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Evaluation of clinical usefulness of reference interval of some selected hematological parameters in canine blood

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Abstract: To estimate the source of variance components for some hematological parameters and assess the utility of the conventional population-based reference interval, this study computed index of individuality for blood samples, which were from 13 dogs drawn once weekly for 4 consecutive weeks. Results were subjected to nested analysis of variance. For all parameters measured between-dog variations were greater than within-dog variation. Except for the parameters RBC and MCHC the index of individuality was <1.4. The low reliability coefficient and high index of individuality of ≤0.8 were found for the majority of hematological parameters. In practical term, the present study indicated that use of hemogram profiles alone in the evaluating clinical state of a single patient should be avoided because of their physiological or natural random variations, and that comparing a single measurement on the blood analytes from an individual dog to the conventional population-based reference range may be too insensitive to detect any significant changes in the blood components of that particular dog. A single measurement may not characterize an individual's average concentration of the parameters even short-term period.

Key words : index of individuality, reference interval, hematology, variability, dog

Introduction

Most clinical laboratory tests are used to aid in the diagnostic process or in monitoring. In order to assess a patient's current state in the course of disease progress, laboratory results can be compared either with a reference interval or with previous results from the same patient. Particularly, in the former case, values of a specific parameter of an animal are compared with a population-based reference interval derived from an observed distribution of measurements of the parameter in representative healthy animals and containing the central 95% of the distribution. Classically, an analytical result outside this reference interval classifies the animal as abnormal indicating an unusual or pathological condition.

Evaluating the degree to which a single measurement is able to distinguish unusual results in a subject has been important issue in monitoring patient. Many alternate statistical expressions relating the relative magnitudes of an analyte's within-person and methodological variances compared with its between-person variance, which has been more widely used in the pathology literature, is the index of individuality [1, 3, 5, 7, 8]. Harris [7] developed an index of individuality and proposed criteria for clinical application. A low index indicates a high degree of individuality, such that, particularly for an index < 0.6, population-based reference intervals will be low utility, both as criteria for detecting a significant change in serial results and as a diagnostic tool. In contrast, when the index is high, the parameter has low individuality; particularly when the index is > 1.4, conventional reference intervals will be of greater utility than for parameters with a low index. With an average ratio between 0.6-1.4, neither conclusion can be drawn.

In veterinary medicine, variance components or index of individuality have been reported for bovine clinical

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chemical parameters [9], canine chemical parameters [10], canine rectal temperatures [11], and canine glycosylated haemoglobin [12], but index of individuality of canine hematological parameters have not been reported. The present study was therefore conducted to estimate the between-dog, within-dog and analytical components of variance for some routinely used canine hematological parameters, and to use these components of variance to assess the utility of the conventional population-based reference interval.

Materials and Methods

Animals and sample collection

Thirteen adult mixed-breed dogs, 4 males and 9 females, with an age range of 2 to 5 years, weighing 4-15 kg were included in the study. The dogs were housed at the animal hospital and used for student training and blood donors. Thus, the dogs were housed and handled in a manner similar to that of hospitalized dogs prior to and during the study. Drugs had not been administered to any of the dogs for 2 weeks prior to and during the study. Throughout the study all dogs were monitored and none showed signs of disease or unusual stress factors that could have interfered with the final results.

The blood was collected once per week from each dog for 4 consecutive weeks. Blood samples were obtained by cephalic venipuncture from each dog between 14:00 and 16:00 hours after being seated for 10 minutes. Two ml of blood was transferred to EDTA tubes and all samples were analyzed within 2 hours after collection. Samples were collected in duplicate from each dog, and thus each dog contributed eight sets of samples to the study, all of which were assigned different identification numbers. The blood-drawing schedule continued unchanged throughout the study.

