Evaluation of a manual technique for detection of neutropenia and thrombocytopenia in dogs receiving chemotherapy

Kenneth M. Wyatt, BVMS, and Gemma L. Wyatt, BSc

Objective—To determine accuracy of a manual technique for detection of neutropenia and thrombocytopenia in dogs receiving chemotherapy.

Design—Masked prospective study.

Animals—11 dogs treated with chemotherapy for neoplasia.

Procedure—124 blood samples from dogs being treated with chemotherapy for various neoplasms were processed through an automated cell counter, and results were compared with those obtained by use of a rapid manual technique for estimating neutrophil and platelet concentrations to determine whether the manual technique could accurately detect dogs with neutropenia or thrombocytopenia.

Results—By use of automated techniques, neutropenia (<3,000 cells/µl) was detected in 17 of 124 blood samples, and thrombocytopenia (<100,000 platelets/µl) was detected in 3 of 124 blood samples. The manual technique correctly identified 16 of 17 (94%) blood samples with neutropenia, with a specificity of 92% (88/107). The manual technique correctly identified 3 of 3 (100%) blood samples with thrombocytopenia, with specificity of 94% (114/121).

Conclusions and Clinical Relevance—Manual estimates of neutrophil and platelet counts are sensitive and specific; however, a full differential cell count is still preferable. (J Am Vet Med Assoc 2002;220:1805–1806)

Dogs receiving cytotoxic medications are often at risk of developing neutropenia and thrombocytopenia. It is therefore recommended that prior to administering these drugs, blood samples should be assessed for these variables.1 Dogs with low neutrophil or platelet counts may require a delay in treatment. Failure to recognize such dogs may result in sepsis because of excessive neutropenia or hemorrhage because of thrombocytopenia. Standard practice at the author’s (KMW) clinic is to submit blood for automated determination of total WBC and platelet concentration and manual differential cell count. Practitioners outside of institutions may find it difficult to obtain rapid assessment of blood samples. Rapid manual estimates of neutrophil and platelet concentrations have been reported.2-7 These methods appear to be inferior to automated methods. However, to the authors’ knowledge, these methods have not been used to identify neutropenic and thrombocytopenic dogs by applying a cut off value, rather than attempting to calculate a neutrophil or platelet concentration. Weiss8 estimated that platelet concentrations were within reference range or abnormal on the basis of a range of platelet numbers per HPF but not with the intent of specifically identifying a group of animals with estimated concentration low enough to warrant specific clinical concern. The purpose of the study reported here was to determine accuracy of manual techniques for detection of neutropenia and thrombocytopenia in dogs receiving chemotherapy.

Materials and Methods

Blood smears from 11 consecutive canine cancer patients from 1 author’s (KMW) practice that were receiving chemotherapy from July 1998 until January 1999 were included in the study. A medical scientist with 5 years of experience in veterinary hematology (GLW) assessed all smears without knowledge of any information regarding the dogs, including previous hematology results and any medications used. Clinical decisions regarding each dog were made on the basis of results of a CBC and differential cell count, rather than results of the manual method of this report.

For comparison with results of the manual technique, hematology results were obtained by use of an automated cell counter,7 which provided the total WBC, hematocrit, and platelet concentrations. The mature neutrophil concentration was obtained by use of a manual differential count.

The microscope used for the manual technique had ocular lenses with a field number of 18; this number describes the size of the field of view, such that the field number divided by the degree of objective magnification equals the diameter of the field of view in millimeters.⁄ The smears were examined initially under 100X magnification to select a monolayer field. Under low power (200X magnification), mature neutrophils in 10 fields were counted. At this degree of magnification, the diameter of the field of view is 0.9 mm. Dogs from which smears had <100 mature neutrophils/10 low-power fields were considered neutropenic. Dogs from which smears had >100 mature neutrophils/10 low-power fields were considered to have an adequate neutrophil concentration. Under 1,000X magnification, with a field of view of 0.18 mm, platelets were counted in 10 fields. Dogs from which smear had <100 platelets/10 HPF were considered thrombocytopenic, whereas dogs from which smear had >100 platelets/10 HPF were considered to have an adequate platelet concentration that would allow cytotoxic drugs to be administered.

The cut off values of 100 neutrophils or platelets per 10 low or HPF, respectively, were determined by evaluating 10 smears with known mature neutrophil concentrations between 2,000 and 3,000 cells/µl and 10 smears with known

From the Murdoch Animal Cancer Care Unit, Murdoch University Veterinary Hospital, Department of Veterinary Clinical Science, Murdoch University, Perth, Australia 6150 (K. Wyatt); and St John of God Pathology, Hollywood Hospital, Monash Ave, Nedlands, Australia 6009 (G. Wyatt)

Results of this study were presented at the Australian College of Veterinary Scientists Conference in Sydney, Australia, Jul 8-10, 1999.

