Vinblastine/prednisolone chemotherapy leads to hematological toxicity in dogs with high-grade or metastatic mast cell tumors

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OBJECTIVE
To determine the myelosuppressive effects/hematological toxicities, other general toxicities, and when these occur during vinblastine/prednisolone chemotherapy in dogs bearing high-grade or metastatic cutaneous/subcutaneous mast cell tumors (MCTs).

METHODS
Medical records were retrospectively reviewed between November 1, 2016, and March 1, 2023. Thirty client-owned dogs with histopathologically confirmed cutaneous high-grade MCTs/metastatic subcutaneous MCTs and that subsequently completed a 12-week vinblastine/prednisolone chemotherapy protocol were included. Hematology was assessed before commencing chemotherapy and before each vinblastine treatment. The effect of each treatment upon hematological values was evaluated. Measured outcomes included the type, frequency, and severity of hematological and other more general toxicities.

RESULTS
24 of 30 dogs experienced at least 1 hematological toxicity, 6 experienced gastrointestinal toxicity, and 4 experienced lethargy. The most common toxicity was anemia (15/30 [50%]), with 93.3% (14/15 dogs) classified as Veterinary Cooperative Oncology Group–Common Terminology Criteria for Adverse Events grade I and 6.6% (1/15) classified as grade II. The second most common toxicity was neutropenia (14/30 [46.6%]), with 71.4% (10/14) classified as grade I and 28.6% (4/14) as grade III. The least common hematological toxicity was thrombocytopenia (4/30 [13%]), all grade I. Neutropenia mainly occurred during weeks 2 and 3; however, there was no significant decrease in neutrophil count relative to baseline. Neutrophil count increased and Hct decreased during weeks 6 to 12 of treatment when compared to baseline. No change in platelet count was observed.

CLINICAL RELEVANCE
Vinblastine/prednisolone chemotherapy leads to hematological toxicity; however, this was mostly low-grade and did not require major intervention. Vinblastine/prednisolone chemotherapy is well tolerated in dogs bearing high-grade or metastatic MCTs.

Keywords: toxicity, vinblastine, chemotherapy, mast cell tumor, dog

Vinblastine is a vinca alkaloid used in veterinary medicine for the treatment of canine mast cell tumors (MCTs). A conventional chemotherapy protocol for treating high-grade or metastatic canine MCTs consists of 8 vinblastine doses in combination with daily oral prednisolone therapy over a 12-week period. Vinblastine is administered by IV infusion at a dosage of 2 mg/m², once weekly for the first 4 weeks and then every 2 weeks for the final 8 weeks. Prednisolone is administered at a dosage of 1 mg/kg PO once daily and is tapered to a complete stop by the end of week 12.1-7 High-risk MCTs encompass high-grade or metastatic MCTs, which usually carry a worse prognosis; therefore, vinblastine/prednisolone (VP) chemotherapy is often used in conjunction with surgical tumor excision and/or radiotherapy (RT) in order to improve survival in patients with MCTs considered to be high-risk. When combined with surgical excision, VP chemotherapy has demonstrated improved survival rates in high-grade MCT patients compared to surgery alone.8

Vinblastine toxicity is the result of nonspecific destruction of rapidly dividing cells, through binding to tubulin, which inhibits microtubule polymerization essential to mitosis.2 In veterinary medicine, the recognized
dose-limiting toxicity of vinblastine is neutropenia, although other toxicities including gastrointestinal upset have also been reported. The neutrophil nadir is variable, with a reported time range of approximately 5 to 10 days after administration.

One study investigated the efficacy of the standard VP chemotherapy protocol for surgically excised grade III canine cutaneous MCTs. Comparable with reported rates, adverse events were recorded following 5.6% (6/108) of vinblastine doses and in 28.6% (4/14) of dogs treated. This degree of tolerance was suggested to be a result of the low dosages the standard VP chemotherapy protocol utilizes alongside the prophylactic use of gastroprotectants. Episodes of neutropenia were reported in 2 out of 14 (14%) dogs. Two further dogs experienced gastrointestinal adverse effects, one of which was Veterinary Cooperative Oncology Group–Common Terminology Criteria for Adverse Events (VCOG-CTCAE v2) grade I diarrhea, while the other exhibited VCOG grade III colitis (likely resulting from exacerbation from a long history of chronic colitis). While the findings corroborated similar rates of gastrointestinal toxicity, the number of neutropenic cases were relatively low compared to other studies, the study was restricted to a smaller population of dogs and did not compare hematological values over time relative to baseline.

