Evaluation of coated platelets, a subset of highly procoagulant platelets, in healthy dogs and dogs with neoplasia

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OBJECTIVE
To determine if dogs with neoplasia produce more coated platelets, a subpopulation of activated platelets generated by dual stimulation with thrombin and convulxin, a glycoprotein VI agonist, than healthy control dogs.

ANIMALS
Client-owned dogs diagnosed with lymphoma (n = 19) or solid tumors (14) and healthy control dogs (14).

PROCEDURES
Platelets were stimulated ex vivo with thrombin and convulxin. Flow cytometry was used to quantify the percentage of coated platelets based on high levels of surface fibrinogen. To compare the percentage of coated platelets between the three groups, an ANOVA was performed followed by pairwise 95% confidence intervals (CI) adjusted for multiple comparisons using Tukey’s method.

RESULTS
We observed a greater mean percentage of coated platelets in dogs with solid tumors, compared with healthy control dogs, by 10.9 percentage points (95% CI: −1.0, 22.8), and a mean percentage of coated platelets in dogs with lymphoma that was less than healthy control dogs by 0.3 percentage points (95% CI: −11.4, 10.8).

CLINICAL RELEVANCE
This study provides the first data-based evidence that dogs with solid tumors may have a greater mean coated platelet percentage when compared with healthy control dogs, although there is overlap between groups. Further studies are needed investigating coated platelets in specific subsets of neoplasia and investigating additional mechanisms of hypercoagulability in dogs with neoplasia.

Dogs and humans with neoplasia have an increased risk of venous thromboembolism (VTE). Human cancer patients have a 4- to 7-fold increased relative risk of VTE compared to healthy controls.1–6 VTE events can be asymptomatic and discovered incidentally during cancer staging,1–4,7–10 or they can be symptomatic and contribute significantly to patient morbidity and mortality. VTE has been identified as one of the leading causes of mortality in human cancer patients.11,12 Indeed, mortality in human cancer patients with a history of VTE was 22%, compared to 4.4% in those with VTE without cancer (although cancer patients in this study had a higher incidence of comorbid conditions, such as elevated creatinine).12 Another study13 of cancer patients in Denmark showed an overall risk of mortality after VTE of 13.6% at 30 days, and 48.3% at 1 year. In a prospective observational study11 in human cancer patients receiving chemotherapy, thrombosis was a common cause of mortality, second only to progression of cancer. Neoplasia is the most common underlying disease in dogs with documented thrombotic events, both venous and arterial.14–19 The exact mechanisms of the hypercoagulability associated with neoplasia are incompletely understood and are likely multifactorial. While a thorough review of hypercoagulability causes in patients with cancer is beyond the scope of this paper, proposed mechanisms include...
thrombocytosis, a hyperinflammatory state, and circulating procoagulant extracellular molecules.\textsuperscript{20-22}

Coated platelets are a subpopulation of platelets that are formed ex vivo after dual stimulation with thrombin and a glycoprotein (GP) VI agonist, such as convulxin.\textsuperscript{21,24} Their surface is coated with procoagulant glycoproteins and alpha granule contents, bridged by serotonin.\textsuperscript{24} In supporting the assembly of the prothrombinase and tenase complexes, coated platelets have an enhanced ability to generate thrombin, making them prothrombotic.\textsuperscript{24} In murine models, coated platelets have been shown to promote platelet-driven fibrin formation,\textsuperscript{22} and decreased coated platelet formation is associated with decreased platelet procoagulant activity in a modified prothrombinase assay.\textsuperscript{26}

