figure 1.** Changes in platelet count, plateletcrit (PCT), mean platelet volume (MPV), and platelet size distribution width (PDW) in response to endotoxin in dogs. Each point is the mean  $\pm$  SEM of 10 dogs. \*\* $P < .01$  and \*\*\* $P < .001$ , compared with baseline values. + $P < .001$ , compared with the saline control group.

platelet production and reactivity, and platelet indices may have potential value in the diagnosis and monitoring of dogs and humans with endotoxemia.

Thrombocytopenia is a common acquired disorder in dogs, which can occur as a result of sequestration, utilization (consumption), destruction, and decreased or ineffective production of platelets.<sup>5</sup> Endotoxin can cause thrombocytopenia through 1 or more of these mechanisms.<sup>1</sup> In this study, consistent with our previous studies reporting circulating platelet changes in response to endotoxin, platelet count decreased dramatically in parallel with PCT within 0.5 hour following the endotoxin injection, and both remained low for 24 hours.<sup>6,9</sup> One possible reason for marked thrombocytopenia and decreased PCT in response to endotoxin may be that endotoxin induces platelet adhesion and aggregation by increasing the release of various platelet agonists and by stimulating the expression of adhesion molecules on endothelial cells.<sup>1,15,16</sup> Increased rolling and adherence of platelets within liver sinusoids during end-

otoxemia has been demonstrated,<sup>15</sup> and we previously showed that platelet closure times were shortened in dogs 1 hour after intravenous endotoxin injection.<sup>6,9</sup> Moreover, decreased survival of circulating platelets and increased removal of platelets from circulation due to phagocytosis by the liver macrophages, as described in a rat model, might enhance the severity of thrombocytopenia in dogs with endotoxemia.<sup>17</sup>

While platelet count and PCT decreased, MPV and PDW increased for at least 24 hours after endotoxin injection, suggesting that platelet counts are correlated positively with PCT, but correlate negatively with MPV and PDW during early endotoxemia in dogs. This result conflicts with the conclusions of a prospective clinical study on the relation between platelet count and MPV in adult humans, in which a parallel trend in MPV and platelet count was observed during the course of sepsis.<sup>18</sup> This contradiction may be explained in part by the limited size of the study population (only 6 patients were evaluated), and also by differences in

the patients' clinical conditions. Our observation on the inverse relationship between MPV and platelet count in dogs with endotoxemia agrees with other data from humans with sepsis,<sup>11,14,19</sup> and from dogs with inflammation.<sup>7,8</sup> In a retrospective study, increased MPV was reported in human patients with septic shock.<sup>20</sup> In another study involving humans, a higher MPV was observed in septic patients than in patients with a localized infection.<sup>21</sup> In this study, the magnitude of decrease in platelet count (by 93%) was more severe than that of the increase in MPV (by 45%), suggesting that thrombocytopenia may be a more consistent laboratory finding than increased MPV in dogs with endotoxemia.

Recently published studies suggest that platelet indices, particularly MPV and PDW, provide clinical information about the underlying mechanism of thrombocytopenia.<sup>7,10-13</sup> PDW, but not MPV, was reported to be a reliable sign for distinguishing hyperdestructive thrombocytopenia from hypoproduative thrombocytopenia in humans.<sup>10</sup> Inflammatory mediators released during endotoxemia could contribute to increased thrombopoiesis and therefore to increased MPV.<sup>22</sup> Thus, the increase in MPV and PDW in dogs in this study may be related to the bone marrow response to endotoxin within 24 hours.<sup>13</sup> This observation is consistent with the findings in a study of dogs with acute inflammatory disease, in which increases in MPV and PDW were interpreted as the release of large platelets from bone marrow in response to greater demand for platelets.<sup>7</sup> Increased MPV also has been reported in dogs with babesiosis, indicating the stimulation of megakaryopoiesis.<sup>12,23</sup> Studies of humans<sup>11,24</sup> and rats<sup>25</sup> with sepsis found that platelets with a greater volume are functionally more active and hypersensitive, with a lower threshold for aggregation and release activity, likely because large platelets contain more dense granules and produce more thromboxane. Early circulatory responses to a bolus injection of endotoxin have been correlated with the release of thromboxane, which incites platelet aggregation.<sup>1,2,5,26</sup> Both platelet hyperaggregability and peripheral damage/consumption could eventually over 24 hours lead to positive feedback to the bone marrow, resulting in production of larger and more active platelets.<sup>11</sup> Our observations on the behavior of platelets and platelet indices in endotoxic dogs could be indirect evidence of disturbances in both platelet production and activity, in agreement with other studies.<sup>7,10,11</sup> We did not observe clinical signs of bleeding (petechiae, ecchymoses, etc) in the dogs in this study, possibly because increased MPV may enhance the hemostatic potential of platelets in thrombocytopenic states.<sup>27</sup>

In this study, platelet aggregates or clumps could not be excluded as a contributing factor to the thrombocytopenia, as peripheral blood smears were not examined. Mild to moderate platelet clumping was reported to contribute to a decrease in platelet concentration when measured by the impedance method.<sup>28</sup> However, in a recent study, platelets in canine blood collected in EDTA were minimally clumped in most blood smears and the interpretation of platelet count and platelet indices was not significantly affected, as compared with blood collected in citrate.<sup>29</sup> In addition, the changes in platelet count in this study were similar to those observed in previous studies using this model.<sup>6,9</sup> The presence of microcytic RBCs may result in erroneously high estimations of platelet count when an impedance counter is used, with small RBCs counted as platelets<sup>29,30</sup>; however, this phenomenon did not likely occur in the present study, as MCV did not change significantly after endotoxin treatment.

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