Hematology analyzer

The hemacyte® hematology analyzer (CDC Ltd., USA) was used for immediate analysis of the sample. The instrument was operated with manufacturer's reagents and protocols for calibration and maintenance. In each dog, the following parameters were measured and logarithmically transformed in subsequent analyses: white blood cell (WBC) count, neutrophil, lymphocyte, monocyte, red blood cell (RBC) count, hematocrit (HCT), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), RBC distribution width (RDW), platelet count and mean platelet volume (MPV).

Statistical analysis

(1) Decomposition of variance components

For a statistical analysis, a nested random effects analysis of variance model (ANOVA) was used, assuming a standard constant variance; i.e., both within-person and analytical variances were constant at any level of the parameter [6]. With this model, the total variance (S^2_{total}) can be break down into three components – between-dog variance (S^2_{inter}), within-dog variance (S^2_{intra}), and analytical variance (S^2_{analy}) – and calculate as follows: $S^2_{total} = S^2_{inter} + S^2_{intra} + S^2_{analy}$ [1, 13-15].

(2) Reliability coefficient

The reliability coefficient (R) is the ratio of between-dog variance to total observed variance [16] and was computed using the following formula: $R = S^2_{inter} / S^2_{total}$. Thus, the R can be interpreted as the correlation between repeated measurements for a dog, and as such it could be estimated by the usual Pearson correlation coefficient if only two samplings were being considered. This value near unity thus indicates that a single measurement can be used to well classify a dog with respect to the parameter. The clinical equivalent to a low reliability coefficient is that a single individual's laboratory result tends to move around throughout the population-based reference range on repeated samplings.

(3) Index of individuality

Index of individuality was calculated using the following formula: $(CV^2_I + CV^2_A) / S^2_G$, where S^2_G is between-subject variance. This index has been used for assessing the usefulness of reference values objectively [3].

(4) Statistical models

Weekly differences in the same dog were assessed using the nonparametric Friedman ANOVA test, with dogs serving as blocking factors, followed by Duncan's multiple comparison. The variance component analysis was performed using PROC NESTED procedure of the Statistical Analysis System Version 8.1 (SAS, Cary, NC), and if negative variance components resulted, values of zero were used. A value of $P < 0.05$ was considered significant for all tests.

Table 1. Sample means, components of variance (expressed as percent coefficient of variation, CV %), index of individuality, coefficient of reliability (R) of some selected hematological values with normal duplicate blood samples using automated hematology analyzer

Analyte* (units)	No. sample	Sampled mean	Variance component (CV %)			Index of individuality	R
			Between-dog	Within-dog	Method		
WBC ($10^3/\mu l$)	156	12.5	12.7	5.1	16.1	0.91	0.45
Neutrophil ($10^3/\mu l$)	156	8.0	25.5	9.3	22.4	0.91	0.45
Lymphocyte ($10^3/\mu l$)	156	2.7	49.3	22.8	35.9	0.75	0.46
Monocyte ($10^3/\mu l$)	156	1.0	31.6	25.8	25.8	1.33	0.38
RBC ($10^6/\mu l$)	156	6.8	12.1	11.0	11.6	1.76	0.35
Hemoglobin (g/d*l*)	156	15.0	14.9	3.4	5.3	0.18	0.63
Hematocrit (%)	156	45.6	14.7	8.1	8.1	0.60	0.48
MCV (fL)	156	65.0	11.2	0.4	2.3	0.04	0.80
MCHC (g/d*l*)	156	33.0	6.1	4.8	6.7	1.82	0.35
RDW (%)	156	16.2	9.6	1.5	2.8	0.11	0.69
Platelet($10^4/\mu l$)	156	34.0	23.5	8.5	16.8	0.64	0.48
MPV (fL)	156	12.7	21.6	4.4	7.8	0.17	0.64

WBC, white blood cell count; RBC, red blood cell count; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; RDW, red blood cell distribution width; MPV, mean platelet volume.