The ability of coated platelets to support prothrombinase activity and thrombin generation, both central to coagulation, may enable coated platelets to participate in VTE development.\textsuperscript{27} Increased coated platelet potential, ie, the percent of platelets converted to coated platelets ex vivo after dual stimulation, is associated with an increased risk of thromboembolic disease in humans.\textsuperscript{28} Humans with ischemic stroke history produce a greater percentage of coated platelets when compared with healthy controls.\textsuperscript{29-31} Conversely, decreased coated platelet potential is associated with increased clinical bleeding in humans and in dogs.\textsuperscript{28} Humans with hemorrhagic stroke history form decreased coated platelets compared with healthy controls.\textsuperscript{32} Dogs with canine Scott syndrome have a decreased ability to form coated platelets, which may contribute to the increased clinical bleeding observed with this syndrome.\textsuperscript{23} Additionally, humans receiving serotonin-specific reuptake inhibitors have a tendency to form fewer coated platelets.\textsuperscript{33} These patients also have a mild increase in bleeding tendency\textsuperscript{34-36} and a mildly reduced risk of VTE,\textsuperscript{37} which may be partially due to the role of serotonin in the formation of coated platelets.

Human cancer patients have been shown to have increased subsets of procoagulant platelets expressing phosphatidylserine when compared with healthy controls.\textsuperscript{38,39} However, the role of coated platelets in the development of VTE in dogs with neoplasia is not known. If coated platelets are found to play a role in VTE in dogs with neoplasia, they could represent a potential therapeutic target.

The primary objective of this study was to evaluate coated platelet potential for the first time in dogs with neoplasia compared with healthy control dogs. It was hypothesized that dogs with neoplasia would produce a greater percentage of coated platelets when compared with healthy control dogs.

Materials and Methods

Dogs

Client-owned dogs presenting to a veterinary teaching hospital with a cytologic or histopathologic diagnosis of either lymphoma or solid tumors (eg, carcinoma, sarcoma, mast cell tumor, etc) were recruited for inclusion in the study. Enrollment occurred over a 3-month period from September to December 2015. Staff- and student-owned dogs that were free of disease based on history and physical examination findings, and not receiving medications other than standard preventative treatments, were recruited as healthy control dogs. Dogs were excluded from enrollment in any group if body weight was less than 5 kg or if they had received any antiplatelet medications (eg, nonsteroidal anti-inflammatory medications) within the 96 hours preceding sampling. For cancer patients, all diagnostic and therapeutic plans were at the discretion of the attending clinician. Dogs were separated into groups based on their clinical remission status for data analysis (either “remission – yes”, or “remission – no”). Dogs in the lymphoma group were classified with regard to clinical stage and substage according to the World Health Organization (WHO) clinical staging system for lymphoma in domestic animals.\textsuperscript{40} Dogs were categorized by stage and substage according to available clinical information. Stage was determined based on physical examination and diagnostic testing, performed at the discretion of the attending clinician. Substage was determined by the attending clinician. Dogs were included independent of whether they were receiving chemotherapy treatment. Informed owner consent was obtained for all dogs before enrollment in the study, and all procedures were approved by the Institutional Animal Care and Use Committees of Iowa State University (8-14-7840K) and the University of Minnesota (1312-31131A), and the Institutional Biosafety Committee of the University of Minnesota (1509-33048H).

Sample collection procedure

Venous blood (1.8 ml) was collected into a syringe containing 0.2 ml acid-citrate dextrose (Sigma-Aldrich), to a total volume of 2 ml. To generate platelet-rich plasma (PRP) for flow cytometry, the acid-citrate dextrose-anticoagulated blood was combined with an equal volume (2 ml) of buffered saline glucose citrate (129 mM NaCl, 14 mM Na\textsubscript{2}Citrate, 11 mM glucose, and 10 mM Na\textsubscript{2}HPO\textsubscript{4}, pH 7.3) and centrifuged at 170 X \textit{g} for 8 minutes at room temperature (25°C). The supernatant PRP was harvested and coated platelet assay reactions were performed within 4 hours.

Automated platelet counts were obtained on ADVIA 2120 hematology analyzers at both institutions.

