STANDARD ARTICLE

Neutropenia in dogs receiving vincristine for treatment of presumptive immune-mediated thrombocytopenia

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Abstract

Background: Neutropenia is an adverse effect of vincristine when used in multidrug chemotherapy protocols.

Objective: To determine the incidence of neutropenia, identify potential risk factors for neutropenia, and determine the effect of neutropenia on outcome, in dogs receiving vincristine for treatment of immune-mediated thrombocytopenia (ITP).

Animals: One hundred twenty-seven client-owned dogs presumptively diagnosed with ITP.

Methods: In this retrospective cohort study, medical records were reviewed to identify dogs presumptively diagnosed with ITP, and treated with vincristine, over a 15-year period. Logistic regression was used to identify risk factors for the development of neutropenia in dogs receiving vincristine. Time to platelet count ≥40 000 platelets/μL, survival, and duration of hospitalization were compared between neutropenic and non-neutropenic dogs.

Results: Vincristine was administered to 127 dogs with presumptive ITP; 19 became neutropenic. Administration of cyclosporine was significantly ($P < .001$) associated with the development of neutropenia (odds ratio: 12.97, 95% confidence interval: 4.17, 40.35). There was no difference in median time to ≥40 000 platelets/μL between neutropenic dogs (4 days; range, 1-14 days) and non-neutropenic dogs (3 days; range, 0-48 days). Percentage survival to discharge was 95% in both groups, but median duration of hospitalization was significantly longer in neutropenic dogs (6 days; range, 3-22 days) compared to non-neutropenic dogs (4 days; range, 2-15 days).

Conclusions and Clinical Importance: Cyclosporine administration was associated with the development of neutropenia in dogs receiving vincristine, which might be related to effects on metabolism of vincristine. Neutrophil counts should be monitored in dogs receiving vincristine treatment for ITP, particularly if administered in conjunction with cyclosporine.

1 | INTRODUCTION

Neutropenia and myelosuppression are documented complications of treatment with vincristine, particularly when used with other myelosuppressive drugs in the treatment of neoplasia in dogs.[1-3] Neutropenia occurs in toxicity studies of vincristine in healthy dogs, when administered as a single agent at doses of 0.1 or 0.2 mg/kg.[4] However, this adverse effect has not been reported when vincristine is used in the treatment of immune-mediated thrombocytopenia (ITP).[5,6]

Vincristine is used in multidrug chemotherapy protocols, particularly in the treatment of hemolymphatic neoplasms, at doses of 0.5 to 0.75 mg/m^2 IV, and induces cytotoxicity through inhibition of microtubule assembly and subsequent disruption of the mitotic spindle.[7] Adverse effects include signs of gastrointestinal disease, myelosuppression, and less commonly neurotoxicosis. Vincristine is less myelosuppressive than other vinca alkaloids such as vinblastine;[8] however, myelosuppression, particularly neutropenia, remains a potential adverse effect.[1-3]

Treatment of ITP in dogs involves immunosuppression with glucocorticoids alone or in combination with other immunomodulatory agents.[9,10] Vincristine, at a dose of 0.02 mg/kg IV, when administered in conjunction with prednisone, is associated with faster recovery of platelet counts and shorter duration of hospitalization for dogs with ITP when compared to prednisone alone.[5] Mechanisms proposed for the improvement in platelet count observed with vincristine treatment include fragmentation of megakaryocytes, inhibition of platelet phagocytosis, interference with formation of antiplatelet antibodies, inhibition of antibody binding, and stimulation of thrombopoiesis.[5,11,12]

Identifying the incidence of neutropenia, and associated risk factors, in dogs receiving vincristine for the treatment of ITP could influence treatment and monitoring decisions for individual dogs. Neutropenic dogs are potentially at increased risk for infection or sepsis, and dogs concurrently immunosuppressed with other medications for the treatment of ITP might be at increased risk for complications such as opportunistic infections.[13,14] While platelet counts are typically closely monitored during the treatment of ITP, complete blood counts might not be performed as frequently, therefore it is important to identify risk factors for the development of neutropenia in these dogs.

The primary goals of this retrospective study were to determine the incidence of neutropenia in dogs treated with vincristine for presumptive ITP and to identify potential risk factors for the development of neutropenia. A secondary goal was to determine if case outcome, specifically time to increase in platelet count, duration of hospitalization, and percentage survival, differed between dogs that did and did not develop neutropenia.