A more recent study investigated the frequency of hematological adverse events in dogs treated with VP chemotherapy combined with RT in comparison to VP chemotherapy alone. Concurrent RT did not increase the risk of neutropenia, which occurred in 18.6% (8/43) and 23.3% (10/43) of dogs in the RT and control groups, respectively. In line with previous studies, neutropenia was the most common hematological toxicity and was usually mild. Thrombocytopenia and anemia were relatively uncommon, with only 1 dog experiencing a VCOG grade I anemia. The frequency of gastrointestinal and other common types of adverse events was not reported.

Thus, while myelosuppressive effects of vinblastine are reported, the frequency, severity, and degree of myelosuppression over time, along with other hematological and more general toxicities, have not been fully investigated in a population of dogs with high-grade or metastatic MCTs undergoing conventional VP chemotherapy. The aim of this study was to investigate the myelosuppressive effects, hematological toxicities, and other general toxicities of VP chemotherapy in dogs bearing high-grade or metastatic cutaneous/subcutaneous MCTs over time.

**Methods**

The medical database of the Queen’s Veterinary School Hospital, University of Cambridge, was searched retrospectively for dogs with MCTs treated between November 1, 2016, and March 1, 2023. Dogs with histopathologically confirmed cutaneous grade III/high-grade MCTs or metastatic subcutaneous MCTs and that underwent surgical excision of the MCT and/or lymph node extirpation and subsequently completed a 12-week VP chemotherapy protocol were included. Regional lymph node metastasis was confirmed via cytology and/or histopathology. Dogs that did not complete the full 12-week protocol at our hospital were excluded due to incomplete data. All dogs underwent clinical staging with 3-view thoracic radiographs and abdominal ultrasound, together with fine-needle aspirates of regional lymph nodes, spleen, and liver. Cytology of lymph nodes and internal organs was considered positive for metastatic disease as described elsewhere.

Pretreatment hematology and biochemistry were also assessed. Hematology parameters were analyzed with a benchtop analyzer (XN1000V; Sysmex Corp) according to manufacturer guidelines, and all counts were verified by manual blood smear examination. Hematology was assessed before commencing chemotherapy (week 1) and subsequently before each vinblastine treatment on weeks 2, 3, 4, 6, 8, 10, and 12. The effect of each treatment upon hematological values was evaluated by use of the results of CBCs from weeks 2 to 10. Weeks 6, 8, 10, and 12 were assessed rather than weeks 5, 7, 9, and 11 because there was no chemotherapy administration as per the protocol. Our laboratory reference intervals were as follows: neutrophil count (3 X 10^9 to 11.5 X 10^9/L), Hct (0.37 to 0.55 L/L), reticulocyte count (0 X 10^9 to 70 X 10^9/L), and platelet count (175 X 10^9 to 500 X 10^9/L). All patients that commenced chemotherapy had normal ALT and AST values prior to administration (due to the potential impact of liver dysfunction on vinblastine toxicity).

**Vinblastine/prednisolone chemotherapy treatment**

Vinblastine was administered as an IV bolus injection at 2 mg/m^2 once weekly for 4 weeks, then every 2 weeks for an additional 4 treatments as previously described. A dose of 1 mg of prednisolone/kg PO daily was administered for the first 2 weeks, which was then tapered to a dose of 0.5 mg/kg every second day for 10 weeks before being discontinued. Complete blood counts were performed before each vinblastine treatment. Hematological adverse events (AEs) were graded according to the VCOG-CTCAE v2.