Coated platelet assay

All activation experiments were performed in a 100 \mu l total assay volume containing 1 \mu l PRP and the following reagents (final concentrations): 2.5 mM Ca\textsubscript{2+}, 1.25 mM Mg\textsubscript{2+}, 1 \mu g/ml biotin-fibrinogen (Sigma-Aldrich), 2.5 \mu g/ml rattlesnake toxin (Miami Serpentarium), 0.5 U/ml bovine thrombin (Sigma-Aldrich), 0.1 mM gly-pro-arg-pro-NH\textsubscript{2}, 150 mM NaCl, and 10 mM HEPEs, pH 7.5. The reaction tubes were incubated at 37°C for 5 minutes. Following incubation, labeling to detect platelet membrane GP IIb,IIIa
(CD41/CD61; 0.8 μg fluorescein isothiocyanate-
abciximab [FITC-abx]) and biotinylated fibrinogen (0.5 μg phycoerythrin-streptavidin (PE-SA); Streptavidin, R-Phycocerythrin Conjugate; Molecular Probes) was performed to identify platelets and to label surface bound biotinylated fibrinogen characteristic of coated platelets, respectively, as previously described.\(^23,41–44\) After incubating an additional 5 minutes at 37 °C, the reaction was stopped by fixation with 200 μl of 1.5% formalin in HEPES-saline (10 mM HEPES and 150 mM NaCl, pH 7.5). Samples were incubated at room temperature (25 °C) for 15 minutes, while protected from light, then diluted with 200 μl of HEPES-saline (10 mM HEPES and 150 mM NaCl, pH 7.5).

Samples were collected with a FACSCanto (ISU; FACSCanto, BD Biosciences) or an LSR II (UMN; LSR II/Fortessa; BD Biosciences) cytometer and analyzed with FlowJo software (FlowJo, v10, Tree Star Inc). After initial optimization and compensation on each cytometer, the same settings were used throughout the study. Samples were gated to collect 10,000 platelet positive events, based on FITC-abx positivity as a function of forward scatter. Platelets were first identified by forward (FSC) and side scatter (SSC) properties, and platelet gates were fine-tuned based on FITC-abx positivity (Figure 1). Within the platelet population, coated platelets were identified and gated based on their PE-SA positivity. Results are reported as the percentage of platelets that were converted to coated platelets (PE-SA positive).

### Statistical analysis

Sample sizes were calculated before the study initiation to detect a 10% difference between cases and controls, a difference approximate to that previously detected between healthy people and those with ischemic stroke,\(^29–31\) with 80% power and an alpha of 0.05. Calculations were performed using the standard deviation of a published coated platelet assay.\(^45\) Based on these calculations, it was determined that each of the three groups (healthy control dogs, dogs with lymphoma, and dogs with solid tumors) needed to contain 13 dogs to detect a significant difference.

Three of the reaction tubes, containing identical reagents, were considered technical replicates and were averaged. Results were discarded if one of the technical replicates was considered an outlier compared to the other two (defined as a difference of > 10% coated platelets) or if assay failure occurred (defined as either a lack of stimulation above resting levels, or maximal stimulation, > 95%, above resting levels). The overall intra-assay coefficient of variation (CV) was calculated by averaging the CVs from the triplicate measurements of each sample analyzed.

Normality was confirmed using qq-plots and boxplots. To compare the percentage of coated platelets between the three groups, an ANOVA was performed followed by pairwise 95% confidence intervals adjusted for multiple comparisons using Tukey’s method.

To see how the relationship between percent coated and group depends on total platelet count, an analysis of covariance (ANCOVA) model was fit with total platelet count as covariate. This model was performed only on the dogs with cancer. No visual evidence of an interaction was seen so the interaction was not included in the model. Total platelet count was missing for one dog in the solid group, so for comparison with the ANOVA, the initial ANOVA model was refit on this subset of the data. The mean difference between groups, with a 95% confidence interval (CI) still adjusted for three comparisons, is reported for both the refit ANOVA model and the ANCOVA model. The coefficient for total platelet count, with 95% CI, is also reported from the ANCOVA model.
Results

Dogs

In total, 55 dogs were screened for enrollment in the study and 47 were enrolled. Clinical characteristics of enrolled dogs are summarized (Supplementary Tables S1–S3). Dogs were excluded due to assay failure early in the course of the study (n = 4, 2 from the healthy control group and 2 from the lymphoma group), emergence of significant underlying disease within 2 months of sampling, making them ineligible to be included as healthy controls (2; 1 case was diagnosed with prostatic adenocarcinoma and 1 case was diagnosed with a severe submandibular abscess), and lack of a definitive diagnosis (n = 2). Healthy dogs were followed for a period of 6 months to ensure they remained clinically healthy. The 47 dogs included in the study consisted of healthy controls (n = 14), dogs with a diagnosis of lymphoma (19), and dogs with solid tumors (14).

