Comparison of platelet count recovery with use of vincristine and prednisone or prednisone alone for treatment for severe immune-mediated thrombocytopenia in dogs

Elizabeth A. Rozanski, DVM, DACVECC, DACVIM; Mary Beth Callan, VMD, DACVIM; Dez Hughes, BVSc, DACVECC, Nancy Sanders, DVM, DACVIM, DACVECC; Urs Giger, Dr med vet, DACVIM

Objective—To evaluate the effect of prednisone alone, compared with a combination of prednisone and vincristine, on platelet counts in bleeding dogs with severe primary immune-mediated thrombocytopenia (IMT).

Design—Prospective case study.

Animals—24 dogs with severe primary IMT.

Procedure—All dogs received immunosuppressive doses of prednisone (1.5 to 2 mg/kg [0.7 to 0.9 mg/lb] of body weight, PO, q 12 h). In addition, 12 dogs received a single dose of vincristine (0.02 mg/kg [0.01 mg/lb], IV). Platelet count, transfusion requirement, and outcome were monitored. A response was defined as an increase in platelet count to ≥ 40,000/µl. Dogs in the prednisone group that failed to respond received 1 dose of vincristine on day 7.

Results—Dogs that received prednisone and vincristine had a significantly faster increase in platelet count to ≥ 40,000/µl than dogs that received prednisone alone (mean ± SD, 4.9 ± 1.1 vs 6.8 ± 4.5 days, respectively). A similarly rapid response was observed in dogs that received vincristine on day 7 after treatment with prednisone alone failed. Furthermore, duration of hospitalization was reduced in the vincristine group, compared with the prednisone group (5.4 ± 0.3 vs 7.3 ± 0.5 days, respectively). No adverse effects attributable to vincristine were observed in any dog.

Conclusions and Clinical Relevance—Administration of combined vincristine and prednisone is associated with more rapid increase in platelet numbers and shortened duration of hospitalization in dogs with IMT, compared with use of prednisone alone. Early use of vincristine seems warranted in dogs with severe primary IMT. (*J Am Vet Med Assoc* 2002; 220:477–481)

Thrombocytopenia, a common cause of surface bleeding in dogs, may result from decreased thrombopoiesis or increased destruction, consumption, or sequestration of platelets. **Immune-mediated thrombocytopenia IMT**, also referred to as **idiopathic thrombocytopenic purpura (ITP)**, may be a primary condition in which there is no apparent inciting cause for the production of antiplatelet antibodies or secondary to an antigenic stimulus such as neoplasia, drug exposure (eg, trimethoprim-sulfonamide), infectious diseases (eg, ehrlichiosis), or vaccines.[1-5] Dogs with IMT have increased antiplatelet antibodies bound to the platelet surface, and disappearance of platelet-bound antibodies has been detected after immunosuppressive treatment, along with an increase in platelet count.[6,7,a] Although there is a risk of life-threatening hemorrhage such as bleeding into the CNS or respiratory tract, many dogs with IMT have minimal bleeding in the form of petechiae and ecchymoses despite severe thrombocytopenia, with platelet counts typically < 40,000/µl and often < 15,000/µl. The prognosis for primary IMT varies from good to guarded, with an approximate 30% mortality rate in dogs during the initial hospitalization for IMT or after relapse.[1] Clinical experience suggests that as soon as platelet numbers return to ≥ 40,000 platelets/µl, the risk of bleeding becomes minimal, and the prognosis improves considerably, albeit relapses may occur.[7]

Ideally, treatment for IMT would rapidly and reliably increase platelet counts, with minimal adverse effects and costs. Because a platelet count < 40,000 platelets/µl increases morbidity and mortality rates, it is desirable to shorten the duration of severe thrombocytopenia. Unfortunately, platelet transfusions (platelet-rich plasma, platelet concentrate, or fresh whole blood) may not be effective in increasing platelet counts, because platelets may be rapidly destroyed within minutes to hours of transfusion. However, in thrombocytopenic patients with severe, uncontrolled, or life-threatening bleeding (eg, pulmonary, CNS), platelet transfusions are indicated and may provide short-term hemostasis despite the lack of a measurable increase in platelet count assessed 1 and 24 hours after transfusion.[8,9]