The authors thank Drs. Mary McConnell, Ian Robertson, and Philip Clark for technical assistance.

Address correspondence to Dr. Kenneth Wyatt.
platelet concentration between 50,000 and 100,000 platelets/µl in an unmasked fashion. For each smear, 10 fields were evaluated at their respective magnification, and the results were used to determine the cut off values used in this study. The use of 10 fields was chosen to provide a rapid test. The authors have not evaluated the accuracy of the technique when performed by use of different numbers of fields of view.

Results

The 11 dogs provided 124 blood samples for evaluation. By use of the manual estimates, 25 smears had neutrophil counts < 100 cells/10 low-power fields, and 10 smears had platelet counts < 100 platelets/10 HPF. Of the 25 samples considered neutropenic, 16 were confirmed by results of the automated and differential cell counts. One dog with neutropenia (900 cells/µl) was not detected by use of the manual method. The manual estimates therefore correctly identified 16 of 17 neutropenic dogs. Of the 25 samples considered neutropenic via the manual estimate, 9 were found to have neutrophil concentrations within the reference range by use of the automated cell count and differential cell count. These data equate to sensitivity of 94% (16/17), specificity of 92% (98/107), positive predictive value of 64%, and negative predictive value of 99%.

Among the 10 smears suspected to have low platelet concentrations by use of the manual estimate, results were confirmed in 3 by use of the automated CBC and differential cell count. All dogs with low automated platelet counts were detected by use of the manual estimates. Results from 7 smears incorrectly suggested thrombocytopenia; platelet concentrations in these samples were > 100,000/µl as determined by use of the automated method. These data equate to sensitivity of 100% (3/3), specificity of 94% (114/121), positive predictive value of 30%, and negative predictive value of 100%.

When results were grouped according to manual estimates of neutrophil concentrations, hematocrit was 36 ± 5.4% for samples with neutrophil estimates less than the cut off value and 38 ± 5.6% for those with neutrophil estimates greater than the cut off value.

Discussion

Use of the manual estimate described here permitted correct identification of 3 dogs with substantial thrombocytopenia. The manual estimate identified 16 of 17 neutropenic dogs, for which chemotherapy would have been delayed and decreased to allow recovery of the neutrophil concentration; 1 neutropenic dog (900 neutrophils/µl) was not identified. Despite this failing, the manual estimate did accurately enable detection of most dogs undergoing chemotherapy that required a delay before continuing drug administration. The fact that the manual estimate did not detect 1 dog with low neutrophil concentration is of concern. It is therefore the authors’ opinion that obtaining a full differential cell count is of concern. It is therefore the authors’ opinion that obtaining a full differential cell count is preferable to using the manual estimate because an inappropriate region of the slide was examined. This error is likely to increase when the test is performed by inexperienced operators.

However, hematocrit of our neutropenic dogs (as determined by use of the manual estimate) was not greater than that of the nonneutropenic dogs; therefore, this did not appear to influence our results.

Secondly, manual estimates are potentially influenced by the choice of area of the smear that is examined and by the skill of the operator. It is possible that 1 neutropenic dog was not detected by use of the manual estimate because an inappropriate region of the smear was examined. This error is likely to increase when the test is performed by inexperienced operators.

If the manual estimate was used in practice, it would be important to note that the method does not differentiate between dogs with mild neutropenia that only require a delay in treatment and dogs with severe neutropenia that require close monitoring and a dose reduction of the cytotoxic agent as well. Therefore, for any dog suspected to have neutropenia or thrombocytopenia by use of this method, cytotoxic treatment should be delayed and a sample of blood submitted for a CBC.

The number of dogs with substantial thrombocytopenia was small, yet use of the cut of value reported here appeared to adequately detect dogs with platelet counts < 100,000 platelets/µl.

Importantly, the method described here used particular sizes of field of view in a microscope with a field number of 18. Many microscopes will have a higher field number, which could cause substantial error if the authors’ cut off values were used. For this method to be used, it is necessary for each individual to develop the technique with their own microscope on dogs with known neutrophil and platelet concentrations. Use of this method should only be considered when a manual differential cell count is not readily available and the practitioner is confident regarding identification of neutrophils and platelets. In that setting, a neutrophil or platelet estimate that is less than the cut of value would indicate the need to delay treatment. Awaiting the results of a full CBC would enable decision making regarding whether dose reduction was also required.

References