The Veterinary Journal 201 (2014) 406–411

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Platelet parameters from an automated hematology analyzer in dogs with inflammatory clinical diseases

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ARTICLE INFO

Article history:
Accepted 12 July 2014

Keywords:
Dog
Inflammation
Mean platelet component
Mean platelet volume
Platelet parameters

ABSTRACT

The mean platelet component (MPC) is a proprietary algorithm of an automated laser-based hematology analyzer system which measures the refractive index of platelets. The MPC is related linearly to platelet density and is an indirect index of platelet activation status. Previous investigations of canine inflammatory conditions and models of endotoxemia demonstrated a significant decrease in the MPC, consistent with platelet activation. The purpose of this study was to evaluate the MPC and other platelet parameters in dogs with different diseases to determine if they could show differential platelet activation with different pathologies. The hypothesis was that the MPC would decrease in clinical conditions associated with systemic inflammation or platelet activation. Complete blood counts run on the analyzer from dogs with different inflammatory conditions (primary immune-mediated hemolytic anemia (IMHA) or thrombocytopenia (ITP), pituitary-dependent hyperadrenocorticism, intra-abdominal sepsis, pancreatitis, intravascular thrombus or thromboembolus and hemangiosarcoma) were reviewed retrospectively and compared with those of control dogs presenting for orthopedic evaluation.

Dogs with ITP had a decreased plateletcrit and MPC, with an increased platelet volume and number of large platelets ($P < 0.001$). Dogs with IMHA had an increased plateletcrit and mass, and more numerous large platelets ($P < 0.001$).With the exception of the ITP group, there was no difference in MPC in the diseased groups when compared with the controls. The results of this study suggest the MPC does not change in certain canine diseases associated with systemic inflammation.

Introduction

Platelet activation during systemic inflammatory conditions may contribute to the development of macro- or micro-thrombi, which can result in significant morbidity and mortality in hospitalized patients (Christopherson et al., 2012; Wiinberg et al., 2012; Kidd and Mackman, 2013). There is therefore interest in monitoring the platelet activation status in hospitalized patients. Early detection of patients at risk of thromboembolism might allow initiation of thromboprophylaxis and monitoring of the response of platelets to these therapies. Flow cytometric detection of activation markers and platelet microparticles has been described in dogs, but has not been extensively studied in a clinical setting (Moritz et al., 2003).

The automated ADVIA 120 Hematology System (Bayer Corporation) uses two-dimensional (2D) laser technology to identify and characterize the size and complexity of cells in EDTA-anticoagulated whole blood. The two light scatter signals generated by each platelet are converted into multiple parameters that represent the number, size, and complexity of platelets in humans (Table 1) (Macey et al., 1999; Giacomini et al., 2001). Similarly, the laser technology also allows canine platelets to be distinguished from erythrocytes and generates an accurate platelet count (Welles et al., 2009). The mean platelet volume (MPV) is a measure of the mean platelet size, while the platelet distribution width (PDW) indicates the variation in platelet size and the plateletcrit is the product of the platelet count and MPV, representing the percent of a deciliter of blood occupied by platelets (similar to hematocrit for erythrocytes). The mean platelet component (MPC) is a measure of the refractive index of platelets and is related linearly to platelet density (Barer et al., 1953), generating an indirect index of the platelet activation status (Thompson et al., 1982; van Oost et al., 1983; Corash, 1990; Zelmanovic and Hetherington, 1998). Once activated, platelets release procoagulant substances from alpha and dense granules, reducing platelet density, and therefore decreasing the MPC (Chapman et al., 2003).

Reference values for canine platelet parameters using the ADVIA 120 analyzer have been established by Moritz et al. (2004). Since then, studies have evaluated the effect of storage (Furlanello et al.,

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http://dx.doi.org/10.1016/j.tvjl.2014.07.009
1090-0233/

Table 1
Platelet parameters determined by the ADVIA 120 Haematology System.