The mainstay of immunosuppressive therapy for IMT is the administration of glucocorticoids that impair the immune system's ability to remove antibody-coated platelets from the circulation and that decrease antiplatelet antibody production.[1-7,a] A variety of other drugs have also been suggested for use in com-

From the Department of Clinical Studies, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104-6010 (Rozanski, Callan, Hughes, Sanders, Giger); and the Department of Clinical Sciences, School of Veterinary Medicine, Tufts University, North Grafton, MA 01536 (Rozanski). Dr. Hughes' present address is the Royal Veterinary College, University of London, London, England. Dr. Sanders' present address is Veterinary Referral Centre, 48 Notch Rd, Little Falls, NJ 07424.

Supported in part by a Research Resource Grant from the Department of Clinical Studies-Philadelphia, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pa.

Address correspondence to Dr. Giger.

bination with prednisone for IMT, including vincristine, danazol, cyclophosphamide, azathioprine, cyclosporine, and human intravenous immunoglobulin, although their efficacy has not been documented in clinical trials in dogs.[1-5]

In humans with IMT, vincristine is occasionally administered concomitantly with corticosteroids in the management of this disorder.[10,11] Vincristine is thought to increase platelet counts by several mechanisms.[1,10-14] In healthy dogs, vincristine appears to stimulate thrombopoiesis.[12,14] However, in dogs with IMT, thrombopoiesis is thought to be already maximally stimulated, because giant circulating platelets and increased megakaryocytes within bone marrow aspirates are detected. Other proposed mechanisms of action include accelerated fragmentation of megakaryocytes and impaired platelet destruction.[10,12-14] Vincristine may reduce the degree of phagocytosis by the mononuclear-phagocyte system, decrease synthesis of platelet antibodies, and interfere with antibody binding to platelets.[10,12-14] Some clinicians hesitate to use vincristine early in the course of IMT and reserve its use for "refractory" cases because of a lack of proven efficacy and potential drug toxicity.[1] Adverse effects associated with use of vincristine in dogs are uncommon but include vomiting, diarrhea, perivascular sloughing with extravasation, and, rarely, peripheral neuropathy.[14,15] Limited retrospective surveys do not permit conclusions regarding the efficacy and safety of vincristine in dogs with IMT, and the authors are not aware of any prospective studies evaluating different treatments for IMT in dogs.[3,4,13]

The goal of the clinical trial reported here was to evaluate the effect of daily prednisone alone, compared with a combination of a single dose of vincristine and daily prednisone, on platelet counts in dogs with severe IMT.

Materials and Methods

Dogs—Twenty-four dogs with primary IMT referred to the **Veterinary Hospital of the University of Pennsylvania (VHUP)** between 1995 and 1999 or the **Foster Hospital for Small Animals (FHSA)** at Tufts University between 1996 and 1999 were included. Dogs that had received prior treatment with corticosteroids for > 24 hours or any other immunosuppressive drugs, as well as dogs with relapsing IMT, were excluded. The protocols used in this study were approved by each university's animal care and use committee, and owner consent was obtained.

Diagnosis of IMT—Only dogs with platelet counts < 15,000/µl and no evidence of any underlying disease, determined on the basis of physical examination, CBC, and serum chemistry analyses were included. Serologic tests for infectious disease (*Ehrlichia canis, E equi, Babesia canis*, *Rickettsia rickettsii*), diagnostic imaging (thoracic radiography, abdominal ultrasonography), and bone marrow aspiration or biopsy were performed at the discretion of the primary clinician. Dogs with identifiable underlying diseases or drug exposure that potentially caused IMT were excluded.

Platelet counts—Platelet counts were determined in EDTA-anticoagulated blood by use of automated cell counters.[b] Platelet counts were performed in the clinical pathology laboratories at VHUP and FHSA by laboratory personnel unaware of the treatment group of the individual dogs. Platelet counts < 30,000/µl were confirmed by manually counting platelets with a hemocytometer. Blood smears were evaluated for a platelet estimate and additional confirmation of thrombocytopenia or evidence of platelet clumping.

