

Evaluation of Iron Deficiency Using Reticulocyte Indices in Dogs Enrolled in a Blood Donor Program

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Background: People donating blood more than twice annually are at risk of developing iron deficiency. Little is known about the iron status of dogs enrolled in blood donor programs.

Hypothesis: Dogs donating blood ≥ 6 times annually will show evidence of iron deficiency based on their reticulocyte indices.

Animals: Thirteen dogs enrolled in a blood donor program donating ≥ 6 times over the preceding 12 months and 20 healthy nondonor control dogs.

Methods: Prospective observational study. Mature red blood cell (RBC) indices, reticulocyte indices, serum iron, serum ferritin, and total iron-binding capacity (TIBC) were compared between groups.

Results: Packed cell volume (median 47%, range 40–52%, $P < .01$), hematocrit (median 46.4%, range 40.3–52.5%, $P < .01$), and reticulocyte count (median 16,000/ μL , range 9,000–38,000/ μL , $P < .01$) were significantly lower in the blood donor dogs. No statistically significant differences were noted in the mature RBC indices between groups. Both reticulocyte mean corpuscular volume (median 88.8 fL, range 83.4–95.5 fL, $P = .03$) and reticulocyte hemoglobin content (median 24.6 pg, range 23.1–26.6 pg, $P < .01$) were significantly lower in the blood donor group. Serum iron and ferritin were similar between groups; however, TIBC was significantly higher in the control group (median 403 $\mu\text{g/dL}$, range 225–493 $\mu\text{g/dL}$, $P = .02$).

Conclusions: The findings in dogs donating ≥ 6 times annually suggest the presence of iron-deficient erythropoiesis in this population.

Key words: Canine; Deficient; Donation; Hemoglobin content.

The 3 compartments for iron distribution within the body related to erythroid production are the storage, transport, and functional compartments.^{1,2} Repeated blood donation in people is a significant cause of depleted body iron stores after as few as 3–4 donations annually.^{3,4} The REDS-II Iron Status Evaluation (RISE) study evaluating human blood donors at enrollment recently reported the incidence of iron-deficient erythropoiesis (IDE) in people donating ≥ 3 times annually as 48.7% in men and 66.1% in women.⁵

Currently, there are no specific criteria describing an appropriate blood donor dog; however, most programs require that dogs weigh >23 kg, are aged between 1 and 7 years, and are in good health.⁶ Dogs donating 15–20% total blood volume (TBV) every 3–4 weeks for 1 year showed no evidence of anemia and maintained a normal mean corpuscular volume (MCV), while dogs donating 15–20% TBV every 1–2 weeks developed a

Abbreviations:

CH	hemoglobin content
CH _m	hemoglobin content of mature RBC
CH _r	hemoglobin content of reticulocytes
EDTA	ethylenediaminetetraacetic acid
%Hypo _m	percentage of hypochromic mature RBC
%Hypo _r	percentage of hypochromic reticulocytes
IDE	iron-deficient erythropoiesis
MCV	mean corpuscular volume
MCV _m	mean corpuscular volume of mature RBC
MCV _r	mean corpuscular volume of reticulocytes
RBC	red blood cell
RISE	Retrovirus Epidemiology Donor Study II (REDS-II) Iron Status Evaluation
TBV	total blood volume
TIBC	total iron-binding capacity
UWVC	University of Wisconsin Veterinary Care

microcytic hypochromic anemia suggestive of iron deficiency as quickly as 4 weeks.⁷

Newer generation hematology analyzers have the capacity to directly measure individual red blood cell (RBC) and reticulocyte indices, including the percentage of hypochromic mature RBCs (%Hypo_m) and reticulocytes (%Hypo_r), the hemoglobin content of mature RBCs (CH_m) and reticulocytes (CH_r), and the MCV of mature RBCs (MCV_m) and reticulocytes (MCV_r).⁸ An earlier study evaluating iron deficiency anemia of all causes in women reported the area under the receiver-operator characteristic curve for %Hypo_m and CH_r as 0.98 and 0.86, respectively.⁹ A report evaluating people donating blood found the sensitivity for ferritin detecting iron deficiency was as low as 61.7% and for hemoglobin was as low as 10.6%.¹⁰ In their study population, the RISE study found %Hypo_m to be the superior RBC index for detecting IDE with a sensitivity and specificity of 72 and 68%, respectively.¹¹