Clinical characteristics of dogs in the healthy control group

The healthy control dogs had a mean age of 5.0 years (range, 1 to 9 years) and consisted of spayed females (7/14; 50%) and castrated males (7/14; 50%). Breeds represented in the healthy control group were mixed breed (3/14; 21.4%), Australian Shepherd (2/14; 14.3%), American Staffordshire (Pit Bull) Terrier (2/14; 14.3%), Labrador Retriever (2/14; 14.3%), and 1 each (1/14; 7.1%) of German Wirehaired Pointer, Rough-Coated Collie, Golden Retriever, Greyhound, and Border Collie.

Clinical characteristics of dogs in the lymphoma group

Of the dogs with a diagnosis of lymphoma, 12/19 (63.2%) were considered to have clinical disease at the time of enrollment and 7/19 (36.8%) were considered to be in clinical remission. Dogs in the lymphoma group had a mean age of 9.2 years (range, 3 to 15 years) and consisted of spayed females (8/19; 42.1%), castrated males (9/19; 47.4%), 1 intact female (5.3%), and 1 intact male (5.3%). Breeds represented in the lymphoma group included Beagle (2/19; 10.5%), Golden Retriever (2/19; 10.5%), and 1 each (1/19; 5.3%) of Vizsla, Bernese Mountain Dog, mixed breed, Doberman, Jack Russell Terrier, Border Collie, Boxer, Newfoundland, Standard Poodle, Cairn Terrier, Chow Chow, Cocker Spaniel, German Shepherd Dog, Basset Hound, and Irish Setter. Diagnosis of lymphoma was based on a combination of cytology and clonality testing (12/19; 63.2%), histopathology (5/19; 26.3%), or cytology alone (2/19; 10.5%). The most common WHO Stage at the time of diagnosis of lymphoma was Stage III (9/19; 47.4%), followed by Stage V (5/19; 26.3%), Stage II (3/19; 15.8%), and Stage IV (2/19; 10.5%). At the time of diagnosis, 10 dogs were considered to be stage b (10/19; 52.6%), and 9 dogs were stage a (9/19; 47.4%). Immunophenotyping was consistent with B-cell neoplasia in 10 cases and T-cell neoplasia in 5 cases. Four dogs with lymphoma did not have immunophenotyping performed. Twelve of the dogs with lymphoma were receiving chemotherapy at the time of enrollment, including 5 of the 12 dogs that were not in clinical remission and all 7 of the dogs in clinical remission. The most common chemotherapy protocol was the CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) protocol (7/12; 58.3%). The following chemotherapy protocols were used in one dog each: modified MOPP (mechloretamine, vincristine, prednisone, procarbazine); chlorambucil, vincristine, and cyclophosphamide; doxorubicin and lomustine; and doxorubicin alone. Ten of the dogs were treated with systemic glucocorticoids (prednisone [9/10; 90%] and prednisolone [1/10; 10%]) as part of the CHOP protocol (7/10; 70%) and in 1 dog each (1/10; 10%) as part of the MOPP protocol, concurrently with doxorubicin, and as the only treatment, at the time of enrollment. Comorbidities are summarized (Supplementary Table S2). None of the comorbid conditions present are known to be associated with coated platelet formation based on extrapolation from the human literature, and none are known to be associated with hypercoagulability in dogs.

