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ORIGINAL RESEARCH

Interpretation of canine and feline blood smears by emergency room personnel

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Key Words
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Background: Interpretation of blood smears is commonly used to provide rapid laboratory evaluation of animals in veterinary emergency practice, but the accuracy of results of blood smear interpretation by emergency room personnel (ERP) compared with evaluation by trained veterinary clinical pathology personnel is unknown.
Objective: The goal of this study was to compare blood smear evaluation by ERP with that of clinical pathology personnel.
Methods: All animals that had a CBC determined by a diagnostic laboratory and had blood smears evaluated by personnel at the Foster Hospital for Small Animals Emergency Room between September 2008 and July 2009 were eligible for study inclusion. ERP who evaluated blood smears completed standardized forms with estimates of the WBC and platelet counts and evaluation of RBC and WBC morphology. Results from point-of-care assessment were compared with automated or manual results reported by the veterinary diagnostic laboratory.
Results: One hundred and fifty-five blood smears were evaluated. There was moderate agreement (κ value, 0.63; 95% confidence interval [CI]: 0.52, 0.74) between estimated platelet counts by ERP and automated counts. Poor agreement was found between estimated WBC counts by ERP and automated counts (κ value, 0.48; 95% CI: 0.37, 0.60). Specific abnormalities with a high likelihood of clinical significance, eg, toxic change, nucleated RBCs, spherocytes, hemoparasites, and lymphoblasts, were not predictably identified by ERP.
Conclusions: ERP interpretation of canine and feline blood smears should be used cautiously and should not replace evaluation by a veterinary diagnostic laboratory.

Introduction

Emergency medicine is a rapidly growing field of veterinary practice. Accurate laboratory testing is vital to appropriate patient assessment and therapy. Owing to the nature of emergency medicine, evaluation of animals presented after hours, when clinical pathology services may not be immediately available, depend on the capabilities of the institution or emergency facility. In these situations, emergency clinicians often perform evaluations of blood smears to estimate nucleated cell and platelet counts, assess cellular morphology, and look for other abnormalities, eg, blast cells, infectious agents, and inclusions. A previous veterinary study of platelet and leukocyte estimates by clinical pathologists and medical technologists evaluating peripheral blood smears documented high correlation ($r = .937$) between platelets per oil immersion ($\times 1000$) field and automated platelet counts and moderate correlation ($r = .75$) between leukocyte numbers per low-power ($\times 100$) field and the automated WBC count.[1] Feline automated platelet counts have been considered to be unreliable due to an inability of some electronic cell counters to distinguish between RBC and platelets because of an overlap in size and a tendency for feline platelets to clump leading to falsely lower counts.[2–5]

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A moderate correlation ($r = .776$) was found between platelets per oil immersion field and manual hemocytometer counts of feline platelets.[6]

To the authors' knowledge a comparison of blood smear interpretation by emergency room personnel (ERP) with evaluation by personnel in a veterinary diagnostic laboratory has not been reported previously. If there is good agreement between the initial blood smear evaluation and the final interpretation determined by trained personnel in the diagnostic laboratory, then the on-going evaluation of blood smears in the emergency setting may be supported. The goal of this study was to evaluate the ability of ERP to evaluate blood smears from cats and dogs presented to an emergency service.

Materials and Methods

Animals

All cats and dogs presented for evaluation at the Foster Hospital for Small Animals Emergency Room, Tufts Cummings School of Veterinary Medicine (TCSVM), between September 2008 and July 2009 and had blood submitted to the TCSVM Clinical Pathology Laboratory for a CBC were eligible for study inclusion.

Blood smear evaluation

Peripheral blood smears were made by ERP using fresh whole blood or an aliquot of EDTA-anticoagulated blood collected for CBC. ERP were recruited from the emergency service; individuals with extensive past training, such as medical technologists, were ineligible. Additionally, individuals who self-reported that they were unskilled at evaluating blood smears were permitted to not participate. ERP were instructed to interpret blood smears using their normal technique. Individuals may have submitted multiple or no samples for inclusion in the study. The individual's identity other than level of training was anonymous to increase participation in study. The smears were air-dried and stained with Hema 3 Stain Set (Fisher Diagnostics, Middletown, VA, USA). ERP completed a standardized data-reporting form with the following information:

(1) Level of training: veterinary student, small animal rotating intern, or emergency and critical care (ECC) resident
(2) Estimated number of smears interpreted by reader per week
(3) Estimated WBC count including a value and an estimated category (high, normal, low)
(4) Estimated platelet count including a value and an estimated category (high, normal, low)
(5) Erythrocyte morphology (specific categories were not offered)
(6) Leukocyte morphology (specific categories were not offered)
(7) Presence of any other abnormalities