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Dogs with a low CH_r have evidence of iron deficiency with a significantly lower hematocrit, MCV, serum iron, and percent saturation of transferrin compared to dogs with a normal CH_r .¹² In dogs fed an iron-deficient diet, CH_r is one of the markers superior to conventional RBC indices for diagnosing iron deficiency.¹³ Blood donor dogs do not appear to have iron deficiency with total iron-binding capacity (TIBC) significantly increased and percent transferrin saturation significantly decreased with repeated blood donations.¹⁴ Furthermore, blood donation induces bone marrow regenerative responses that can restore depleted blood cells within 10 days after collection.¹⁵

The purpose of our study was to evaluate dogs enrolled in a blood donor program for evidence of iron deficiency by evaluating both standard markers of iron stores as well as RBC and reticulocyte markers. Our hypothesis was that dogs donating at least 6 times annually would have RBC and reticulocyte markers showing evidence of iron deficiency whereas serum ferritin would fail to demonstrate iron deficiency.

Methods

Inclusion Criteria

Dogs participating in the University of Wisconsin Veterinary Care (UWVC) blood donor program and that donated ≥ 6 times of the prior 12 months were enrolled in a prospective observational study. The cut-off of 5 donations over 12 months was selected because people have progressive development of iron deficiency following the third, fourth, and fifth donations in a 12-month period.³⁻⁵ To participate in UWVC blood donor program, dogs were required to be >23 kg, between 1 and 9 years of age, and deemed healthy on the basis of physical examination and annual CBC, serum chemistry profile, and infectious disease screening.

Healthy control dogs owned by veterinary students or staff of the UWVC or client-owned dogs presenting for routine preventative health care through the primary care service were recruited. In order to be considered, dogs had to fill the age and weight requirements of the blood donor program. In order to be enrolled, all control dogs had to be healthy on the basis of history, physical examination, CBC, and serum chemistry profile.

Exclusion Criteria

Any dog receiving iron supplementation was not included in either group. The blood donor population did not include any breed with known microcytosis (Akitas, dogs of Korean descent),^{16,17} and these breeds were excluded from the control population. Any dog with a history of receiving a transfusion could not be included in the UWVC blood donor program and was excluded from the healthy control population.

Data Collection

At the time of a regularly scheduled blood donation, each of the blood donor dogs had 5 mL additional blood drawn before donation: 1 mL into an ethylenediaminetetraacetic acid (EDTA)-containing tube for a CBC and RBC and reticulocyte indices evaluation, and 4 mL into a red-top tube for collection of serum for measurement of serum iron, serum ferritin, and TIBC. The healthy control dogs also had 5 mL blood drawn: 1 mL into an EDTA-containing tube for a CBC and RBC and reticulocyte indices eval-

uation, and 4 mL into a red-top tube for collection of serum for chemistry analysis. Sera from control dogs with values within reference intervals on their screening CBC and serum chemistry profile were subsequently used to measure serum iron, serum ferritin, and TIBC.

A new-generation hematology analyzer^a was used to measure hematocrit, MCV, and CH as well as %Hypo_m, %Hypo_r, MCV_m, MCV_r, CH_m, and CH_r. EDTA samples were maintained at room temperature and analyzed within 60 minutes. Because hemolysis falsely increases serum iron in people,¹⁸ any sample with gross evidence of hemolysis in either the plasma or serum was redrawn. Sera were harvested and frozen at -20°C for a maximum of 4 days and shipped overnight on dry ice to the Kansas State University Comparative Hematology Laboratory for measurement of serum iron, serum ferritin, and TIBC.

Statistics

Sample size calculations were performed using previously established data for both mean and standard deviation of canine RBC and reticulocyte indices.¹³ A sample size of 8 dogs would provide 83% power to find, as significant, a decrease of 5 fL in MCV between donor and control dogs, with $\alpha = 0.05$, and would provide 93% power to find, as significant, a decrease of 6 pg in CH_r . Twelve dogs would provide adequate statistical power to find a decrease of 160 ng/mL in serum ferritin (81% power at $\alpha = 0.05$). Based on these calculations, we enrolled 13 active blood donors that met the inclusion criteria.

All data are presented as median with observed ranges. Because of the low number of enrolled dogs, all analyses performed were of nonparametric nature. All comparisons between groups were performed using a Mann-Whitney *U*-test with $P < .05$ considered significant.

