ORIGINAL ARTICLE

Prevalence of Low Automated Platelet Counts in Cats: Comparison with Prevalence of Thrombocytopenia Based on Blood Smear Estimation

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Abstract: **True thrombocytopenia is uncommon in cats; however, low platelet counts frequently are found using automated cell counters. Although this discrepancy is a well known problem, the prevalence of low automated platelet counts in feline blood samples has not been documented. We retrospectively compared the prevalence of low automated platelet counts with low blood smear-estimated platelet counts in feline blood samples. Results of blood sample analysis from 359 cats during a 1-year period at the University of Glasgow Veterinary Haematology Laboratory were examined. Smear estimates of platelet number were done in those cases in which records did not indicate adequate platelet numbers. Platelet counts obtained with an impedance counter (Minos Vet, Abx Hematologie) were $<200\times10^9$ cells/L in 256 samples (71%) and $<50\times10^9$ cells/L in 43 samples (12%). However, based on estimation of platelet numbers from blood smears, only 11 samples (3.1%) had platelet counts of $<200\times10^9$ cells/L and 9 samples (2.5%) had counts of $<50\times10^9$ cells/L. Four cats with thrombocytopenia estimated by blood smear evaluation had clinical signs of a bleeding disorder. Disorders associated with thrombocytopenia included neoplasia, cytotoxic chemotherapy, and infectious diseases. There was no evidence that delay due to mailing of samples was associated with lower automated platelet counts than would have been obtained on the day of sampling. The high prevalence of apparent thrombocytopenia in automated platelet counts was attributed to a combination of platelet aggregation and the impedance method of cell differentiation by size. Vigilance and careful examination of blood smears is required to identify the few cats with true thrombocytopenia. (*Vet Clin Pathol.* 2001;30:137-140)**

Key Words: **Feline, hematology, platelet, thrombocytopenia**

Thrombocytopenia is uncommon in cats, with a reported prevalence of 1.2% of 3300 cats admitted to a veterinary teaching hospital.[1] Clinical signs of abnormal hemostasis were detected in only 0.42% of cats.[1] However, laboratory results suggesting thrombocytopenia are a common finding when automated cell counters are used. Impedance counters differentiate cells by size alone. In cats, RBCs and platelets overlap in size, such that settings that exclude RBCs from the platelet count also will exclude a proportion of platelets. An additional problem is caused by in vitro aggregation of platelets, which occurs readily and often in feline blood.[2-4] Aggregation of platelets into large clumps may cause them to be counted as one large cell by impedance cell counters, underestimating the platelet count and falsely increasing the counts of other cell types. Falsely decreased platelet counts also occur with laser cell counters because platelet aggregates have a different light scatter pattern than do individual platelets, such that aggregates are not counted as platelets.[2] Aggregation of platelets also interferes with manual counting.

The frequency of occurrence of low automated platelet counts in feline samples in a diagnostic laboratory setting has not been reported. Laboratory experience at the University of Glasgow Veterinary Haematology Laboratory suggested that low counts were so common that an automated platelet count rarely could be relied upon. This study was undertaken to retrospectively examine the prevalence of low automated platelet counts compared with low blood smear-estimated platelet counts in feline blood samples over a 12-month period in our laboratory.

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Materials and Methods

Records of automated hematology counts for all feline blood samples on which complete blood counts were done at the University of Glasgow Veterinary Haematology Laboratory were retrospectively examined for the period from April 1, 1997, to March 31, 1998. Data were collected from the records and, if necessary, by examination of stored blood films. When an individual cat was sampled on more than 1 occasion, only the results from the first blood sample were included in the study.

Blood was submitted in EDTA for hematologic analysis using any one of a variety of commercially available EDTA tubes. Samples collected from patients hospitalized at the University of Glasgow Veterinary Hospital (internal samples) were stored at room temperature until analyzed within 24 hours (in most cases within 8 hours). Samples also were received from veterinarians elsewhere by first-class mail (external samples) and were held at room temperature until analyzed on the day of receipt. Notation was made if clots were seen or if the amount of sample in the tube was grossly inadequate such that a disproportionately high concentration of EDTA would be present; these samples were excluded from the study.