Results from the standardized form were compared with results determined by the TCSVM Clinical Pathology Laboratory. CBCs were analyzed the same day as blood smear evaluations by ERP if submitted during the diagnostic laboratory's hours of operation or the following day if submitted after hours. In the diagnostic laboratory, blood smears were air-dried and stained using an Aerospray 7120 hematology slide stainer (Wescor Inc., Logan, UT, USA) with an aqueous Romanowsky stain (Hemaspray, Thermo Fisher Scientific, Waltham, MA, USA). Cell counts were determined using a Cell Dyn 3700 (Abbott Laboratories, Abbott Park, IL, USA) and differentials verified manually by either an experienced medical technologist or a medical laboratory technician. Criteria for review by an ACVP board-certified clinical pathologist were the presence of the following:

(1) Left shift (band neutrophils $> 5\%$)
(2) Leukopenia $< 1000/\mu L$
(3) Nucleated RBCs > 20 per 100 WBC without increased polychromasia
(4) Monocytosis $> 4000/\mu L$
(5) Lymphocytosis $> 15{,}000/\mu L$
(6) Spherocytes
(7) Lymphoblasts, atypical lymphocytes, unclassified cells
(8) Hypochromasia
(9) Eosinophilia $> 10{,}000/\mu L$
(10) Questionable blood parasite
(11) Mast cells
(12) Bacteria

All blood smears marked for pathologist review were examined by 1 of 2 clinical pathologists (J.K. or Perry Bain) at TCSVM Clinical Pathology Laboratory. Toxic change in canine neutrophils was identified when the cytoplasm showed increased cytoplasmic basophilia with or without foaminess or contained Döhle bodies. The first 2 criteria were also used to identify toxic change in feline neutrophils. A few small Döhle bodies were considered an incidental finding in feline neutrophils and were insufficient to report toxic change. Toxic change was subjectively graded as mild, moderate, or marked depending on the degree of cytoplasmic basophilia, the presence or absence

of cytoplasmic vacuolization, and the percentage of neutrophils affected. Samples were considered positive for nRBC if the diagnostic laboratory reported ≥ 1 nRBC per 100 leukocytes. Samples were considered positive for spherocytosis if > 1 spherocyte was consistently seen per oil immersion ($\times$ 1000) field.

Laboratory categories for WBC count were based on the diagnostic laboratory's reference intervals for the CellDyn 3700. A low category was assigned if the WBC count was $< 4900/\mu L$ in dogs and $4500/\mu L$ in cats. A normal category was assigned if the WBC count was between 4900 and 16,900/μL for dogs and between 4500 and 15,700/μL for cats. A high category was assigned if the WBC count was $> 16,900/\mu L$ for dogs and 15,700/μL for cats.

Categories for platelet counts for the diagnostic laboratory were based on reference intervals for automated platelet counts. A low category was assigned if the platelet count was $< 180,000/\mu L$. A normal category was assigned if the automated platelet count was between 180,000 and 525,000/μL. A high category was assigned if the automated platelet count was $> 525,000/\mu L$. Cats ($n = 11$) were excluded from platelet analysis due to unreliability of automated platelet counts in cats.[2–5]

The Tufts Institutional Review Board approved the study protocol for human investigation; the Clinical Studies Review Committee waived the requirement for client consent.

Statistical analysis

The primary outcomes were discrepancies between the diagnostic laboratory's analyzer-determined and ERP-estimated WBC and platelet counts. Box plots were used to summarize these discrepancies across education level of the observer. As both outcome variables were highly skewed, we compared them across groups using the Kruskal–Wallis test. If the overall P-value was significant ($P < .05$), we examined all possible pairwise comparisons to determine which groups were different. To minimize multiple testing errors, $P < .01$ was considered statistically significant for the pairwise comparisons analysis.

The κ statistic was used as a measure of agreement for the diagnostic laboratory categorization of WBC count (low, normal, high) and the ERP categorization (low, normal, high). We also used the κ statistic to measure agreement between the diagnostic laboratory and ERP categories of platelet count (low, normal, high). All statistical analyses were run in SAS 9.2 (SAS Institute Inc., Cary, NC, USA). Bland–Altman graphs were plotted to visualize the discrepancy between the diagnostic laboratory WBC and platelet counts and estimated WBC and platelet counts performed by ERP. Sensitivity and specificity were calculated using these formulas:

$$\text{Sensitivity} = \text{true positives}/(\text{true positives} + \text{false negatives})$$
$$\text{Specificity} = \text{true negatives}/(\text{true negatives} + \text{false positives})$$

The corresponding 95% confidence intervals (CI) were determined using the online calculator found at http://www.measuringusability.com/wald.htm. Except as noted above, $P < .05$ was considered statistically significant.