2 | MATERIALS AND METHODS

The electronic medical records database at the North Carolina (NC) State University Veterinary Hospital was searched for dogs diagnosed with thrombocytopenia between July 2003 and June 2018. Inclusion criteria were: (a) platelet count ≤15 000 platelets/μL, (b) documented results of a minimum of 2 CBCs with at least 1 performed at NC State University, and (c) documented results of additional diagnostic testing, including a serum biochemistry profile, vector-borne disease agent testing, thoracic radiographs, and abdominal ultrasound examination. Dogs were excluded if they had a confirmed diagnosis of neoplasia, presented with neutropenia, did not receive vincristine after admission, or were treated with vincristine before admission to NC State University. A flow chart of the study design is presented in Figure 1. Dogs were presumptively diagnosed with ITP based on a platelet count of ≤15 000 platelets/μL and no evidence of any other cause of severe thrombocytopenia, as determined by history and physical examination, and the results of a CBC, serum biochemistry profile, thoracic radiographs, and abdominal ultrasound examination.[5] The final study group consisted of dogs presumptively diagnosed with ITP that were treated with vincristine.

Data extracted manually from medical records included signalment, weight (in kg), type of immunomodulatory agent(s) administered, administration of human intravenous immunoglobulin (hIVIG), prescribed dose of vincristine, platelet count, neutrophil count, presence of hyperbilirubinemia (serum bilirubin above the upper limit of the laboratory reference range), and vector-borne disease agent testing methods and results. Dogs were considered positive for vector-borne disease agents based on a positive result on a SNAP 3Dx, 4Dx, or 4Dx Plus ELISA (IDEXX Laboratories, Westbrook, Maine), positive immunofluorescent antibody assay (IFA) serology (titer ≥1 : 64), or positive PCR result. Hyperbilirubinemia was included as a potential risk factor because serum bilirubin concentration is an indicator of liver function, and vincristine is primarily metabolized by the liver.[8] Data from CBCs were extracted for up to 14 days from the time of presentation, when available, for the detection of neutropenia. If neutropenia was detected during that time, CBCs were evaluated for as long as available, to determine if or when the neutropenia resolved.

Neutropenia was defined as a segmented neutrophil count <2800 cells/μL (the laboratory's lower reference limit for segmented neutrophil count in dogs). When neutropenia was detected it was classified as grade 1 (1500-2799 cells/μL), grade 2 (1000-1499 cells/μL), grade 3 (500-999 cells/μL) or grade 4 (<500 cells/μL).[15]