After thorough mixing of each blood sample on an automated mixer for 10 minutes, a complete automated blood count was performed using an impedance cell counter (Minos Vet, Abx Hematologie, Montpellier, France), which was maintained and calibrated as recommended by the manufacturer. A WBC differential count and smear cytologic analysis also was performed on each sample. Thin air-dried blood smears made after thorough mixing of the sample were stained with a modified May-Grünwald-Giemsa stain and examined under light microscopy. Platelet numbers were reported to be adequate when aggregates were seen or, subjectively, based on the experience of the laboratory technicians. Where the record did not note the results of smear evaluation for platelets, the slides were re-examined by one of us for platelet aggregates. If no aggregates were found, platelet count was estimated by averaging the number of platelets in 5 oil-immersion fields in the monolayer of the smear. An Olympus BX50 microscope was used with a ×100 oil-immersion lens and an ocular field number of 22. Mean platelet number per oil-immersion field was multiplied by a factor of 15.8 to give an approximate count $\times 10^9$ cells/L.[5,6] Thrombocytopenia was defined as a platelet count of $<200\times10^9$ cells/L.

Statistical analysis was performed using Minitab for Windows software (release 10.2, 1994, Minitab, State College, Penn). All counts were log transformed, and comparisons were made using an unpaired *t*-test. A *P* value of <.05 was considered significant.

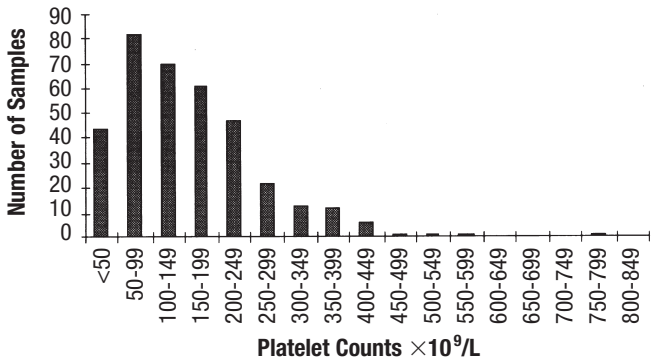

Figure 1. Frequency distribution of automated platelet counts from 359 cats during a 1-year period.

Results

A total of 583 feline blood samples were submitted during the study period. Of these, records were incomplete in 14 cases for various reasons, including cancellation of the request by the submitting veterinarian. In another 4 samples, substantial underfilling of the EDTA tube was noted, and in 26 samples gross clotting of the sample had occurred, making the sample unsuitable for further analysis. These samples were excluded from the study, leaving a total of 539 samples from 359 cats, comprising 325 internal samples from 227 cats and 214 external samples from 132 cats.

In 256 of the 359 cats sampled (71%), automated platelet counts indicated thrombocytopenia ($<200\times10^9$ cells/L) (Figure 1). In 43 of these cats (12%), platelet counts were severely decreased ($<50\times10^9$ cells/L), and in 7 cats (1.9%) counts were $<20\times10^9$ cells/L. Based on evaluation of smears, 11 of 359 cats (3.1%) had platelet counts of $<200\times10^9$ cells/L. Platelet counts were markedly decreased ($<50\times10^9$ cells/L) in 9 cats (2.5%), and in 8 cats (2.2%) counts were $<20\times10^9$ cells/L. In all samples with blood smear-estimated thrombocytopenia, the automated platelet count was $<200\times10^9$ cells/L. In only 4 of the 11 samples was thrombocytopenia mentioned in the final hematology report to the submitting veterinarian.

Of the 11 samples with blood smear-estimated thrombocytopenia, 4 cats had histories that suggested a hemostatic defect. In all 4 cats, both the automated and estimated platelet counts were $<20\times10^9$ cells/L. One of the cats had pemphigus foliaceus and was being treated with myelosuppressive drugs. One cat had feline immunodeficiency virus–related disease. In 2 cats, an underlying disease was not reported. Of the remaining 7 thrombocytopenic samples, 1 cat was receiving chemotherapy for lymphosarcoma, 1 cat had a positive feline coronavirus titer and intracranial disease of suspected nutritional origin, and 1 cat each had renal neoplasia, haemobartonellosis, and hepatic disease. In 2 cats an

underlying disease was not reported.

When external samples were compared with internal samples, there was no significant difference ($P = .23$) in the automated platelet counts.

Discussion

Thrombocytopenia as evidenced by smear examination was present in only 3.1% of cats in this study. Jordan et al.[1] reported a prevalence of thrombocytopenia of 1.2%. These figures are broadly similar, and differences are likely due to sampling error. Jordan et al. had a much larger sample size over a 5-year period, and where hemocytometer counts were not performed, the average platelet count in 25 oil-immersion fields was used to estimate platelet numbers.[1] The higher number of fields counted compared with the present study would have lessened any inaccuracy resulting from uneven distribution of platelets on the blood smear.