Results

A total of 155 blood smears, including samples from 139 dogs and 16 cats, were evaluated. Of these, students evaluated 52, interns evaluated 50, and ECC residents evaluated 53. There were a total of 80 students, 15 interns, and 16 residents on the ECC service during the period of data collection.

WBC counts were estimated on 145 smears (15 feline and 130 canine) by 47 students (32%), 47 interns (32%), 9 first-year ECC residents (6%), 32 second-year ECC residents (22%), and 10 third-year ECC residents (7%). The discrepancy between the automated WBC count and the WBC estimate count among the different ERP groups was calculated and plotted (Figure 1). Pairwise comparisons between groups by level of education found that there was a significant difference in median discrepancy between students and interns ($P = .002$). No other significant differences in median discrepancy between groups were found. A Bland–Altman plot (Figure 2) indicated that there were wide discrepancies in data between ERP-estimated and automated WBC counts from the diagnostic laboratory and that these differences increased as the WBC count increased. A κ value of 0.48 (95% CI: 0.37, 0.60) was calculated between the leukocyte category (low, normal, high) based on ERP estimation and that based on the automated WBC, indicating poor agreement.

ERP estimates of absolute platelet counts and automated platelet counts were performed on 121 canine smears. ERP and laboratory personnel estimates of platelet categories (low, normal, high) were performed on 132 canine blood smears. ERP did not report an absolute platelet estimate for 14 of the 139 canine blood smears owing to platelet clumping in 3 cases and unspecified reasons in the other 11. The diagnostic laboratory did not report an absolute platelet count in 6 of

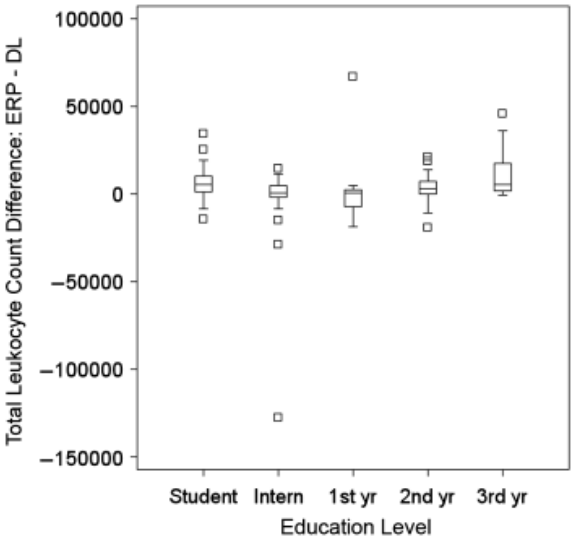

Figure 1. Box plot showing the distribution of the discrepancy between automated WBC counts performed by the diagnostic laboratory (DL) and estimates of WBC counts by different groups of emergency room personnel (ERP). The boxes represent the interquartile intervals from the 25th to the 75th percentiles. The solid horizontal bars through the boxes represent the medians, and the 10th–90th percentiles are represented by the capped vertical bars. Outliers fall above and below the vertical bars. Groups included senior veterinary students, interns, and emergency and critical care residents in years 1–3. Significant differences were found only between students and interns ($P < .02$).

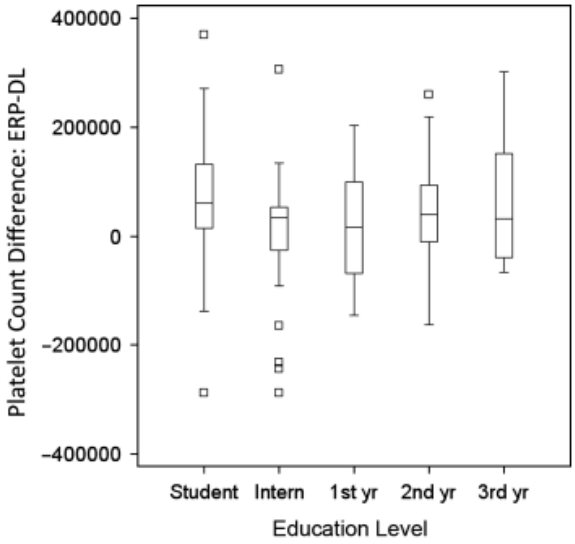

Figure 3. Box plot showing the distribution of the discrepancy between automated platelet counts performed by the diagnostic laboratory (DL) and estimates of platelet counts by different groups of emergency room personnel (ERP). The boxes represent the interquartile intervals from the 25th to the 75th percentiles. The solid horizontal bars through the boxes represent the medians, and the 10th–90th percentiles are represented by the capped vertical bars. Outliers fall above and below the vertical bars. Groups included senior veterinary students, interns, and emergency and critical care residents in years 1–3. No significant differences among ERP groups were found.