Additional data extracted included number of days from vincristine injection to platelet count ≥40 000 platelets/μL, duration of hospitalization (days), percentage survival to discharge, and percentage survival 30 days after admission. When a dog died, or was euthanized,
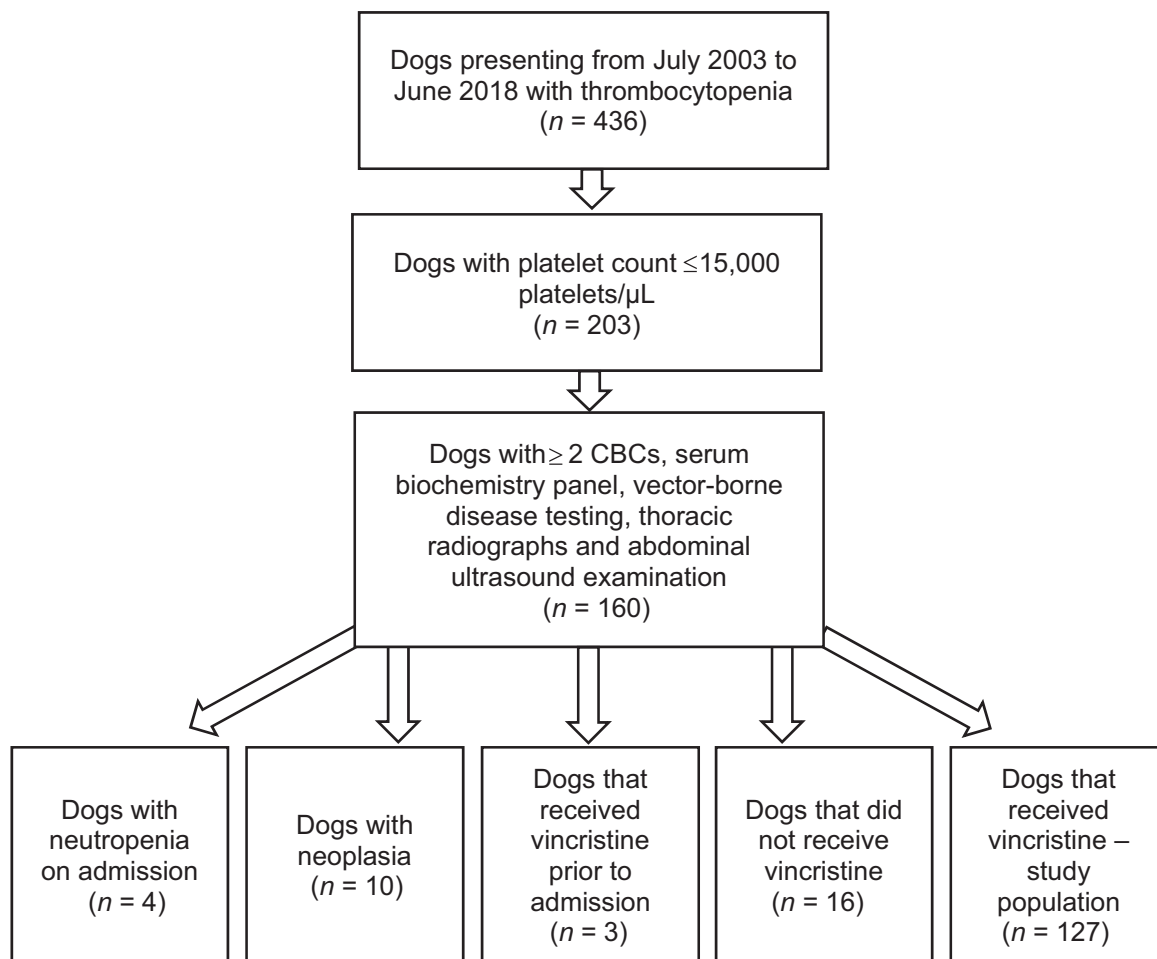
FIGURE 1 Flow chart of study design

the presumptive cause of death or reason for euthanasia was recorded. Necropsy data were recorded when available.

2.1 | Statistical analysis

Logistic regression models were used to determine the effect of the following factors on the outcome of neutropenia in dogs treated with vincristine: presence of hyperbilirubinemia, administration of individual immunomodulatory agents in the hospital (azathioprine, cyclosporine, leflunomide, mycophenolate mofetil), administration of hIVIG, and vector-borne disease agent status (ie, positive test for 1 or more vector-borne disease agents). Relevant factors were identified by use of the Bayesian Information Criteria for backward model selection. To determine if dogs that became neutropenic had received a higher dose of vincristine, the prescribed dose, expressed both as mg/kg and mg/m^2, was compared between neutropenic and non-neutropenic dogs using the Mann-Whitney test. The outcomes of days to platelet count ≥40 000 platelets/μL and duration of hospitalization were compared between neutropenic and non-neutropenic dogs that received vincristine with the Mann-Whitney test. Rates of survival to discharge

and of survival 30 days after admission were compared between the neutropenic and non-neutropenic dogs with Fisher's exact test.

Statistical analyses were performed using commercial software (Logistic regression: R, version 3.6.1, R Foundation for Statistical Computing, Vienna, Austria; Mann-Whitney test and Fisher's exact test: GraphPad Prism, version 8.3.0, GraphPad Software Inc, San Diego, California). A P value <.05 was considered significant.

3 | RESULTS

3.1 | Study sample

One-hundred and sixty dogs were identified based on the search of medical records and inclusion criteria. Four dogs were excluded due to neutropenia on admission, 10 dogs were excluded due to a diagnosis of neoplasia, 3 dogs were excluded due to administration of vincristine before presentation, and 16 dogs did not receive vincristine treatment after admission, resulting in 127 dogs in the study group (Figure 1). The median age was 8 years (range, 0.5-14.2 years). Three dogs were intact females, 65 were spayed females, 6 were intact

males, and 53 were castrated males. There were 46 breeds represented in the study group. The 7 most common breeds were mixed breed (n = 22), Labrador Retriever (n = 10), American Cocker Spaniel (n = 10), Maltese Terrier (n = 8), Standard Poodle (n = 7), Jack Russell Terrier (n = 6), and Miniature Schnauzer (n = 5).