Treatment—Dogs were allocated into 2 treatment groups: prednisone alone or prednisone and vincristine, on the basis of the primary clinician's preference. Primary clinicians included internal medicine and emergency and critical care residents and board-certified faculty and staff members in either or both colleges. All dogs were treated with prednisone[c] (1.5 to 2 mg/kg [0.7 to 0.9 mg/lb] of body weight, PO or SC, q 12 h). Dogs in the prednisone group with platelet counts < 40,000/µl after 7 days also received vincristine. A single dose of vincristine[d] (0.02 mg/kg [0.01 mg/lb]) was administered to dogs via an IV catheter that was carefully flushed with saline (0.9% NaCl) solution before and after infusion in order to ensure proper placement and to prevent any extravasation of vincristine. Treatment with prednisone or prednisone and vincristine was begun the first day of hospitalization after laboratory results confirmed severe thrombocytopenia. Independent of treatment group, doxycycline[e] (10 mg/kg [4.5 mg/lb], PO, q 24 h) was administered until tick-borne diseases were excluded, judged on the basis of negative results of serologic tests or attending clinician preference, but not before 1 week of treatment. No other immunosuppressive agents were allowed, but supportive care, including stored **packed red blood cell (PRBC)** transfusions and crystalloid fluids, was provided as needed to correct anemia and dehydration, respectively. One transfusion was defined as approximately 10 ml of PRBC/kg (4.5 ml of PRBC/lb). Use of synthetic colloid solutions was discouraged as they could potentially exacerbate bleeding. All dogs were monitored for clinical evidence of cessation in bleeding and for changes in PCV and platelet count. A response was defined as an increase in platelet count from < 15,000 to ≥ 40,000/µl. In addition, dogs were monitored for bleeding and adverse effects of drugs.

Statistical analyses—Platelet counts, including the lowest recorded platelet count, number of PRBC transfusions, days to a platelet count ≥ 40,000/µl, and duration of hospital stay were recorded. Treatment groups were compared by use of a Student *t*-test for normally distributed data and a Mann-Whitney rank sum test for data with nonnormal distribution. A value of $P < 0.05$ was considered significant.

Results

Dogs—Twenty-four dogs fulfilled the entry criteria and were enrolled in the study. Half the dogs received prednisone, and half received prednisone and vincristine. Four dogs in the prednisone group also received vincristine after 7 days, because their platelet count had not reached 40,000/µl. There were 9 mixed-breed dogs and 15 purebreds, with 1 to 3 dogs/breed. All 15 female dogs were spayed, whereas 7 of 9 male dogs were neutered. Mean age was 6.8 years, with a range of 1 to 17 years. No significant differences were observed between groups with respect to breed and age distributions. All dogs had petechiae and ecchymoses on physical examination, and some had other evidence of epithelial bleeding (epistaxis, melena). No dogs had signs of intracranial, urogenital, or pulmonary bleeding.

Platelet count and response to treatment—The lowest pretreatment platelet count ranged from 1,000

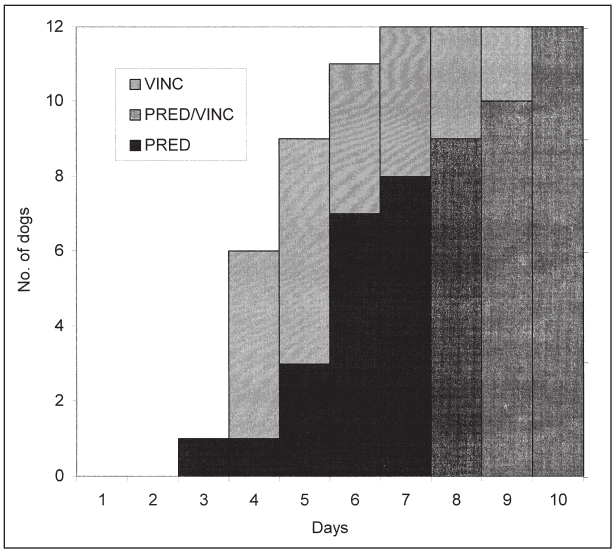

Figure 1—Cumulative No. of dogs that responded to treatment (ie, platelet count > 40,000/µl) with prednisone alone (n = 12), prednisone and vincristine (VINC; 12), or initial treatment with prednisone followed by treatment with vincristine on day 7 because of poor response (PRED/VINC; 4) during each day of treatment.