Clinical characteristics of dogs in the solid tumor group

Of the dogs with solid tumors, 12/14 (85.7%) were considered to have residual disease at the time of enrollment and 2/14 (14.3%) were considered to be in clinical remission. Dogs in the solid tumor group had a mean age of 9.4 years (range, 5 to 13 years) and consisted of castrated males (9/14; 64.3%), spayed females (4/14; 28.6%), and 1 intact male (7.1%). Breeds represented in the solid tumor group included mixed breed (4/14; 28.6%), English Springer Spaniel (2/14; 14.3%), Golden Retriever (2/14; 14.3%), and 1 each (7.1%) of Boxer, Boston Terrier, English Setter, German Shepherd Dog, Labrador Retriever, and Australian Shepherd. Diagnosis of neoplasia in dogs with solid tumors was based on histopathology (8/14; 57.1%), cytology (3/14; 21.4%), or both (2/14; 14.3%). One dog was enrolled that had a presumptive diagnosis of an insulinoma based on clinical signs, documented hypoglycemia with concurrent elevated blood insulin levels, and evidence of a pancreatic mass on computed tomography.

Dogs with solid tumors had a variety of cytologic or histopathologic tumor types, including anal gland adenocarcinoma (2/14; 14.3%), insulinoma (2/14; 14.3%; one presumptive and one confirmed based on histopathology), osteosarcoma (2/14; 14.3%), chondrosarcoma (2/14; 14.3%), and 1 each (1/14; 7.1%) of mast cell tumor, nasal adenocarcinoma, hemangiosarcoma, oligodendrogial ganglioglioma, and clear cell adenocarcinoma. One dog had an oral melanoma that had previously been surgically excised with incomplete margins, and an anal gland adenocarcinoma diagnosed on the day of study enrollment that had therefore not been treated. Two dogs (2/14; 14.3%) were receiving chemotherapeutic treatment at the time of enrollment, including one
that had previously received a full course of fractionated radiation therapy that was receiving carboplatin for an anal gland adenocarcinoma, and one dog with hemangiosarcoma that was receiving doxorubicin and a bispecific ligand-targeted angiototoxin as part of a clinical trial. The dog that was receiving carboplatin was also receiving metoclopramide and metronidazole for chemotherapy-related side effects. Three dogs (3/14; 21.4%) were receiving systemic glucocorticoids. Comorbidities are listed in Supplementary Table S3. None of the comorbid conditions present are known to be associated with coated platelet formation based on extrapolation from the human literature, and none are known to be associated with hypercoagulability in dogs.

Coated platelet technical replicates

Results of one of the technical replicate reactions were discarded in 6 instances (6/141; 4.3%). This occurred due to consideration as an outlier (defined as a difference of >10% coated platelets compared to the other 2 technical replicate tubes) in 5/141 reactions (3.5%) and due to assay failure in 1/141 reaction (0.7%).

Comparison of coated platelet percentage between healthy dogs and dogs with solid tumors or lymphoma

When all dogs were included regardless of remission status, the mean percentage (± standard deviation) of coated platelets produced by healthy control dogs was 30.8% (±11.8%; range, 15.1 to 49.5%), the mean percentage of coated platelets produced by dogs with solid tumors was 41.7% (±9%; range, 26.3 to 56.5%), and the mean percentage of coated platelets produced by dogs with lymphoma was 30.5% (±15.9%; range, 4.5 to 63%). There was overlap of the distribution of data between the three groups (Figure 2).

We observed a greater mean percentage of coated platelets in dogs with solid tumors, compared with healthy control dogs, by 10.9 percentage points (95% CI: −1.0, 22.8; \( P = .07 \); Figure 2). We observed a mean percentage of coated platelets in dogs with...
lymphoma that was less than healthy control dogs by 0.3 percentage points (95% CI: −11.4, 10.8; \(P = .99\)).

When dogs in clinical remission were excluded, the mean percentage of coated platelets produced by dogs with solid tumors was 41.4% (range, 26.3 to 56.5%) and the mean percentage of coated platelets produced by dogs with lymphoma was 26.7% (range, 4.5 to 63%). We similarly observed a greater mean percentage of coated platelets in dogs with solid tumors, compared with healthy control dogs, by 10.6 percentage points (95% CI: −2.2, 23.4; \(P = 0.1\)). We observed a mean percentage of coated platelets in dogs with lymphoma that was less than healthy control dogs by 4.1 percentage points (95% CI: −16.9, 8.6; \(P = .7\); Figure 2).

The average intra-assay CV for coated platelets in this study was 6.9%. No CV was greater than 20%, which is the threshold often used as the limit of acceptable precision for assays.