All dogs were tested for vector-borne disease agents with at least 1 test, including ELISA, IFA, or PCR. Testing with a commercially available ELISA (SNAP 3 Dx, IDEXX Laboratories, Westbrook, Maine) for *Ehrlichia*, *Borrelia*, and *Dirofilaria* was performed in 3 dogs. Testing with a commercially available ELISA (SNAP 4 Dx or 4 Dx Plus, IDEXX Laboratories, Westbrook, Maine) for *Ehrlichia*, *Anaplasma*, *Borrelia*, and *Dirofilaria* was performed in 105 dogs. One additional dog was tested for *Dirofilaria* with an ELISA, and 16 dogs were not tested for *Dirofilaria*. Additional vector-borne disease diagnostic tests were performed based on availability of testing at the time and at the discretion of the primary clinician. Additional testing included: *Babesia canis* IFA, *Babesia gibsoni* IFA, *Ehrlichia canis* IFA, *Rickettsia* IFA, *Bartonella vinsonii* IFA, *Bartonella henselae* IFA, *Bartonella kohlerae* IFA, *Anaplasma* PCR, *Babesia* PCR, *Bartonella* PCR, *Ehrlichia* PCR, *Rickettsia* PCR, and *Bartonella* alpha-proteobacteria growth medium (BAPGM) with PCR.

Twenty dogs were positive for 1 vector-borne disease agent, 4 were positive for 2, and 1 was positive for each of 3, 4, and 5 vector-borne disease agents. Vector-borne disease agents for which dogs tested positive included *B. canis* (n = 1), *Ehrlichia* spp. (n = 5), *E. canis* (n = 3), *Ehrlichia ewingii* (n = 1), *Rickettsia* (n = 18), *Borrelia* (n = 2), *Anaplasma* (n = 2), *B. vinsonii* (n = 2), *Bartonella koehlerae* (n = 3), and *B. henselae* (n = 3). No dogs tested positive for *Dirofilaria*. Of the 27 dogs that had a total of 40 positive test results for vector-borne disease agents, only 2 dogs had a positive PCR test, and each for only a single disease agent; all the remaining positive results were from antibody tests. The 2 positive PCR tests were for *Ehrlichia ewingii* and *B. henselae*, after BAPGM enrichment. Both dogs were in the non-neutropenic group.

3.2 | Treatment for presumptive ITP

All but 1 dog in the study group received glucocorticoids, including either injectable dexamethasone, or prednisone or prednisolone orally, or both injectable and oral glucocorticoids, according to clinician preference. The dog that did not receive glucocorticoids was diagnosed on endoscopy with an ulcer in the pyloric antrum before presentation. All dogs in the study group received a single dose of vincristine. The prescribed dose was 0.02 mg/kg IV in 119 dogs. Seven dogs were prescribed 0.01 mg/kg and 1 dog received 0.04 mg/kg. The latter dose was administered in error. Of the dogs receiving 0.01 mg/kg, the reasons for the dose reduction were obesity (n = 1), hepatopathy (n = 1), and not recorded (n = 5).

Eighty-nine dogs received 1 additional immunomodulatory medication during hospitalization, 13 received 2 additional immunomodulatory medications, and 2 dogs received 3 additional immunomodulatory medications. Additional immunomodulatory medications included azathioprine (n = 63), cyclosporine (n = 34), mycophenolate

mofetil (n = 23), and leflunomide (n = 1). Forty-one dogs received hIVIG. Immunomodulatory medications reportedly administered before presentation to NC State University included: prednisone and azathioprine for 4 days (n = 1), azathioprine for 1 day (n = 1), azathioprine for 3 days (n = 2), azathioprine long term (n = 1), cyclosporine long term (for Sudden Acquired Retinal Degeneration Syndrome) and azathioprine for 3 days (n = 1), cyclosporine for 2 days (n = 1), and cyclosporine long term for atopy (n = 2). These medications were continued during hospitalization, and included in the analysis, with the exception of 1 dog in which azathioprine was discontinued on admission.