to 14,000/µl, with a mean of 6,000 platelets/µl, and was not significantly different between groups. All dogs' platelet counts increased in response to treatment (**Fig 1**). Overall lag time from the start of treatment until platelet count increased to ≥ 40,000 /µl ranged from 3 to 10 days; the time span did not correlate with the initial platelet count. The prednisone and vincristine group's platelet count increased sooner than that of the prednisone group ($P = 0.018$). The range, median, and mean (± SD) values for duration for response in the prednisone group were 3 to 10, 6.5, and 6.8 ± 4.5 days, respectively; for the prednisone and vincristine group, these values were 3 to 7, 4.5, and 4.9 ± 1.1 days, respectively. All dogs in the prednisone and vincristine group responded by day 7, whereas 4 dogs in the prednisone group failed to respond to prednisone alone by 7 days and were also treated with vincristine on day 7. After vincristine administration, platelet counts in these 4 dogs rapidly increased from a mean of 14,500 to ≥ 40,000/µl, with a mean of 105,000/µl and range of 51,000 to 135,000/µl within 1 to 3 days, respectively. Dogs were discharged from the hospital when the platelet count had exceeded 40,000 platelets/µl, and the dogs were doing well clinically. The dogs in the prednisone group stayed in the hospital a mean of 7.3 ± 0.5 days, whereas the dogs in the prednisone and vincristine group had a significantly shorter duration of hospitalization at 5.4 ± 0.3 days ($P = 0.02$).

Transfusion requirements—Epistaxis, subcutaneous bleeding, and gastrointestinal tract hemorrhage were the major sources of blood loss in dogs that required PRBC transfusions. Blood transfusions were not administered in 2 of 12 dogs of the prednisone group and 4 of 12 dogs in the prednisone and vincristine group. The range, median, and mean (± SD) number of transfusions (equivalent to 10 ml/kg) administered was 0 to 5, 1, and 1.4 ± 1.4, respectively, in the prednisone group, and 0 to 3, 1, and 0.8 ± 0.8, respectively, in the prednisone and vincristine group. Thus, dogs treated with prednisone and vincristine received fewer PRBC transfusions than dogs in the prednisone group did, although the difference was not significant ($P = 0.33$).

Adverse effects—All dogs eventually developed expected signs of hyperadrenocorticism, including polyuria or polydipsia and muscle weakness, in response to prednisone treatment. Gastrointestinal tract disturbance, thrombophlebitis, evidence of increased bleeding, and peripheral neuropathy were not observed in any dog that received vincristine.

Outcome—All dogs survived to hospital discharge except for 1 dog that developed respiratory distress on day 9, despite a platelet count of 118,000/µl, and was euthanized the next day. In addition, 1 dog died suddenly at home 2 days after discharge, and 1 dog was euthanized by the referring veterinarian 1 week after discharge (platelet count within reference range) because of severe polyuria and polydipsia. Permission for necropsy was not received from the owners of these dogs. Long-term follow-up was not available for the other dogs.

Discussion

Definitive diagnosis of primary IMT (ITP) may be challenging, as no single test is available to confirm or refute the diagnosis. In the dogs of this study, the diagnosis of primary IMT was made on the basis of finding a very low platelet count (< 15,000 platelets/µl) without signs of any other diseases, despite comprehensive clinical and laboratory evaluations. Antiplatelet antibody tests[6,7,a] can further support an immune basis for thrombocytopenia but were not available for the dogs in our study. A response to treatment with immunosuppressive agents is often used in support of a diagnosis of IMT. Glucocorticoids such as prednisone are generally used in the treatment of IMT in dogs and humans.[1-5,10,11] In addition, a single dose of vincristine (0.02 mg/kg, IV) has been recommended for treatment of refractory or severe IMT in dogs.[1,13,14] The results of the limited prospective study reported here indicate that the addition of vincristine to prednisone in the treatment of primary IMT is well-tolerated and is associated with a rapid increase in platelet counts in dogs with severe thrombocytopenia as an initial treatment regime and when vincristine was added after 1 week of prednisone treatment.