### Comparison of platelet counts in dogs with solid tumors and dogs with lymphoma

The mean platelet count of the neoplasia cases included in this study was 333 X 10^3 platelets/μl (range, 124 to 667 X 10^3/μl). Dogs with lymphoma had a mean (±standard deviation) platelet count of 321 X 10^3/μl (±160 X 10^3/μl; range, 124 to 667 X 10^3/μl). Of the lymphoma dogs, 7/19 (36.8%) had platelet counts outside of the reference range (200 to 500 X 10^3/μl); 4/7 were below the reference range (124 to 192 X 10^3/μl) and 3/7 were above the reference range (506 to 667 X 10^3/μl). Dogs with solid tumors had a mean platelet count of 349 X 10^3/μl (±102 X 10^3/μl; range, 124 to 667 X 10^3/μl). Of the solid tumor dogs, 2/13 (15.4%) had platelet counts outside of the reference range (200-500 X 10^3/μl); 1 had a platelet count below the reference range (167 X 10^3/μl) and 1 had a platelet count above the reference range (545 X 10^3/μl). One dog in the solid tumor group did not have a platelet count performed on the day of enrollment but historically had platelet counts within the normal reference range.

The healthy control dogs had a mean platelet count of 226 X 10^3/μl (±46 X 10^3/μl; range, 153 to 294 X 10^3/μl). Four of the 14 healthy control dogs had platelet counts below the reference range (range, 153 to 183 X 10^3/μl), and no healthy control dog had a platelet count above the reference range.

Using the model with only data for which total platelet count was present, the mean percentage of coated platelets was higher in dogs with solid tumors than dogs with lymphoma by 11.4 percentage points (95% CI: −2.2, 23.4; \(P = .1\)). With the exception of the dog with hematuria, which was presumed secondary to either a urinary tract infection or sterile hemorrhagic cystitis secondary to cyclophosphamide, no dog included in this study had evidence of clinical bleeding.

### Thrombotic and hemorrhagic events

None of the dogs included in this study had evidence of a thrombotic event at the time of enrollment, historically, or for a period of 2 years after enrollment. With the exception of the dog with hematuria, which was presumed secondary to either a urinary tract infection or sterile hemorrhagic cystitis secondary to cyclophosphamide, no dog included in this study had evidence of clinical bleeding.

### Discussion

The primary objective of this study was to evaluate coated platelet potential in dogs with neoplasia compared to healthy controls. Given the large range (15.1 to 49.5%) of coated platelet formation in our healthy dog population, there was overlap between coated platelet percentage between solid tumor dogs, dogs with lymphoma, and healthy control dogs. However, our data are compatible with dogs with solid tumors having a higher mean coated platelet percentage than the healthy control dogs.

Hypercoagulability has been well documented in both humans and dogs with cancer. Neoplasia was found to be the most common underlying disease found in dogs with documented pulmonary thromboembolism,15,16 portal vein thrombosis,17,18 and splenic vein thrombosis.16 Although the data from this study did not document a definitive role for coated platelets in canine neoplasia, it was the first time this subset of procoagulant platelets has been evaluated in dogs with cancer.

There is still much to be learned regarding the underlying causes of the hypercoagulability that is seen in cancer patients. Several studies have documented a tendency toward hypercoagulability in canine cancer patients. Andreasen et al17 showed that 47/71 (66.2%) of canine cancer patients had prothrombotic thromboelastography (TEG) profiles. Carcinoma patients, with the exception of