3.3 | Neutropenia

Nineteen dogs became neutropenic after administration of vincristine. In these dogs, neutropenia was 1st documented between 2 and 14 days (median: 5 days) after vincristine administration, with the lowest count documented between 3 and 14 days (median: 5 days) after vincristine administration. The median lowest neutrophil count was 1040 cells/µL (range, 0-2700 cells/µL). The neutropenia grade (based on the lowest recorded neutrophil count) was grade 1 in 7 dogs, grade 2 in 3 dogs, grade 3 in 2 dogs, and grade 4 in 7 dogs. Of these 19 dogs, follow-up data regarding the neutropenia were available for 12; resolution of neutropenia occurred from 1 to 8 days (median: 3 days) after the lowest neutrophil count in these dogs. In the remaining 7 neutropenic dogs, no CBC data were available to determine when recovery occurred. After backward selection, the logistic regression model identified administration of cyclosporine as significantly associated with increased probability of neutropenia in dogs receiving vincristine for the treatment of ITP (odds ratio: 12.97, 95% confidence interval: 4.17, 40.35, *P* < .001). Hyperbilirubinemia, administration of other immunomodulatory agents, administration of hIVIG, and vector-borne disease agent status were not significantly associated with the development of neutropenia in dogs receiving vincristine for the treatment of ITP and were all removed during model selection. Table 1 summarizes age, sex, body weight, vincristine dose, the presence of hyperbilirubinemia, the additional medications administered, and the vector-borne disease agent test results of dogs that received vincristine. Three of the neutropenic dogs were receiving immunomodulatory medications before presentation, including cyclosporine long term for atopy (n = 1), cyclosporine for 2 days (n = 1), and prednisone and azathioprine for 4 days (n = 1). Bilirubin results were not available for 1 dog that did not develop neutropenia. When neutropenic dogs were evaluated by grade of neutropenia, it was found that 4 of 7 dogs with grade 1 neutropenia had received cyclosporine, 2 of 3 dogs with grade 2 neutropenia had received cyclosporine, 1 of 2 dogs with grade 3 neutropenia had received cyclosporine, and 7 of 7 dogs with grade 4 neutropenia had received cyclosporine.

There was no difference in the prescribed mg/kg dose of vincristine between dogs that became neutropenic (median: 0.02 mg/kg) and dogs that did not become neutropenic (median: 0.02 mg/kg).

TABLE 1 Signalment, body weight, vincristine dose, presence of hyperbilirubinemia, additional medications administered, and vector-borne disease testing results in dogs receiving vincristine for treatment of ITP

Variable		Neutropenic dogs (n = 19)	Non-neutropenic dogs (n = 108)
Age (years)		7.9 (2.4-13)	8.2 (0.5–14.2)
Sex	Intact female	1 (5.3%)	2 (1.9%)
	Spayed female	10 (52.6%)	55 (50.9%)
	Intact male	1 (5.3%)	5 (4.6%)
	Castrated male	7 (36.8%)	46 (42.6%)
Weight (kg)		10.5 (2.2-59.1)	14.1 (2.8-67.8)
Vincristine dose (mg/kg)		0.02 (0.02)	0.02 (0.01-0.04)
Vincristine dose (mg/m^2)		0.43 (0.24–0.78)	0.48 (0.2-0.81)
Hyperbilirubinemia		5 (26.3%)	27 (25.2%)[a]
Medications	Azathioprine	7 (36.8%)	56 (51.9%)
	Cyclosporine	14 (73.7%)[b]	20 (18.5%)
	Leflunomide	0	1 (0.9%)
	MMF	4 (21.1%)	19 (17.6%)
	hIVIG	7 (36.8%)	34 (31.5%)
Vector-borne diseases	Babesia spp.	0	1 (0.9%)
	Ehrlichia spp.	1 (5.3%)	8 (7.4%)
	Rickettsia spp.	3 (15.8%)	15 (13.9%)
	Borrelia spp.	1 (5.3%)	1 (0.9%)
	Anaplasma spp.	0	2 (1.9%)
	Bartonella spp.	0	4 (3.7%)

Note: Age, weight, and vincristine dose are reported as median (range). Sex, hyperbilirubinemia, medications and vector-borne diseases are reported as number of dogs (percent). Medications are only those administered in the hospital. Vector-borne disease testing results are reported as number of positive dogs rather than number of positive test results.

Abbreviations: hIVIG, human intravenous immunoglobulin; ITP, immune-mediated thrombocytopenia; MMF, mycophenolate mofetil.

[a]Serum bilirubin concentration was not available for 1 dog in the non-neutropenic group, therefore percentage is recorded out of 107 dogs. .

[b]Significantly associated with increased probability of neutropenia (P < .001).