Experimental studies in various species have documented the beneficial effects of glucocorticoids in treatment of IMT. It is also generally accepted in clinical practice that prednisone blunts the immune destruction of platelets in humans and dogs with IMT.[1-5,10,11] However, clinical trials assessing the efficacy of prednisone in dogs with IMT have not been reported. In our study, 8 of 12 dogs with severe IMT responded to prednisone within a week, whereas the remaining 4 also reached a platelet count of ≥ 40,000/µl within the second week,

albeit with the addition of vincristine. This platelet response to immunosuppressive doses of prednisone appears to be what is generally expected.

The ability of vincristine to increase the platelet count has been studied experimentally in dogs and has been suggested in dogs with primary IMT in clinical practice.[1,12,13] Because it would be unethical to withhold prednisone to study the effects of another immunosuppressive agent, vincristine was given in addition to prednisone. Thus, it remains unknown whether the effects observed with vincristine and prednisone would have been observed with vincristine alone. All 12 dogs in the prednisone and vincristine group responded with a platelet increase to ≥ 40,000/µl within 1 week. The platelet count recovery time in the vincristine group was significantly shorter. The mechanism for the increase in platelet number in response to vincristine remains unknown in dogs with IMT but may be associated with accelerated platelet production attributable to megakaryocytic fragmentation and impaired destruction of platelets. The mechanism of the platelet response to vincristine in the dogs of this study was not evaluated.

There are some concerns regarding the function of platelets produced in response to vincristine.[12-14] Results of 1 study suggest that in vivo platelet function is not altered by vincristine in clinically normal dogs, whereas another study revealed abnormal in vitro platelet function in dogs with lymphoma treated with vincristine.[12,16] The effect of vincristine on platelet function in dogs with IMT has not been studied because of the inherent difficulties in performing platelet aggregation or other function studies in thrombocytopenic patients (it is difficult to obtain sufficient numbers of platelets for evaluation). However, after an increase in platelet numbers to ≥ 40,000 platelets/µl, no dogs treated with vincristine had any evidence of active hemorrhage, suggesting adequate platelet function and restoration of primary hemostasis. Additionally, an immediate increase in bleeding after vincristine administration, which may have suggested a possible thrombocytopathy, was not detected in any dog.

Limitations of this study included small sample size, lack of randomization, variable diagnostic evaluations, and lack of blinding as to treatment group. The dogs were allocated into groups at the discretion of the primary clinician. This option was chosen to enroll the greatest number of dogs possible. Earlier observations in our hospitals revealed strong personal opinions among various clinicians about the appropriate treatment for IMT, without any prior clinical trial evidence. Finally, despite lack of blinding the clinician to treatment group, the drug selection criterion did not appear to have added any bias to the study, as the groups at study entry were similar, and the end point was an objective value (platelet count) measured by the clinical laboratory.

The transfusion needs of dogs with IMT have not been previously reported. The PRBC transfusion requirements among individual dogs with IMT varied substantially in this study, despite similarly low platelet numbers (< 15,000 platelets/µl). As observed previously, the most common sites of severe hemorrhage were the gastrointestinal tract and nasal cavity, although some dogs additionally appeared to have large losses subcutaneously, as judged by ecchymoses and bruising as well as blood-loss anemia.[4] Dogs that received a single dose of vincristine IV in addition to prednisone had fewer blood transfusions than dogs that did not receive vincristine, although the difference was not significant. This difference was likely associated with the duration of severe thrombocytopenia rather than any other specific effect of vincristine or case selection.

The results of this study may have clinical importance for several reasons. Morbidity rate, mortality rate, and expense of medical care are dependent on the duration of severe thrombocytopenia and are associated with extent of bleeding and duration of hospitalization. The longer an individual dog is thrombocytopenic, the greater the risk of either life-threatening hemorrhage or anemia requiring transfusion. The availability of blood products is limited, and transfusions harbor an inherent risk for transfusion reactions and are associated with additional cost. Administration of vincristine shortened hospitalization, because all dogs (except 1) were discharged when their platelet counts exceeded 40,000/µl. Additionally, dogs that did not respond to prednisone appeared to readily respond to vincristine, although it remains unknown whether these dogs would have responded without vincristine. No associated systemic or local adverse effects attributable to the vincristine administration were observed, but careful attention was given to injection technique to avoid perivascular administration. Furthermore, the degree of immunosuppression and subsequent predisposition to infection with vincristine appeared minor; thus, its clinical use in dogs appears to be simple and safe. Cost containment in health care is also important. The nominal cost of vincristine ($7.66/20-kg [44-lb] dog[f]) is well outweighed by a reduced risk for life-threatening hemorrhage, a shorter hospital stay, and decreased number of transfusions.

