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A Comparison of Complete Blood Cell Count in Canine Blood Samples Obtained from the Jugular Vein, Cephalic Vein and Lateral Saphenous Vein

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Abstract The purpose of this study was to compare the results of complete blood cell count (CBC) of blood samples collected from the jugular vein, cephalic vein and lateral saphenous vein and to find out if there were clinically significant differences. Total of 40 dogs were tested. CBC tests were conducted with blood samples obtained from the jugular vein, cephalic vein and lateral saphenous vein and manual differential count was performed to accurately distinguish the white blood cell (WBC) types. The results were analyzed using Repeated Measures ANOVA and post-hoc test was conducted using the least significant difference method. As a result, there was a statistically significant difference ($P < 0.05$) in the total WBC and monocyte count. The post-hoc test of total WBC counts revealed a significant difference between the jugular vein and cephalic vein, and the jugular vein and lateral saphenous vein. For monocyte counts, a significant difference was observed between the jugular vein and lateral saphenous vein.

***Key words*:** cephalic vein, complete blood cell count, dog, jugular vein, lateral saphenous vein.

Introduction

Complete blood cell count (CBC) is one of the most important parts of the minimum database used to check the patient's medical and physiological status through the quantitative and morphological evaluation of blood cells (5). The database evaluates the clinical state including the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) of red blood cell (RBC), hematocrit (HCT), reticulocyte count, total WBC count, differential white blood cell (WBC) count, and platelet count (5). For patients with a hematologic disease, a regular CBC examination is required and accurate results are important for the purpose of monitoring.

Physiological factors such as signalment, prandial state, exercise, lactation, pregnancy, and growth period are factors that can affect the lab test result. There are other factors such as hemolysis, lipemia, pre-existing medication usage, wrong sampling method, wrong anticoagulant usage, and any delay in the analysis (1,12). For reliable results, these factors should be excluded or the results should be reinterpreted based on previous reports.

Generally, the veins used to obtain blood from dogs are the jugular vein, cephalic vein, and lateral saphenous vein (18). However, clinically accessing these veins can be challenging due to several reasons including intravenous catheterization of the patient, vein damage due to frequent blood sampling, aggressive or uncooperative behavior of the patient and reluctance of the owner. If the results of blood tests are different according to the blood sampling site, it could be unreliable to diagnose or monitor the patient using samples taken from different blood vessels. While several human studies compared the CBC results of different blood collection sites based on convenience and practicality, it is not well explored in veterinary studies.

Therefore, the goal of this study is to compare the CBC results of blood samples obtained from the jugular vein, cephalic vein and lateral saphenous vein, and evaluate whether there are biological variations from the use of different veins that affect the lab results in dogs.

Materials and Methods

Animal preparation

Subjects were client-owned patients that visited Gyeongsang National University Veterinary Medical Teaching Hospital including clinically normal dogs. Twenty three male and seventeen female dogs (nine Beagles, six Maltese, six mongrels, four Shih Tzu, two Chihuahuas, two Pomeranians, two Golden Retrievers, two Yorkshire Terriers, one Cocker Spaniel, Poodle, Pit bull Terrier, Schnauzer, Old English Bulldog, Japanese Chin and Samoyed) with an age range between 1 years and 15 years (median, 9 years; standard deviation [SD], 4.5 years) and a weight range between 2 kg and 30 kg (median, 8 kg; SD, 7.3 kg) were used for the collection of blood samples.

Blood sampling

Blood was collected from the jugular vein, cephalic vein

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and lateral saphenous vein. The order of the venous site from each dog for blood sampling was determined randomly. The same person collected all blood samples through a 26 gauge, 1 cc syringe keeping as low vacuum pressure as possible to prevent hemolysis until all 0.5 ml of blood had been collected. The delay time between blood collections from the different sites was not exceeded by 2 minutes, and immediately after sampling the blood was collected in an EDTA tube.