There was also no statistically significant difference in the mg/m^2 dose of vincristine between the dogs that became neutropenic (median: 0.43 mg/m^2; range, 0.24-0.78 mg/m^2) and the dogs that did not become neutropenic (median: 0.48 mg/m^2; range, 0.20-0.81 mg/m^2) (P = .92; Mann-Whitney test). Although not included in the final study group, medical records were reviewed for the 16 dogs presumptively diagnosed with ITP that did not receive vincristine; neutropenia was not detected in any of the dogs in that group.

3.4 | Outcomes

For dogs receiving vincristine, the median time to reach a platelet count of ≥40 000 platelets/μL was 4 days after administration of vincristine for the dogs that became neutropenic (n = 15; range, 1-14 days), and 3 days for the dogs that did not become neutropenic (n = 101; range, 0-48 days). There was no significant difference between the groups (P = 0.63; Mann-Whitney test). Four neutropenic dogs and 7 non-neutropenic dogs had no recorded platelet count ≥40 000 platelets/μL. The duration of hospitalization was significantly longer for dogs that became neutropenic (median: 6 days; range,

3-22 days) compared to dogs that did not become neutropenic (median: 4 days; range, 2-15 days) (P < .001; Mann-Whitney test).

Eighteen of 19 (95%) neutropenic dogs and 103 of 108 (95%) non-neutropenic dogs survived to discharge. These survival rates were not significantly different (P ≈ 1, Fisher's exact test). Five neutropenic dogs were euthanized or died within 30 days of admission, including 1 that died during hospitalization. Reasons listed in medical records included cardiopulmonary arrest due to suspected pulmonary thromboembolism (n = 1), gastrointestinal bleeding (n = 1), hemoabdomen secondary to hepatic fracture (n = 1), gall bladder rupture and sepsis (n = 1), and unknown (n = 1). Fifteen non-neutropenic dogs were euthanized or died within 30 days of admission, including 5 that died during hospitalization. Reasons listed in medical records included pancreatitis and sepsis (n = 1), secondary to transfusion requirements (n = 1), cardiopulmonary arrest (n = 2), lack of response (n = 3), respiratory distress (n = 1), hepatopathy (n = 1), suspect hepatic abscess (n = 1), recurrent epistaxis (n = 1), financial constraints (n = 1), and unknown (n = 3). When comparing dogs that did or did not become neutropenic, there was no significant difference in the proportion of dogs that died or were euthanized within 30 days of admission (P = .18; Fisher's exact test).

4 | DISCUSSION

This study documents the development of neutropenia in dogs receiving vincristine for the treatment of ITP. Of the 127 dogs in the study that received vincristine for the treatment of ITP, 15% developed neutropenia. This is less than previously reported incidences of neutropenia in 40% of dogs after receiving a combination of vincristine with L-asparaginase and 13 episodes of neutropenia after vincristine administration in 50 dogs.[1,16] However, the latter includes episodes of neutropenia after administration of vincristine throughout the chemotherapy protocol. When considering dogs treated for lymphoma, the reported incidence of myelosuppression after the 1st dose of vincristine is lower than in our study, with only 2 of 40 dogs developing neutropenia.[3]

Small dogs are at higher risk for the development of sepsis after administration of chemotherapy, due to the relative overdosing of smaller dogs and underdosing of larger dogs when dose is calculated based on body surface area.[17] Vincristine for ITP is traditionally dosed based on body weight not body surface area,[5,6] which could lead to relative underdosing of smaller dogs and overdosing of larger dogs. Our study did not identify a significant difference in the mg/kg or mg/m^2 dose of vincristine between dogs that did or did not develop neutropenia, but this study was most likely underpowered for the detection of a difference. In addition, the medical records accessed in this study routinely recorded the dose of vincristine that was prescribed, but as this was a retrospective study, the actual dose administered could not be verified.

Neutropenia was not reported in 2 publications that documented the benefits of vincristine treatment in dogs with ITP.[5,6] However, the numbers of dogs receiving vincristine in those prospective studies were much lower than the 127 dogs included in this retrospective study. Specifically, in previous studies, vincristine was administered to 12 dogs[5] and to 10 dogs[6] with ITP. These 2 prospective studies also appropriately excluded the use of additional immunosuppressive agents. Our retrospective study, while arguably less rigorous than a prospective controlled trial, provided an opportunity to evaluate a range of clinically relevant risk factors that could potentially contribute to the development of neutropenia in dogs administered vincristine for the treatment of ITP.