The primary measured outcome was the type, frequency, and severity of hematological toxicity reported during the 12-week treatment period. Secondary outcomes that were also measured included the frequency and severity of other general toxicities. Statistical analysis was performed with available software (Prism 5.0 for Windows; GraphPad Software Inc). Comparisons of neutrophil count, Hct, and platelet counts at the different time points were made with the Friedman test with post hoc testing of individual time points performed using the Dunn multiple comparisons test. P values < .05 were considered statistically significant.

**Results**

**Population characteristics and clinical information**

In total, 44 dogs received VP chemotherapy at our hospital during the study period. Fourteen dogs...
were excluded due to incomplete data sets (6 dogs continued their protocol at their primary care practice, 3 had evidence of progressive disease, 1 had already received 4 doses of VP chemotherapy prior to arrival, and 4 were lost to follow-up during the protocol). Thirty dogs treated with VP chemotherapy were then included. The median age at diagnosis was 7 years (range, 2 to 12 years), and the median weight was 27.8 kg (range, 9.3 to 51.6 kg). There were 13 spayed females, 16 castrated males, and 1 intact male. The most common breeds included were Labrador Retriever (n = 7), followed by Boxer (4), Staffordshire Bull Terrier (2), Pug (2), Nova Scotia Duck Tolling Retriever (2), crossbreed (2), Golden Retriever (2), and Jack Russell Terrier (2). One each of Bernese Mountain Dog, Saluki, Italian Greyhound, Hungarian Vizsla, French Bulldog, Old English Sheepdog, and Springer Spaniel were also included. All dogs had high-grade or metastatic MCTs; 26 had cutaneous MCTs, and 4 had subcutaneous MCTs with evidence of regional lymph node metastasis.

Chemotherapy protocol
The vinblastine dose used was 2 mg/m². In 4 cases, the chemotherapy treatment was reduced to 1.8 mg/m² during the protocol because of a documented VCOG grade III neutropenia. The dose of prednisolone in the first 2 weeks of chemotherapy was 1 mg/kg once daily. This was then reduced to 0.5 mg/kg every other day and discontinued at week 12.

Hematological toxicity trends
Twenty-five (83.3%) dogs experienced at least 1 VCOG-CTCAE v2 event during the 12-week treatment protocol. Twenty-four of 30 (80%) experienced at least 1 hematological toxicity, 6 of 30 (20%) experienced gastrointestinal toxicity, and 4 of 30 (13.3%) experienced lethargy. The most common hematological toxicity was anemia (15/30 [50%]), with the majority being classified as VCOG grade I (14/15 [93.3%]) and the remainder VCOG grade II (1/15 [6.6%]). Three of these dogs with anemia had an elevated reticulocyte count (median, 179 X 10⁹/L; range 85 X 10⁹ to 286 X 10⁹/L), with the remainder classified as nonregenerative. The second most common was neutropenia (14/30 [46.6%]), with the majority being classified as VCOG grade I (10/14 [71.4%]) and the remainder classified as VCOG grade III (4/14 [28.6%]). Three of 4 (75%) of the grade III neutropenias occurred in week 2, with the remainder in week 3. The least common hematological toxicity was thrombocytopenia (4/30 [13%]), all classified as VCOG grade I.

The Hct was significantly decreased in weeks 6, 8, 10, and 12 compared to week 1 (Figure 1); however, there were no significant differences between the other time points. The highest percentage of dogs with anemia occurred during weeks 8 and 10 at 20% (6/30) and 23.3% (7/30), respectively. The neutrophil count was significantly increased in weeks 6, 8, 10, and 12 compared to weeks 1, 2, and 3, and in weeks 8 and 10 compared to week 4; however, no significant differences were noted between the other time points (Figure 2).

The highest percentage of dogs with neutropenia occurred during weeks 2 and 3 at 33.3% (10/30) and 16.6% (5/30), respectively.