Parameter	Abbreviation	Units	Description
Platelet count	PLT	μL	Identified by cell volume (0–60 fL) and refractive index
Mean platelet volume	MPV	fL	Calculated from the platelet histogram
Large platelets	L-PLT	μL	Two dimensional analysis allows platelets <60 fL to be distinguished from erythrocytes
Plateletcrit	PCT	%	Percent of blood volume occupied by platelets (PLT × MPV)
Mean platelet mass	MPM	pg	Calculated from the platelet dry mass histogram
Platelet distribution width	PDW	%	Distribution width of platelet volume histogram
Mean platelet component	MPC	g/L	Calculated from the platelet component histogram; indicates platelet density (=100 × platelet mass/platelet volume)

2006; Prins et al., 2009), anticoagulants (Stokol and Erb, 2007), and hemolysis (Bauer et al., 2010) on ADVIA platelet parameters. Some studies have also evaluated canine platelet parameters in response to experimentally-induced and spontaneous inflammatory diseases (Moritz et al., 2005; Rafaj et al., 2005; Yilmaz et al., 2008; Flatland et al., 2011).

The purpose of this study was to evaluate platelet parameters in dogs with a large number of disease states that are generally associated with systemic inflammation, and to compare them with a control series of dogs presenting for elective orthopedic surgery. Our hypothesis was that the MPC would be lower in clinical conditions thought to cause platelet activation.

Materials and methods

Study design

The medical records of dogs that presented to the University of Georgia Veterinary Teaching Hospital (UGA-VTH) between 1 January 2007 and 31 August 2009 were reviewed by two clinicians (JRS, KS). Canine coded diagnoses selected were primary immune-mediated hemolytic anemia (IMHA), primary immune-mediated thrombocytopenia (ITP), pituitary-dependent hyperadrenocorticism, intra-abdominal sepsis, pancreatitis, thrombi or thromboembolism (including diagnosis or strong suspicion of pulmonary thromboembolism, PTE), and hemangiosarcoma. Vital parameters, the total white blood cell (WBC) count and percentage of band neutrophils on admission were noted for generation of a patient Systemic Inflammatory Response Syndrome (SIRS) status (Hauptman et al., 1997; Brady and Otto, 2001). The dog was deemed SIRS positive if three or more of the following criteria were satisfied: a heart rate >120/min; a respiratory rate >40/min; a rectal temperate <38 °C or >40 °C; a WBC count <5 or >18.0 × 10^9/L; or ≤10% immature neutrophils.

In addition to securing an appropriate history and noting clinical signs, specific criteria were used to verify the coded diagnoses. Primary IMHA cases had PCV <25%, spherocytosis, either a positive autoagglutination or direct Coombs' test or evidence of erythrocyte maturation arrest on bone marrow examination, and with underlying infectious or neoplastic causes eliminated. Dogs with primary ITP had a platelet count of <20,000 cells/μL, an underlying infectious or neoplastic cause eliminated, and responded to immunosuppressive therapy.

The diagnosis of pituitary-dependent hyperadrenocorticism was based on results of a low dose dexamethasone suppression test or adrenocorticotropin (ACTH) stimulation test, and evaluation of an abdominal ultrasound. Dogs with adrenal tumors were excluded. Diagnostic criteria for pancreatitis included a positive canine pancreatic lipase immunoreactivity test (>400 μg/L) and the presence of a hypoechoic pancreas with hyperechoic mesentery on abdominal ultrasound, or a histopathological diagnosis from surgical biopsy or necropsy. Diagnostic criteria for intra-abdominal sepsis included cytological diagnosis of septic peritonitis or a positive culture of an abdominal effusion.

A PTE was diagnosed by clinical history, hypoxemia, thoracic radiography ruling out another respiratory pathology, arterial blood gas and results of D-dimer and viscoelastic thromboelastography testing being consistent with the presence of a thrombus and hypercoagulable state, respectively. Thrombi causing limb ischemia were diagnosed based on a comparison of blood glucose and lactate concentrations from the contralateral limb, or thrombi were identified via ultrasound, MRI or necropsy. The diagnosis of hemangiosarcoma was confirmed by histopathological evaluation of the affected tissue, identification of a hemorrhagic pericardial effusion and right auricular mass on echocardiogram, or necropsy.