Procedure of CBC

The ProCyte Dx Hematology Analyzer (IDEXX Laboratories Inc.; Westbrook, USA) was used for automatic blood cell count. We used various data from the device including RBC count, hematocrit, hemoglobin, MCV, MCH, MCHC, reticulocyte and total WBC count. Manual differential count was performed to accurately distinguish the WBC types. Manual differential counts were prepared with thin blood smears stained by diff quik method. Using the microscope, two veterinarians counted the WBC and the mean value was used. All analyses were performed within 3 hours after sampling.

Statistical analysis

All CBC results were expressed as mean values ± SD. Since blood was collected three times from different sites of the same dog, Repeated Measures ANOVA was used to compare the CBC results, correlation within an individual and high variance between individuals. Least significant difference was used as post hoc test. Values of $p < 0.05$ were considered statistically significant.

Statistical analyses were performed with Statistical Package for the Social Sciences version 22.0 (IBM Co.; New York, USA).

Results

RBC parameter value

Anemia (hematocrit < 37%) was identified in 8 dogs (severe 1, moderate 1, mild 6). The mean value and SD of hemoglobin, HCT, MCH, MCHC, MCV, RBC counts and reticulocyte counts are listed in Table 1. There were no statistically significant differences.

Platelet value

Thrombocytosis (platelet count > 484×10^3 cells/μL) was identified in 10 dogs and thrombocytopenia (platelet count < 148×10^3 cells/μL) was identified in 2 dogs. The mean value and SD of the platelet counts are listed in Table 2. There were no statistically significant differences.

WBC value

Leukocytosis (total WBC count > 16.8×10^3 cells/μL) was identified in 6 dogs. The total WBC counts from 3 dogs extracted from the jugular vein were within the reference range, while the cephalic or lateral saphenous vein displayed leukocytosis over the reference range (Table 3). The mean value and SD of total WBC, monocyte, lymphocyte, band neutrophil, segmented neutrophil and eosinophil counts are listed in Table 4. Statistically significant results with p values of 0.023 and 0.045 were obtained for total WBC count and monocyte count, respectively. The post-hoc analysis of total WBC counts revealed a significant difference of 658 cells/μL between the jugular vein and cephalic vein, and a significant difference of 510 cells/μL between the jugular vein and lateral saphenous vein (Table 5). For monocyte counts, a significant difference of 138 cells/μL was observed between the jugular vein and lateral saphenous vein (Table

Table 1. Blood cell count results of RBC in each vein, mean ± SD (n = 40)

Hematologic variables	Unit	Jugular vein	Cephalic vein	Lateral saphenous vein	p Value
HB	g/dl	14.78 ± 3.36	14.66 ± 3.22	14.80 ± 3.11	0.445
HCT	%	43.43 ± 9.40	43.16 ± 9.08	43.42 ± 8.60	0.708
MCH	Pg	22.18 ± 1.39	22.15 ± 1.38	22.15 ± 1.36	0.604
MCHC	g/dl	33.90 ± 1.71	33.87 ± 1.71	33.97 ± 1.73	0.518
MCV	fL	65.63 ± 5.89	65.59 ± 5.62	65.46 ± 5.89	0.413
RBC	10^{12}/L	6.69 ± 1.54	6.65 ± 1.51	6.70 ± 1.44	0.616
Reticulocyte	10^3/μL	72.64 ± 84.07	74.45 ± 86.37	77.22 ± 97.15	0.218

HB, hemoglobin; HCT, hematocrit; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; RBC, red blood cell; Pg, picogram; fL, femtoliter.

Table 2. Blood cell count results of platelet in each vein, mean ± SD (n = 40)

Hematologic variables	Unit	Jugular vein	Cephalic vein	Lateral saphenous vein	p Value
Platelet	10^9/L	419 ± 220	407 ± 227	400 ± 252	0.341

Table 3. Total WBC counts in borderline leukocytosis subjects (10^9/L)

	Jugular vein	Cephalic vein	Lateral saphenous vein
Dog A.	14.03	16.07	17.63
Dog B.	14.65	14.8	17.05
Dog C.	16.54	17.23	18.03

Table 4. Blood cell count results of WBC in each vein, mean ± SD (n = 40)