the 139 canine blood smears, citing platelet clumping as the reason for all 6. Absolute platelet counts were estimated by 41 students (34%), 37 interns (30%), 8 first-year ECC residents (7%), 27 second-year ECC residents (22%), and 8 third-year ECC residents (7%). Differences in the discrepancy between the automated platelet count and the platelet estimate by ERP were not found (Figure 3, $P > .05$). A Bland–Altman plot (Figure 4) indicated that there were wide discrepancies between ERP-estimated and automated platelet counts from the diagnostic laboratory and that these differences increased as the platelet count increased. A κ value of 0.63 (95% CI: 0.52, 0.74) was calculated between the platelet category (low, normal, high) based on ERP estimates and that based on the automated platelet count indicating moderate agreement.

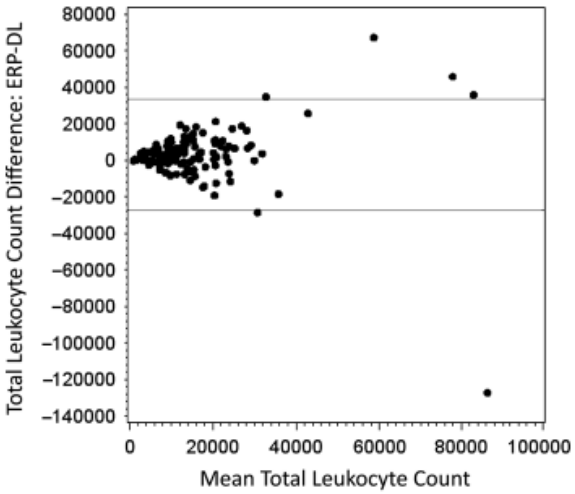

Figure 2. Bland–Altman plot showing the difference between the estimate of WBC count (cells/μL) by emergency room personnel (ERP) and the automated WBC count performed by the diagnostic laboratory (DL) plotted against the mean of the 2 methods (cells/μL). Wide discrepancies are shown, and these differences increase with increasing WBC count.

Other reported abnormalities included toxic change in neutrophils, nRBCs, spherocytes, lymphoblasts, and morulae, presumably *Anaplasma phagocytophila*, within neutrophils. The sensitivity and specificity of ERP interpretation of these abnormalities were calculated with 95% CI for each (Table 1). Interpretations reported by the diagnostic laboratory were used as the reference standard. Other cellular abnormalities such as echinocytes, acanthocytes, and other poikilocytes were reported, but were not statistically evaluated due to low numbers.

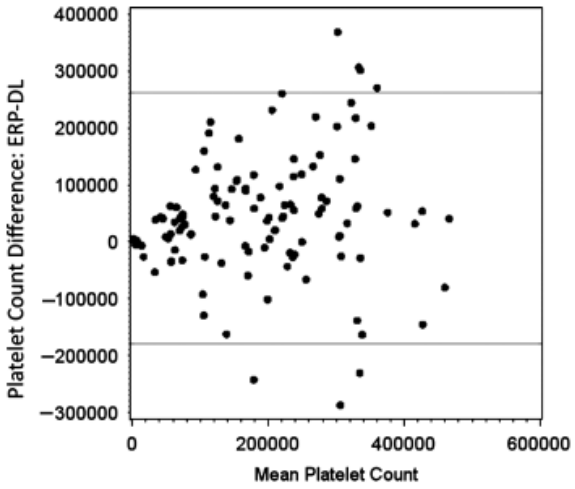

Figure 4. Bland–Altman plot showing the difference between the estimate of the absolute platelet count (platelets/μL) by emergency room personnel (ERP) and the automated platelet count performed by the diagnostic laboratory (DL) plotted against the mean of the 2 methods (platelets/μL). Wide discrepancies are shown, and these differences increase with increasing platelet count.