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**Figure 3**—Percent coated platelets by total platelet count (X 10^3/μl) in dogs with lymphoma and solid tumors, with points colored by group. Lines and bands represent linear fits and 95% confidence intervals for each group.
mammary carcinomas, had increased clot strength on TEG and increased fibrinogen. Dogs with distant metastases had significantly elevated fibrinogen and d-dimer levels compared to those with only local disease.47 Another study48 evaluating TEG in dogs with neoplasia found similar results, with dogs with malignant disease displaying prothrombotic TEG profiles, including decreased time to clot formation and increased clot strength. Adenocarcinomas were the most common neoplasia associated with hypercoagulability in that study, Kol et al49 showed that 17/27 (63%) of dogs with multicentric lymphoma also had prothrombotic TEG parameters, and this tendency persisted throughout treatment and for up to a month after clinical remission was attained. In these lymphoma patients, accelerated clot formation on TEG at the end of the chemotherapy regimen was associated with decreased survival.49 Limited information is known about the correlation between coated platelet formation ex vivo and TEG parameters; however, Scott syndrome dogs (that have a decreased ability to form coated platelets) had prolonged mean clotting times (K) and prolonged time to maximal rate of thrombus generation on non-tissue factor–activated TEG compared with same day healthy control samples.50 Additionally, TEG parameters and percentage of coated platelets have been shown to change over time in stored platelet concentrate samples from healthy human donors.51

Coated platelets have not previously been evaluated in dogs with cancer and were investigated here as a potential mechanism for their documented hypercoagulability. In the current study, we observed a greater mean percentage of coated platelets in dogs with solid tumors compared to healthy controls and dogs with lymphoma. However, a major limitation of this study is that the variability in our patient population was notably larger than that in the data set used to estimate the variability for our power calculation,52 resulting in this study potentially being underpowered and having less precision than planned. Additionally, the sample sizes were calculated to detect a difference of 10% in coated platelet production. This percentage is considered clinically significant in humans.50,51 However, it is unknown if the difference in coated platelet percentage required for clinical significance in humans can be translated to dogs. Another limitation is that inter-assay variability for this assay for coated platelets in dogs has not been established. Our population of healthy control dogs displayed a large range (15.1 to 49.5%) of coated platelets upon ex vivo stimulation. We did confirm that the assay utilized in this study had very low intra-assay variability. However, samples in this study were analyzed on two different cytometers at two different institutions. Attempts were made to minimize other variables when possible. The assays were performed by the same investigators, using the same reagents and antibodies, using the same analysis software. Use of the same reagents and analysis software was shown to improve consistency of results in a flow cytometric study of canine lymphoproliferative disorders.52 This same study also identified a strong agreement across 9 institutions using various cytometers but the same antibodies. However, given these limitations, this study should be considered a hypothesis-generating study that will better inform future targeted studies.

The cases enrolled were very heterogeneous, but all eligible cases were included since coated platelets had not yet been evaluated in dogs with cancer. Cases differed with regards to treatment status, with some dogs having had surgical treatments of their neoplasia, and with some dogs receiving chemotherapy, glucocorticoids, and/or other medications. The effect of each of these treatments on coated platelets is not currently known; however, it must be acknowledged that these treatments may have influenced our results. It was decided to include dogs that were receiving chemotherapy and glucocorticoids in this initial hypothesis-generating study because both the underlying neoplasia and the treatment effects have potential implications for thrombotic outcomes. Endogenous and exogenous glucocorticoids have a documented association with hypercoagulability in both dogs and humans.53–56 Human cancer patients receiving chemotherapy have an increased risk of thrombotic disease compared to human cancer patients that are not receiving chemotherapy.5,11,57,58 Similarly, thrombosis is also a known potential complication after cancer surgery in humans.59 To improve the precision of these findings, coated platelets should be evaluated in a larger or more uniform population of dogs with neoplasia. Coated platelets could also be evaluated in dogs with cancer at the time of diagnosis, before starting therapy, to reduce the potential for confounding variables associated with treatment. The data from our current study can be employed to determine the appropriate sample size for future studies.

In summary, our data are compatible with dogs with solid tumors having a greater mean percentage of coated platelets when compared to healthy control dogs. Further studies investigating coated platelet production in more specific subsets of neoplasia and investigating additional mechanisms of hypercoagulability in dogs with neoplasia are warranted. It may also be warranted to investigate potential therapies to mitigate the formation of coated platelets in dogs at risk of thrombosis, such as serotonin-specific reuptake inhibitors.

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The authors declare that there were no conflicts of interest.

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References


Supplementary Materials

Supplementary materials are posted online at the journal website: avmajournals.avma.org