As has been previously reported, neoplasia and infectious diseases are the most common disorders in cats with thrombocytopenia.[1] In some cats, thrombocytopenia may be a component of disseminated intravascular coagulation (DIC); 38% of cats undergoing coagulation testing have been found to meet some or all of the diagnostic criteria for DIC.[7] No cases of immune-mediated thrombocytopenia were diagnosed during this 1-year survey, in keeping with the low prevalence of this disease in cats.[1,7]

Automated platelet counts performed by an impedance counter were low in the majority of cats sampled (71%), whereas the prevalence of thrombocytopenia based on blood smear estimation was only 3.1%. Thus, apparent thrombocytopenia was a significant problem in automated counts using an impedance cell counter. Although automated counts were low in all cats with blood smear-estimated thrombocytopenia, the frequent occurrence of falsely low automated platelet counts meant that thrombocytopenia commonly was ignored.

The impedance counting method, in which platelets and RBCs are differentiated by size alone, contributes to falsely low automated platelet counts in cats. With the Minos Vet analyzer, RBCs and platelets are analyzed concurrently in a single channel with a 50-µm–diameter aperture. The impedance generated by each particle passing through the sensing zone is plotted against the number of impulses (particles) for analysis. A fixed upper platelet and lower RBC threshold of 17.5 fL for cats has been determined by the manufacturer. In comparison, a threshold of 27.0 fL is used for dogs. In most cases, this threshold cuts the histogram at the trough between platelets and RBCs. However, the platelet and RBC histograms commonly overlap in cats, such that the threshold may exclude larger platelets from the platelet count and smaller RBCs from the RBC count. Feline platelets are larger than those of other species, with a mean volume of 11.0-18.1 fL.[8] Mean platelet volume (MPV) for dogs, pigs, and human beings is 7.6-8.3 fL.[4] The magnitude of error in counting platelets is much greater than for counting RBCs because of the difference in relative numbers of platelets and RBCs. Aggregation of platelets increases this error because clumped platelets appear to the cell counter as a single larger cell. Although other factors such as RBC microcytosis and schistocytosis also contribute to the problem, platelet aggregation is a far more frequent occurrence, affecting at least 50% of the feline blood samples analyzed during this 1-year period. Platelet aggregation occurred in 66.6% of blood samples collected from 48 healthy, anesthetized cats,[2] and in another study, aggregation-induced interference was found in 56% of 41 feline blood samples undergoing automated cell counting.[9]

The use of optical cell counters in which platelets and RBCs are differentiated by their light scattering pattern would be expected to avoid errors associated with impedance counters. However, the light scattering pattern of a platelet aggregate is not the same as that of a single platelet, so aggregates are excluded from the platelet count by optical counters.[2] Manual counting of platelets also is affected by aggregation because individual platelets cannot be counted within aggregates either on smears or in a hemocytometer. Aggregation also is likely to lead to uneven distribution of platelets, with aggregates accumulating on the edges of smears and counting chambers. Hence, aggregation of feline platelets in vitro contributes to technical difficulties in platelet counting, and accurate counting of feline platelets depends on the absence of aggregates.

Platelets are reactive cells that can be stimulated to aggregate by a variety of factors including substances released from activated platelets themselves such as adenosine diphosphate (ADP) and serotonin, circulating substances such as adrenalin and vasopressin, extravascular substances such as collagen, products of the coagulation cascade such as thrombin, physical factors such as shear stress and stirring, and many foreign substances.[10-15] Certain features of feline platelets may cause them to be more reactive than platelets of other species, such as their larger size,[9] their higher concentration of serotonin,[9] and their response to serotonin by irreversible platelet aggregation with granule release, which is unique among domesticated species.[16] Irreversible aggregation occurs at lower concentrations of ADP in cat platelets than in platelets from other species.[17] The small size and imperfectly tractable nature of cats contribute to venipuncture difficulties, which also may increase the likelihood of in vitro platelet aggregation.

Gentle handling of the sample, avoidance of small-bore needles for venipuncture and undue negative pressure on the syringe,[16] use of siliconized glassware or plastic sample containers,[18,19] and discarding the first few drops of the sample[19] have been advocated to reduce aggregate formation. However, the problem appears to be unavoidable even under favorable conditions such as anesthesia. A simple method to consistently avoid platelet aggregation in cats would be valuable. In this study, no significant contribution to platelet aggregation could be attributed to delays and additional handling arising from sample mailing; there was no significant difference in automated platelet counts between external and internal samples.

The findings of this survey demonstrate that although thrombocytopenia is an uncommon occurrence in cats, false indications of thrombocytopenia are common and result from the inability of an impedance counter to accurately quantify platelet counts in feline blood samples. This lack of reliability necessitates examination of individual blood smears for adequate platelet numbers, and vigilance on the part of laboratory workers to detect the few cats in which thrombocytopenia is actually present. In this study, as was previously reported, thrombocytopenia in cats was most commonly associated with neoplasia, chemotherapy, and infectious diseases.◊

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