Hematologic variables	Unit	Jugular vein	Cephalic vein	Lateral saphenous vein	*p* Value
WBC	10^9/L	14.56 ± 11.57	15.21 ± 12.20	15.06 ± 11.68	0.023
Monocyte	10^6/L	990 ± 539	1,074 ± 671	1,128 ± 666	0.045
Lymphocyte	10^6/L	1,663 ± 849	1,833 ± 1,153	1,711 ± 914	0.313
Band neutrophil	10^6/L	567 ± 1,308	448 ± 898	489 ± 1,205	0.397
Segmented neutrophil	10^6/L	10,792 ± 10,200	11,126 ± 11,213	11,123 ± 10,150	0.335
Eosinophil	10^6/L	454 ± 455	504 ± 555	494 ± 522	0.582

Table 5. Post-hoc analysis of total WBC counts

Sampling site		Mean difference (10^9/L)	Significance level
Jugular vein	Cephalic vein	−0.658	0.005
	Lateral saphenous vein	−0.510	0.044
Cephalic vein	Jugular vein	0.658	0.005
	Lateral saphenous vein	0.149	0.580
Lateral saphenous vein	Jugular vein	0.510	0.044
	Cephalic vein	−0.149	0.580

Table 6. Post-hoc analysis of monocyte counts

Sampling site		Mean difference (10^6/L)	Significance level
Jugular vein	Cephalic vein	−84.206	0.190
	Lateral saphenous vein	−138.070	0.005
Cephalic vein	Jugular vein	84.206	0.190
	Lateral saphenous vein	−53.864	0.261
Lateral saphenous vein	Jugular vein	138.070	0.005
	Cephalic vein	53.864	0.261

Table 7. Blood cell count results of WBC and monocyte in each vein from dogs over 7 kg, mean ± SD (n = 22)

Hematologic variables	Unit	Jugular vein	Cephalic vein	Lateral saphenous vein	*p* Value
WBC	10^9/L	16.40 ± 14.74	16.86 ± 15.72	16.53 ± 15.02	0.501
Monocyte	10^6/L	1,034 ± 568	1,147 ± 841	1,122 ± 804	0.336

Table 8. Blood cell count results of WBC and monocyte in each vein from dogs less than 7 kg, mean ± SD (n = 18)

Hematologic variables	Unit	Jugular vein	Cephalic vein	Lateral saphenous vein	*p* Value
WBC	10^9/L	12.30 ± 5.38	13.20 ± 5.34	13.27 ± 5.31	0.000
Monocyte	10^6/L	936 ± 511	985 ± 382	1,136 ± 470	0.027

6). The statistical processing was implemented once again for total WBC and monocyte, by dividing the subjects into a group that is heavier than 7 kg and a group that is lighter than 7 kg. In heavier than 7 kg group, the result of difference according to blood vessels was insignificant (Table 7). And the *P* value turned out to be lower in the lower than 7kg group (Table 8).

Discussion

This study compared the CBC results of blood samples from the jugular, cephalic and lateral saphenous veins. Statistically significant differences were found in the total WBC and monocyte counts. Compared to the 14% difference between the jugular and lateral saphenous vein for monocytes, only 4.5% (658 cells/μl) and 3.5% (510 cells/μl) difference was found between the jugular and cephalic veins, and between the jugular and lateral saphenous veins, respectively for WBC. In the biological variability study, it was reported that the maximum allowable analytical imprecision was 6.1% for the WBC counts in normal dogs (8). Generally, any difference of less than 5% was interpreted as an allowed error caused by the blood analyzer equipment. It is hard to consider a difference of less than 5% in WBC counts as a factor to influence the degree and existence of leukocytosis. Additionally, only 3 out of 40 dogs in the present study showed a total WBC count from the jugular vein within the reference range, while the cephalic or lateral saphenous vein

displayed leukocytosis over the reference range (Table 3). Results from the jugular vein were within the high margin of the reference range while results from the other veins were within the low margin range that indicates leukocytosis (Table 3). Considering all these factors, there is relatively less relevant difference that can be deduced clinically in the total WBC counts across all veins.