Reference intervals for platelet parameters have not yet been established at the UGA-VTH and so a control population was derived from dogs that presented for orthopedic surgery for repair of a cranial cruciate ligament rupture or for a total hip replacement. These dogs were deemed otherwise healthy on the basis of results of a physical examination and complete blood count (CBC), biochemical and urinalysis results, and only pre-surgical samples were evaluated.

Blood sample collection and analysis

Blood was collected by jugular or cephalic venepuncture using minimal stasis, except in cases of suspected ITP when the cephalic or lateral saphenous veins were used, into tubes containing potassium EDTA (Vacutainer tubes, Becton–Dickinson). Samples were stored at 4 °C and analyzed on the ADVIA 120 analyzer within 8 h of collection; a daily quality control was performed. Results of the initial CBC were retrieved. In addition to recording conventional hematological parameters, specific platelet parameters collected included absolute platelet count, MPV, absolute large platelet count, plateletcrit, PDW, mean platelet mass (MPM) and MPC.

Statistical analysis

Statistical analysis was performed using commercial software (SigmaStat, version 3.5, Systat Software). Data sets were assessed for normality using the Kolmogorov–Smirnov test. Normally distributed data are listed as means ± SD, and non-parametric data listed as median and range. Data were compared between groups using a one-way ANOVA or a Kruskal–Wallis one way ANOVA on ranks, depending on the distribution of the data. Adjustments for multiple comparisons were made using Dunn's method when significant results were obtained for initial comparisons. The comparison of MPC between dogs with SIRS and those without SIRS was performed using either a Student's *t* test (un-paired and two tailed) or a Mann–Whitney rank sum test, depending on the distribution of data. Correlation between the MPC and leukocyte counts was determined using Spearman rank order correlation. A P value < 0.05 was deemed statistically significant.

Results

A total of 228 dogs were included in the study, grouped by diagnosis into control dogs ($n = 19$), and those with hyperadrenocorticism ($n = 47$), hemangiosarcoma ($n = 27$), IMHA ($n = 37$), ITP ($n = 32$), pancreatitis ($n = 32$), sepsis ($n = 25$), and thrombotic disease ($n = 9$) (Table 2). The hemangiosarcoma animals were older than the control group ($P < 0.001$) (Table 2). Dogs with hyperadrenocorticism, IMHA and pancreatitis weighed less than the control dogs ($P < 0.001$) (Table 2). CBC analysis showed WBC counts were greater in the IMHA, ITP, pancreatitis, sepsis and thrombus groups than the control group ($P < 0.001$) (Table 2). Increased numbers of segmented neutrophils were seen in dogs with IMHA, ITP and sepsis compared with the control dogs ($P < 0.001$) and more band neutrophils were seen in dogs with IMHA and ITP than the control dogs ($P < 0.001$) (Table 2). There was no difference in the MPC between dogs characterized as having SIRS (i.e., three or greater criteria) and those without SIRS, both within individual disease groups (all $P > 0.05$), and when all dogs were evaluated together ($P = 0.107$).

Reflecting the preselection of patients with low platelet counts, these were lower in the ITP group than all other groups, except for those dogs with a thrombus ($P < 0.001$) (Table 3). Dogs with IMHA and ITP had a larger MPV than the control dogs ($P < 0.001$). Dogs with ITP had fewer large platelets ($P < 0.001$) and a smaller plateletcrit than the control dogs ($P < 0.001$), while the number of large platelets and MPM were greater in dogs with IMHA compared with the control dogs ($P < 0.001$) (Table 3). The PDW was increased in dogs with hemangiosarcoma compared with the control dogs ($P < 0.001$) (Table 3). The MPC was decreased in the ITP groups when compared with all other groups ($P < 0.001$) (Table 3). With