Discussion

Estimation of WBC and platelet counts may be important in the assessment of critical cases by ERP as disease processes, such as severe thrombocytopenia or severe leukopenia, that need immediate therapy may be detected. Platelet and leukocyte estimates from peripheral blood smears in dogs and cats have been described previously using conversion factors multiplied by the average number of cells per oil immersion field.[1,6,7] Good correlation between platelet and leukocyte estimates performed by clinical pathologists or medical technologists and hematology analyzers or manual hemacytometers has been reported.[1,6] In the current study we found that agreement between canine platelet estimates and automated platelet counts was moderate and the degree of error by ERP increased as the platelet count increased. The agreement between ERP-estimated and automated WBC counts, including both canine and feline samples, was poor. Similar to platelet estimates, the degree of error by ERP increased as the WBC count increased. Other abnormalities, such as toxic change in neutrophils and the presence of nRBCs, spherocytes, circulating lymphoblasts, and morulae within neutrophils were detected with variable success. Possible reasons for discrepancies between ERP and laboratory personnel may include, but are not limited to poor smear preparation technique, different stain methodologies, poor smear interpretation technique, and varying sensitivity between manual and automated techniques. It is difficult to determine the best explanation for these difficulties based on the results of this study.

Table 1. Sensitivity and specificity with 95% confidence interval (CI) were calculated for each morphologic abnormality.

Abnormality	Sensitivity (95% CI)	Specificity (95% CI)
Toxic change in neutrophils ($n = 30$)	30% (17–48%)	80% (72–86%)
Nucleated RBCs ($n = 64$)	39% (28–51%)	96% (89–99%)
Spherocytes ($n = 19$)	74% (51–89%)	83% (76–89%)
Morulae (presumably *Anaplasma phagocytophila*) ($n = 6$)	83% (42–99%)	99% (95–100%)
Lymphoblasts ($n = 5$)	40% (12–77%)	99% (96–100%)

Difference in smear preparation and slide-staining techniques may account for some of the variability between ERP and the diagnostic laboratory. Comparison between slide making techniques has been investigated between automated and manual wedge-pull technique; automated smears had a larger optimal area for cell counting, and, generally, determination of WBC differentials was better using automated smears when compared with a gold standard reference instrument.[8] In our study, all smears (prepared by ERP and laboratory personnel) were made by manual methods, and individual variation may have been a source of error; the quality of individual smears in this study was not assessed. Differences in cellular component staining have been reported between methanolic and aqueous Romanowsky stains and between different types of rapid staining techniques.[9,10] In our study, aqueous Romanowsky stains were used by both ERP and laboratory personnel; therefore, the impact of different staining methods was probably not great. It must also be noted that ERP-prepared blood smears, even if made the same sample submitted to the laboratory, may have been made at different times relative to the time of blood sampling. Temporal factors, such as settling of cellular components of blood, may have contributed to discrepancies if samples were not appropriately processed. For the purposes of this study, specific sources of error were not investigated and future studies may be warranted.

Poor interpretation techniques, for example, evaluating cellular morphology in an area that is too thick or thin, may also contribute to error in evaluating blood smears. In this study, it is possible that students and clinicians over-reported spherocytes because they were evaluating RBCs too close to the feathered edge where they are more likely to lose central pallor and mimic spherocytes. RBCs in inappropriately thick areas of the smear may also lose central pallor because

crowding does not allow them to flatten out on the smear in a proper fashion.

Another factor that may have contributed to error could be varying sensitivity between manual and automated techniques. The diagnostic laboratory uses automated cell counts that are then verified when the blood smear is examined.

In-clinic hematology analyzers are becoming increasingly popular in many veterinary clinics. Comparability between in-clinic and commercial hematology analyzers for canine and feline CBCs has been studied, and excellent to good correlation for WBC and platelet counts has been reported for many types of in-clinic hematology analyzers; differential leukocyte counts by in-clinic hematology analyzers had variable correlation with commercial laboratory analyzers.[11,12] Examination of peripheral blood smears is often recommended to support results based obtained from in-clinic hematology analyzers. In combination with these analyzers, estimates of WBC and platelet counts could have clinical value. However, detection of cellular abnormalities such as toxic change in neutrophils, spherocytes, nRBC, lymphoblasts, and morulae require experience not replaced by most in-clinic analyzers. Use of these hematology analyzers without review of peripheral blood smears by a medical technologist, medical laboratory technician, or clinical pathologist could lead to failure to identify clinically relevant abnormalities.

The results of this study provide useful information to emergency veterinary facilities and practice managers as well as to diagnostic laboratories. Evaluation of blood smears for severe thrombocytopenia and severe leukopenia may still have clinical utility as the data of this study suggest there is better agreement between ERP and the diagnostic laboratory personnel at lower values. However, ERP-generated platelet and leukocyte estimates in animals without severely low counts should be used cautiously and not replace evaluation by diagnostic laboratory personnel or an in-clinic hematology analyzer. Also, qualitative cytopathologic analysis by ERP had variable results and further studies investigating the ability of ERP to interpret specific cellular abnormalities and to perform leukocyte differential counts are warranted.

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