Of the potential risk factors evaluated in our study, the administration of cyclosporine was found to be significantly associated with an increased probability of neutropenia. Fourteen of the 19 dogs (74%) that became neutropenic had received cyclosporine, compared with 20 of the 108 dogs (19%) that did not become neutropenic. In addition, all of the 7 dogs that developed severe (grade 4) neutropenia had received cyclosporine. Vincristine is primarily metabolized by the liver and excreted in bile.[18] In humans, the CYP3A (CYP3A4 and CYP3A5) subfamily of enzymes are involved in the metabolism of vincristine.[19] The specific enzymes involved in metabolism of vincristine in dogs have not been fully evaluated; however, the CYP3A enzymes might be involved. There is increased neurotoxicosis with vincristine in people receiving medications that inhibit cytochrome P450 enzymes or P-glycoprotein pumps, such as cyclosporine, azoles,

macrolide antibiotics, and calcium channel blockers.[20] Cyclosporine might reduce the activity of CYP3A enzymes and P-glycoprotein pumps, thereby increasing the incidence of vincristine associated toxicity.[19,20] In addition, high dose cyclosporine has been evaluated in conjunction with vincristine in people with relapsed or refractory primary or metastatic malignancies to modulate the effects of multidrug resistance due to P-glycoprotein pumps. In these patients, the dose of vincristine was reduced by 50%, and adverse effects included myelosuppression and acute reactions.[21]

P-glycoprotein efflux pumps are responsible for excretion of vincristine, which explains the increased risk of hematological toxicoses, including neutropenia in dogs with ABCB1-1Δ mutations.[22] Our study had only a small number of breeds identified as homozygous or heterozygous carriers for ABCB1-1Δ, so breed-associated risk factors could not be evaluated.[23] In addition, we did not test for this mutation in dogs in our study group. However, breed associations with vincristine toxicosis have been identified independent of ABCB1-1Δ mutations, which could suggest alternative mechanisms.[24]

In the treatment of neoplasia in dogs, vincristine dose reductions of 50% in dogs with abnormal hepatic function or hyperbilirubinemia (serum bilirubin concentration >1.5-2.0 mg/dL) have been recommended.[1,25] Hyperbilirubinemia did not appear to be associated with an increased probability of neutropenia in dogs receiving vincristine for the treatment of ITP; however, the failure to detect an association could potentially be due to the low prevalence of hyperbilirubinemia, in this study, and to the deliberate vincristine dose reduction in 1 of the dogs.

While development of neutropenia has been associated with longer remission times and longer survival times in dogs receiving chemotherapy for lymphoma,[16] our study did not detect that the development of neutropenia improved outcome in dogs receiving vincristine for the treatment of ITP. The number of days to ≥40 000 platelets/μL, survival to discharge, and survival at 30 days postadmission to the hospital were similar in both neutropenic and non-neutropenic dogs. In addition, neutropenic dogs were hospitalized for a significantly longer period of time (median: 6 days) when compared to non-neutropenic dogs (median: 4 days). The causal relationship between development of neutropenia and duration of hospitalization cannot be determined from this retrospective study. One potential explanation is that neutropenic dogs were hospitalized for longer due to concerns for risk of sepsis, to facilitate IV administration of antibiotics, or for monitoring for resolution of neutropenia. Conversely, it is also possible that there was an increased probability of detection of neutropenia in dogs that were hospitalized for a longer period of time, potentially associated with an increased opportunity to detect a later nadir in those dogs.

The diagnosis of ITP should be considered presumptive in the dogs in this retrospective study. There is no single test that confirms the diagnosis of ITP,[26] and platelet autoantibodies were not measured in dogs in this study group. Reported values for platelet count used to support the diagnosis of ITP in other studies range between 15 000/μL[5] and 20 000/μL.[6,26,27] The inclusion criteria that were used in our study included a platelet count of ≤15 000 platelets/μL, as this was the

most conservative value that was also consistent with other published studies in dogs. When considering a positive response to treatment, we used a cutoff of 40 000 platelets/μL. While a count of 40 000/μL is unlikely to represent full remission of disease, it is sufficiently different from 15 000/μL to suggest improvement. A value of 40 000/μL is also a target that is likely to be achieved during hospitalization for the treatment of ITP. In contrast, had we selected a target of 100 000 platelets/μL, for example, this would have been more difficult to detect as many dogs are likely to be discharged from the hospital before their platelet counts reach this number. In addition, a platelet count of at least 40 000/μL has been used as evidence of positive response in several other studies evaluating management of ITP in dogs.[5,6,28]