Previous veterinary studies explored the comparison of blood gases between the venous and arterial blood in dogs, and the comparison of CBC and chemistry results of blood from the jugular vein and cephalic vein (7,9). The latter study found that comparison between the jugular and cephalic veins in 23 dogs did not show any significant differences in CBC, which contradicts the findings in our study (9). Also, the study reported a higher level of creatinine and potassium in the jugular veins (9). Other veterinary studies reported higher hemoglobin concentration, total erythrocyte count and packed cell volume in the peripheral blood from the ear compared to blood from the femoral vein of African green monkeys and infant baboons (2). It also explored the difference in blood from a bovine coccygeal vein and jugular vein (14).

There are a lot of human studies comparing the capillary and venous blood since it is easy to access the capillaries at sites such as the fingers. While the sample characteristic and blood collection method differed slightly across studies, the overall results showed high hemoglobin, HCT, RBC count, MCV and total WBC count, and a low number of platelets in capillaries (3,4,10,11,16,17,19). The differential counts of WBC were shown to be high for large leukocytes including granulocytes and monocytes, and low for lymphocytes in capillaries (3,13,16,19). While there are no studies that support this finding, each study introduced several theories. Firstly, hemoconcentration could occur when fluid leaks into the tissue (3). In situations like this, WBC and RBC counts may increase. Secondly, platelets are activated during blood sampling as the tissues receive external pressure (11). Thirdly, during blood sampling the blood vessels are disrupted and WBCs marginated on the blood vessel wall come out (15). Lastly, laminar flow occurs when collecting blood with a more rapid and central stream of concentrated WBC and RBC in capillaries, while platelets are relatively displaced along the vessel wall (3). Our study is unique as it focuses on the venous blood collected from the jugular vein, cephalic vein and lateral saphenous vein, compared to previous studies that used peripheral capillary and venous blood in human trials, and studies with infant baboons and African green monkeys. Unlike findings in previous studies, most of the parameters in this study, including RBC and platelet counts showed no significant differences. However, the total WBC and monocyte counts supported the findings in previous studies which showed significantly higher counts in the vessels with a smaller diameter compared to those of the jugular vein. Although this study does not provide a clear basis for these findings, we estimate that it is due to the possibility of disruption of the marginated WBCs during the blood sampling process, as proposed by a previous study (15). In practice, it is easier for WBC margination to occur in smaller vessels (6).

A previous study reported by Jensen *et al.* compared the jugular and cephalic veins and showed a lack of differences in the CBC results. One possible explanation could be that the previous study involved large breed dogs (eight Beagles, seven mongrels, two German Shepherds, one German Short Hair, one Irish Terrier, one Bull Terrier, one Poodle, one Newfoundland and one Cocker Spaniel), which could have a small influence on different vein sizes, compared to the current study involving mainly small to medium sized dogs (9). In order to check this, the statistical processing was implemented for total WBC and monocyte, which had a significant outcome, by dividing the subjects into a group that is heavier than 7 kg and a group that is lighter than 7 kg. As expected, it was confirmed that the outcome difference according to blood vessels was insignificant in heavier-than-7 kg group (Table 7), and the p value turned out to be lower in the lower-than-7 kg group (Table 8). It is understood that the outcome difference according to blood vessels is more significant in small-sized dogs, and the effect due to the sampling site will need to be considered more for small-sized dogs in actual clinical practice as well.

This study had several limitations as follows. Many of the subjects were small breed dogs like Maltese, Yorkshire Terrier, Chihuahua and Pomeranian, and they were client-owned. Since drawing blood from the lateral saphenous vein with a large gauge needle can cause problems including injury to the blood vessels resulting in contusion, and the need for a longer time to achieve hemostasis, small gauge needles were used instead. Although the blood was drawn slowly to minimize pressure, we cannot completely rule out the possibility that it impacted the result due to factors including hemolysis of RBC. Additionally, we could not provide a clear explanation for the differences in our results, and since this study involved three different types of veins, there was a limitation in obtaining the result using various statistical analyses.

The present study reports a comparison of the CBC results of canine blood samples from the jugular vein, cephalic vein, and lateral saphenous vein. Statistically significant differences were observed in the total WBC and monocyte counts. Interpreting the CBC results considering that there may be differences in the WBC count and monocyte count when taken from the jugular vein, cephalic vein and lateral saphenous vein is reliable.

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