Platelet count did not change significantly during the course of chemotherapy (Figure 3), with the highest percentage of dogs presenting with thrombocytopenia occurring during weeks 2 and 3 at 3.3% (1/30) and 6.6% (2/30), respectively.
Clinical signs secondary to histamine production and gastrointestinal ulceration, which can lead to hemorrhage. Given that there was no reported hematemesis or melena, overt gastrointestinal ulceration and hemorrhage is considered unlikely; however, subclinical gastrointestinal ulceration (either secondary to mast cell neoplasia and/or prednisolone administration) could reflect the degree of anemia observed. In addition, it is possible a hostile vascular environment secondary to neoplasia could have increased RBC fragility leading to mild hemolysis and the regenerative anemia seen in 3 dogs. Lastly, the slower turnover rate of RBCs compared to neutrophils and platelets, together with the significantly later onset of anemia, could reflect chemotherapy-induced suppression of erythropoiesis.

Neutropenia was the second most common toxicity observed, affecting 46.6% patients treated with VP chemotherapy which is a higher prevalence than previously reported. Neutropenia most commonly occurred in week 2, which is consistent with the expected nadir from previous reports. Interestingly, the neutrophil count significantly increased after week 6 (compared to weeks 1 to 3), which may be due to a rebound effect in neutrophil production following the dose-intensive phase of chemotherapy. The effects of stress as well as prednisolone administration may also have contributed to neutrophilia. While serious toxicities pose obvious threats to the health and welfare of canine MCT patients, myelosuppression is suggested to be predictive of a positive tumor response and hence a mild neutropenia may actually be a desirable sign during chemotherapy.

Thrombocytopenic events were rare and sporadic, which is consistent among previous studies, except for a small study that found thrombocytopenia to be the most common toxicity, affecting 75% of dogs. There was no significant change in platelet count noted in the present study, which is consistent with this chemotherapy protocol having a minimal effect on platelet production.

More general toxicities were largely mild and self-limiting, consisting of mild gastrointestinal upset and lethargy. These toxicities did not influence the treatment regime and were sporadic in nature, which can positively influence an owner’s perception on VP chemotherapy.

One major limitation of this study related to the pharmacokinetic profile of vinblastine. The VP chemotherapy protocol uses body surface area to determine vinblastine dosage, which poorly correlates with the pharmacokinetics and pharmacodynamics of this drug in the dog. The pharmacological behavior and toxicity of vinblastine is more dependent on individual metabolism and genetics. Contributing factors to this may include prior or current medications, hepatic function, and breed-specific MDR-1/ABCB1-1Δ polymorphisms, although veterinary research is lacking in this area. Another limitation of this study was that no dogs were screened for ABCB1-1Δ polymorphisms, nor was hepatic function assessed beyond a routine biochemistry profile. As the ABCB1-1Δ polymorphism mutation can be found in various breeds,
including crossbreeds,\textsuperscript{23} this could have influenced the toxicity profile seen\textsuperscript{24,25} and could explain the multiple neutropenic events identified in 4 dogs. Lastly, given the neutrophil nadir is variable and reported to be within 5 to 10 days,\textsuperscript{9-11} it is possible that blood collected at only a single time point on day 7 could have missed the true nadir and influenced the degree of neutropenia observed. Given the retrospective nature of this study, in addition to time and financial constraints related to veterinary medicine, it is often impractical to collect blood at each time point during the nadir. Nonetheless, this could be employed for any future prospective studies.

This study supports that maximum myelosuppression is likely to occur between weeks 1 and 3 of treatment. Increasing blood sampling frequency between weeks 1 and 3 may determine the exact time point of maximum myelosuppression when intervention may be indicated. In addition, clinicians and owners may elect to monitor dogs more closely during these time points.

In conclusion, our study revealed that VP chemotherapy leads to hematological toxicity; however, this was typically low-grade and did not require major intervention in most cases. Anemia was the most common toxicity noted, followed by neutropenia, whereas thrombocytopenia was uncommon. All other general toxicities were mild and self-limiting. There was a statistically significant decline in Hct and increase in neutrophil count during the treatment period, although neither are likely to be clinically significant. These findings can help influence clinician and owner perceptions around this chemotherapy protocol given its success as an adjuvant treatment and the relatively low-grade toxicities noted.

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