Examination of Inherent Variation in Leukocyte Estimates from Peripheral Blood Smears

MS Camus and DR Pope

Department of Pathology, College of Veterinary Medicine, University of Georgia, Athens, GA, USA

ABSTRACT

Leukocyte (WBC) estimation from peripheral blood smears is performed on hematologic specimens submitted to the UGA Clinical Pathology Laboratory, to visually confirm the accuracy of the automated analyzer. It is occasionally the only option for determining WBC concentration, when a usable sample is unobtainable. There is currently no established normal allowable error (Tea) for hematologic parameters in veterinary medicine. However, the WBC count in human medicine is deemed acceptable when it is within 20% of the estimated count. In an attempt to standardize the WBC estimation procedure and minimize CV, an internal review and a prospective evaluation were conducted. It was hypothesized that, though there is variation in estimation technique, the CV would fall within 25%. Ten routinely processed peripheral blood smears representing all levels of severity (leukopenia to profound leukocytosis) were chosen: 6 canine, 3 feline, and 1 equine. Seven certified laboratory professionals (CLPs) performed a WBC estimate on each sample and were asked to provide average WBC/field, objective used, formula used, and microscope model. CV was determined for each sample. All CLPs counted at 40X magnification and used the same estimation formula. CV varied between 13% and 44%, with highest CVs in leukopenic samples. It was concluded that there is marked variation in WBC estimation, despite using the same slides and estimation formula. When compared with the gold standard, WBC concentration determined by the ADVIA® 120, only 49% of estimates were within the 20% acceptability variance. Sources for error include different counting areas, counting broken cells, and variation in microscope aperture size. As a result of our study, WBC estimates are now interpreted qualitatively in our laboratory (e.g. decreased/adequate/increased) and quantitative estimates are no longer given.

INTRODUCTION

Performing a leukocyte estimate from a peripheral blood smear is a commonly requested procedure in our laboratory, particularly for exotic species, where it can be difficult to acquire an adequate volume of well-preserved blood to perform a CBC. It also becomes necessary with occasional samples submitted through the laboratory for analysis. During an internal review of processes, done largely in preparation for AAJLD accreditation, the leukocyte estimation process utilized in our laboratory was re-evaluated. There was marked variation in the estimation, believed to be due in part to different estimation formulas. Suspecting that the variation was multifactorial, a prospective study was designed to investigate our internal CV and determined potential sources of variation.

MATERIALS AND METHODS

Ten routinely processed peripheral blood smears were selected by the Quality Manager. These samples were previously made by laboratory staff as a component of a CBC. Samples from six dogs, three cats, and one horse were reviewed. Leukocyte counts, as determined by the ADVIA® 120 hematology analyzer, ranged from <2000/µL to 203,300/µL. All seven certified laboratory professionals (CLPs) employed by our lab performed a WBC estimate on each sample. At the time they performed the estimates, they were asked to provide the average number of WBCs per field, the objective magnification used, and the estimated leukocyte count per sample. They were also asked to provide the manufacturer and model number for the microscope they utilized to perform the differential, as well as their estimation formula. This data was evaluated by the Quality Manager, who calculate a coefficient of variation (CV) for each sample and investigated potential sources of variation.

RESULTS

Numbers indicated in red are the values generated by the ADVIA® 6120. The seven numbers in each column represent the estimated values obtained by each CLP. The CV is given at the bottom and is based solely on the estimates.

The CV is a numerical measure of precision. It is defined as the ratio of the standard deviation to the mean. CVs typically vary with an analyte’s concentration, as CV values are expressed as percentages. In this case, the CV was greatest (44.4%) in the most leukopenic sample and was least (13.1%) in the sample with the most profound leukocytosis. Only one sample had a CV less than the accepted 20% and the hypothesized 25%. As a comparison to the gold standard, only 49% of estimates (of the 9 samples with numerical values) were within the mean +/-2SD.

DISCUSSION

Numerous factors affect the accuracy of blood smear leukocyte estimates. To minimize the effects of smear preparation technique and the density of the monolayer, estimates in this study were done off of premade smears. When CLPs were asked to perform these estimates, there were numerous variations in technique, from acceptable counting area to which cells (intact versus disrupted) to count. Additionally, there is inherent variation in the aperture size of microscopes, even with the same manufacturer. Therefore, it is essential to standardize the estimation technique, as described by CAP, for each microscope in the clinical pathology core. In our laboratory, six CLPs are licensed medical technologists (MLT) and one is a licensed medical laboratory technician (MLT). All have gained experience with veterinary species by a minimum of seven years experience in a veterinary specific laboratory.

According to the College of American Pathologists (CAP), WBC estimation should not be done in the absence of correlation. The suggested correlation process involves derivation of a standardized WBC estimation factor. Steps for factor derivation, which should be performed for each individual microscope, are as follows:

1. Perform automated WBC counts on 30 consecutive specimens from fresh patient blood samples.
2. Prepare and stain one peripheral blood smear for each sample.
3. Count the WBCs in 10 consecutive fields for each smear. Either 40X or 100X magnification can be used, as long as it is used consistently throughout the procedure and all subsequent procedures.
4. Divide the total number of WBCs by 10 to obtain the average per field.
5. Divide the automated WBC count by the average number of WBCs per field to obtain the conversion factor for each sample.
6. Add the conversion factor for each sample (determined in step 5) and divide by 30 (the number of samples analyzed) to obtain the average ratio of the automated WBC count to the WBC per high power field. Round this number to the nearest whole number to obtain the WBC estimation factor.

The WBC estimation factor determined via this method is equivalent to the number of peripheral WBCs represented by a single WBC per microscopic field. Once this has been determined for each individual scope, the CLP should calculate the average number of WBCs per field on subsequent patient specimens and multiply this value by the estimation factor to obtain an accurate WBC estimate.

OUTCOME

As a result of our internal process review, the decision was made to stop reporting leukocyte estimates in instances where an analyzer generated WBC count is not available or is believed to be inaccurate. Blood smears are evaluated to assess cell morphology and identify infectious agents. CLPs perform an internal semi-quantitative WBC estimate (decreased, adequate, increased) simply to verify the analyzer generated leukocyte count and determine if potential sample anomalies (e.g. increased nRBCs) warrant amendment of the automated leukocyte count. Similarly, the clinical pathology faculty has changed the way that we teach students, teaching the white cell estimate only as a tool for confirming sample identity through the analyzer generated leukocyte count. We have stopped providing an estimation formula, although we are aware that many other diagnostic services teach the clinical utility of leukocyte estimation.

FUTURE DIRECTIONS

Given the need for a standardized leukocyte estimation method, we will attempt to derive a WBC estimation factor, as described by CAP, for each microscope in the clinical pathology laboratory. If utilization of the derived factor(s) proves to be within acceptable precision limits (CV < 20%) and an acceptable number are within the mean +/- 2SD, we may explore utilizing the method on diagnostic cases and teaching this estimation method to students for use on their in-house microscopes. Similarly, discovery of such inherent variation in leukocyte estimation technique has raised internal questions about other estimation methods, such as platelet estimation and has spawned a generalized internal review of processes.

REFERENCES