Results

The individual components of variability for the various analytes and the reliability coefficients in four sequential measurements of the blood components are shown in Table 1. The indexes of individuality are also included as percentages of total variances, along with the CVs. The analytes in order of the increasing reliability coefficient and the decreasing index of individuality were as follows: MCHC, RBC, monocyte count, WBC, neutrophil count, lymphocyte count, platelet count, HCT, Hb, MPV, manual HCT, RDW, and MCV. Weekly differences for all analytes in the same dogs were not statistically significant although some variation between dogs was noted.

The index of individuality for all analytes except for the parameters RBC (Index = 1.76) and MCHC (Index = 1.82) were less than 1.4. The R ranged 0.38-0.46 for WBC parameters, 0.35-0.80 for RBC parameters, and 0.48-0.64 for platelet parameters.

Discussion

The high indices of individuality for the RBC and MCHC (i.e., the low degree of individuality) indicate that observed values could be compared usefully with population-based reference intervals [3]. In contrast, the parameters Hb, MCV, RDW and MPV were <0.6, such that comparing a single measurements on blood components from a dog to the conventional population-based reference range may be too insensitive to detect small but important changes in the blood components of that particular dog. In other words, some hematological values that are abnormal for those particular dogs may still be within the reference range. One solution to solve this problem is that the reference range could be subdivided according to sex, age and breed in order to more closely resemble the within-dog variation of blood analytes, although this approach may be too cumbersome. On the other hand though, a low index means that the index being measured could find value in tracking of a disease progression or the effectiveness of the treatment. The other parameters were ranged between 0.6-1.4 so that caution is warranted in comparing these hematological values to reference ranges in tracking an individual's values. Fraser and Harris [3, 4] indicated that most quantities of interest in laboratory medicine do have indices of individuality < 1.4, which is one of the reasons why many procedures are not very useful in detecting disease in screening programs; individuals may have values that are very unusual for them but that still fall within the reference limits.

Since within-dog and methodological variability simply are unwanted noise, it is desirable that these variations be small. Nevertheless, variability does exist and can affect the interpretation of the relation between a

particular risk factor and clinical course of the disease occurrence, therefore making it important to quantify the magnitude of these variance components. As an alternative ways of evaluating an animal's current status critical difference may be used [10, 11], in which the patient serves as its own reference using a comparison of analytical results from samples obtained serially at appropriate intervals. The critical difference allows consecutive analytical results to be compared and assists in determining whether the difference between two consecutive results can be safely ascribed to natural variation or whether it is caused by other factors such as disease, therapy or experimental procedures.

The R is numerically equivalent to the correlation coefficient for repeated measurements made on blood collected and analyzed in a laboratory at multiple time points. In this study, for the parameters Hb, RDW, manual HCT and MPV with relatively intermediate coefficient (range 0.63-0.69), a single measurement will moderately classify the participants with respect to his or her short-term average analyte concentration. Except for MCV (R=0.8) the coefficient of the remaining parameters were very low (range 0.35-0.48), suggesting that a single measurement of the analyte may not predict the real concentration or change in analyte concentration and thus may not predict clinical course of the disease. These findings were similar to the R (range 0.6-0.9) for commonly used chemistry analytes in human medicine [2]. Overall, the short-term reliability coefficients for hematological parameters in the present study were low ($R \leq 0.8$), suggesting that, single hematological measurements may not classify dogs with respect to their average concentrations of hematological parameters quite well. In other words, a typical individuals hematological results appear to variable within a relatively broad range even for one month of study period. This situation is also delineated by the high individuality between dogs, because the R and index of individuality move in opposite directions.

In conclusion, the overall intermediate R for WBC parameters (ranges 0.38-0.45) shows that these values in an individual are relatively unstable, such that a single measurement may not well characterizes an individual's average values. The indices of individuality for the parameters Hb, MCV, RDW and MPV were low (ranges 0.04-0.18). Therefore, the need for subject-based reference intervals may be necessary. On the other hand, this may attributable to the user's technical errors, inherent biological variation of the subjects or the limited efficiency of the analyzer.

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