[a]Kohn B, Engelbrecht R, Giger U, et al. Platelet-bound antibodies in dogs with thrombocytopenia and change with treatment (abstr). *J Vet Intern Med* 2000;14:361a.
[b]Cell Dyne 3500 System, Abbott Diagnostics, Abbott Park, Ill.
[c]Prednisone, Schein Pharmaceutical Inc, Florham Park, NJ.
[d]Vincristine, Faulding Pharmaceutical Co, Elizabeth, NJ.
[e]Doxycycline, Schein Pharmaceutical Inc, Florham Park, NJ.
[f]Tufts Pharmacy, North Grafton, Mass.

References

1. Lewis DC, Meyers KM. Canine idiopathic thrombocytopenic purpura. *J Vet Intern Med* 1996;10:207–218.
2. Northern J Jr, Tvedten HW. Diagnosis of microthrombocytosis and immune-mediated thrombocytopenia in dogs with thrombocytopenia: 68 cases (1987–1989). *J Am Vet Med Assoc* 1992;200:368–372.
3. Thomason KJ, Feldman BF. Immune-mediated thrombocytopenia: diagnosis and treatment. *Compend Contin Educ Pract Vet* 1985;7:569–576.
4. Williams DA, Maggio-Price L. Canine idiopathic thrombocytopenia: clinical observations and long-term follow-up in 54 cases. *J Am Vet Med Assoc* 1984;185:660–663.
5. Mackin A. Canine immune-mediated thrombocytopenia—part II. *Compend Contin Educ Pract Vet* 1995;17:515–535.

6. Lewis DC, Meyers KM, Callan MB, et al. Detection of platelet-bound and serum platelet-bindable antibodies for diagnosis of idiopathic thrombocytopenia purpura in dogs. *J Am Vet Med Assoc* 1995;206:47–52.

7. Kohn B, Engelbrecht R, Leibold W, et al. Klinische Befunde, Diagnostik und Behandlungserfolge bei der primären und sekundären immunbedingten Thrombozytopenie beim Hund. *Kleintierpraxis* 2000;45:893–907.

8. Levine SP. Thrombocytopenia caused by immunologic platelet destruction. In: Lee GR, Foerster J, Lukens J, et al, eds. *Wintrobe's clinical hematology.* 10th ed. Baltimore: The Williams & Wilkins Co, 1999:1583–1611.

9. Zucker MB, Lundberg A. Platelet transfusions. *Anesthesiology* 1966;27:385–398.

10. Ferrara F, Copia C, Annanziata M, et al. Vincristine as salvage treatment for refractory thrombotic thrombocytopenic purpura. *Ann Hematol* 1999;78:521–523.

11. Chamouni P, Lenain P, Buchonnet G, et al. Difficulties in the management of an incomplete form of refractory thrombotic thrombocytopenic purpura, the usefulness of vincristine. *Transfus Sci* 2000;23:101–106.

12. Mackin AJ, Allen DG, Johnstone IB. Effects of vincristine and prednisone on platelet numbers and function in clinically normal dogs. *Am J Vet Res* 1995;56:100–108.

13. Greene CE, Scoggin J, Thomas JE, et al. Vincristine in the treatment of thrombocytopenia in five dogs. *J Am Vet Med Assoc* 1982;180:140–143.

14. Golden DL, Langston VC. The uses of vincristine and vinblastine in dogs and cats. *J Am Vet Med Assoc* 1988;93:1114–1117.

15. Wohl JS, Cotter SM. Approach to complications of anticancer therapy in emergency practice. *J Vet Emerg Crit Care* 1995;5:61–76.

16. Grau-Bassas ER, Kociba GJ, Couto CG. Vincristine impairs platelet aggregation in dogs with lymphoma. *J Vet Intern Med* 2000;14:81–85.