The inclusion of dogs that tested positive for vector-borne disease agents could be considered a weakness of this study. However, the inclusion of these cases was deliberate, as we sought to investigate any factors in this group that could potentially increase the risk of development of neutropenia when vincristine is used in the treatment of presumptive ITP. It is possible that some of the dogs had secondary ITP associated with vector-borne disease, but the severity of the thrombocytopenia was such that all dogs were treated for ITP, typically before the results of vector-borne disease agent testing were available, and regardless of the possibility that the ITP could be secondary. Furthermore, in this study, of 27 dogs that tested positive for at least 1 vector-borne disease agent, only 2 dogs tested positive by PCR, and those 2 dogs did not develop neutropenia. All the remaining positive results were antibody tests. We cannot prove or disprove that the thrombocytopenia or neutropenia in the dogs in this study were associated with vector-borne disease; however, this study was performed in an area where dogs are commonly exposed to ticks, serological evidence of exposure to vector-borne disease agents is relatively common, even in healthy dogs,[29] and a positive antibody test would not be regarded as diagnostic for vector-borne disease. Finally, excluding dogs that tested positive for vector-borne disease agents would have yielded a study group that was not representative of dogs that are typically treated for ITP, and would have precluded the evaluation of vector-borne disease agent test results as potential risk factors for the development of neutropenia.

Vincristine treatment in dogs with ITP, in combination with prednisone, is associated with more rapid increases in platelet counts and shorter duration of hospitalization compared to the use of prednisone alone.[5] In light of these advantages of vincristine treatment, and given the relatively low incidence of severe or life threatening neutropenia (grade 3 or 4) and lack of demonstrable effects on survival in our study group, there is little evidence to discourage the use of vincristine for the treatment of ITP in dogs. However, close monitoring for neutropenia with serial CBCs in the days after vincristine administration should be considered. Our study demonstrated longer duration of hospitalization in dogs that developed neutropenia after administration of vincristine, so measures to reduce the risks of neutropenia should be considered. Alternative 2nd agent immunomodulatory medications or delay in the initiation of cyclosporine treatment might be prudent when using vincristine. There is little published data regarding the pharmacokinetics of vincristine in dogs; however, an elimination half-life of 23 hours was reported in a small group of healthy

Beagles.[30] Thus it might be reasonable to suggest a delay of 5 days between vincristine administration and initiation of cyclosporine in dogs with ITP. However, there are currently no pharmacokinetic data from dogs with ITP to inform this recommendation. In addition, in dogs chronically receiving cyclosporine before presentation for ITP, vincristine dose reduction should be considered.

Several limitations of this study are associated with its retrospective nature. The frequency and timing of follow-up CBCs in the dogs was variable and inconsistent between dogs. However, this lack of standardization could not have produced any false positive results; in fact, the variable timing and duration of follow-up would be more likely to underdetect the development of neutropenia. While this retrospective study is certainly less likely to determine the true incidence of neutropenia when compared to a standardized prospective study, it also cannot overestimate the incidence of this adverse effect. Similarly, accurate documentation of time to resolution of neutropenia was also inhibited by the variable duration and frequency of longer-term monitoring of CBCs. Therefore, we can only provide an estimate of the time frame in which neutropenia occurs, and an estimate of how long it takes to resolve. Finally, the retrospective nature of this study dictates that there is a lack of standardization of the additional immunosuppressive medications that were used in these dogs. However, this provided an opportunity to determine whether these additional commonly used medications were risk factors for the development of neutropenia.

Neutropenia is a known adverse effect of vincristine administration, but the prevalence in dogs being treated for ITP has not been previously described. In this study of a large group of dogs that received vincristine and other immunomodulatory medications for the treatment of clinically suspected ITP, we were able to demonstrate that cyclosporine administration was associated with an increased risk of development of neutropenia, potentially related to effects on vincristine metabolism. Other medications and disease processes not evaluated in our study might also play a role in increasing the risk of vincristine-associated toxicosis.

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
Authors declare no IACUC or other approval was needed. All dogs were treated at the North Carolina State University Veterinary

Hospital. The Hospital Board approved use of anonymous dog data for this study.

HUMAN ETHICS APPROVAL DECLARATION

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

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