Results

Enrolled Patients

Thirteen dogs enrolled in the UWVC blood donor program were included in this study; the median age and weight of included dogs were 4.2 years (range, 1.9–8.7 years) and 28.8 kg (range, 25.5–39.1 kg), respectively. There were 9 male dogs (MI, 2/9; MC, 7/9) and 4 female dogs (FI, 1/4; FS 3/4). Major breeds represented included: Doberman pinchers (4), Labrador retrievers or Labrador retriever mixed breed (3), and Golden retrievers or Golden retriever mixed breed (3). The median number of blood donations performed in the last 12 months was 8 (range, 6–9).

Twenty dogs were included in the control population. All CBC and chemistry values were within reference intervals in all control dogs with the exception of a single dog having an increased creatinine (1.6 mg/dL; reference interval: 0.5–1.5 mg/dL). This dog was included after a urine specific gravity of >1.040 indicated adequate renal function. The median age and weight of control dogs were 4.2 years (range 2.0–7.4 years) and 29.3 kg (range, 23.2–50.0 kg), respectively. There were 15 male dogs (MI, 1/15; MC, 14/15) and 5 female dogs (FS, 5/5). Major breed representation included: Labrador retrievers or Labrador retriever mixed breed (5) and Golden retrievers or Golden retriever mixed breed (3). No statistically significant difference was found in age ($P = .50$) or weight ($P = .65$) between groups.

RBC, Reticulocyte, and Iron Indices

Dogs in the donor group had significantly lower PCV and hematocrit than the control group ($P < .01$); no dog in either group had a PCV or hematocrit outside the reference interval. There were no differences in conventional RBC indices between the donor or control groups (Table 1). No significant difference was found between groups for mature RBC indices; however, the reticulocyte count ($P < .01$), MCV_r ($P = .03$), and CH_r ($P < .01$) were significantly lower for the donor group than for the control group (Table 1). Total iron-binding capacity was significantly lower ($P = .02$) in the donor group compared to the control group (Table 1). There were no significant differences in serum iron or serum ferritin between the groups (Table 1).

Discussion

Based on decreased reticulocyte indices, the results of our study suggest that iron deficiency or IDE might occur in dogs donating blood ≥ 6 times annually. Notably, traditional markers of iron deficiency (including MCV, serum ferritin, and serum iron) were not different between the donor and control groups. Although both previous studies examining iron status in blood donor dogs have reported changes suggesting iron deficiency,^{14,15} our study uses RBC and reticulocyte indices support the diagnosis of iron deficiency and IDE.

The %Hypo_m is the most sensitive RBC or reticulocyte marker to detect iron deficiency in people.⁹⁻¹¹ %Hypo_m was not assessed in earlier studies but, relative to conventional indices, CH_r appeared to be a more sensitive RBC or reticulocyte index for detection of iron deficiency.^{12,13} In our study, we found CH_r to be significantly lower in the donor group, whereas there was no difference in %Hypo_m between groups. MCV_r was significantly lower in the blood donor group in our study, as in an earlier report.¹²

In addition to being relatively sensitive markers, thus permitting earlier detection of iron deficiency, the indices assessed in this study are routinely obtained by the newer generation hematology analyzers. These values are measured whenever a CBC and reticulocyte count is performed, making this data readily available. Serum iron, ferritin, and TIBC, by contrast, generally are associated with both a longer turnaround time and an increased cost.

Although both the hematocrit and PCV were within our reference intervals for all donor dogs, the median values for both variables were significantly lower in donor dogs compared to control dogs. Depleted blood cells can be restored within 10 days of donation,¹⁵ suggesting that the interval between donations is adequate. However, repeated donation leading to IDE might permit only a blunted regenerative response. Further study comparing serial reticulocyte counts after donation could better elucidate the regenerative response in dogs

Table 1. Red blood cell (RBC) measurements and indices, mature RBC indices, reticulocyte count, reticulocyte indices, and iron indices for both the blood donor group and the control group. All data are presented as median (minimum, maximum). Reference intervals (RI) are included. UWVC RI is included for the reticulocyte count.

Variable	Donor group	Control group	P-value
PCV (%)	47.0 (40.0, 52.0)	50.5 (46.0, 56.0)	<.01*
RI: 40-59			
Hematocrit (%)	46.4 (40.3, 52.5)	50.6 (44.9, 57.5)	<.01*
RI: 39-57			
MCV (fL)	67.4 (65.8, 72.0)	68.6 (65.0, 74.4)	.10
RI: 61-73			
CH (pg)	24.5 (23.0, 25.5)	24.8 (23.5, 26.9)	.20
RI: 22-26			
MCV_m (fL) ^a	71.5 (69.0, 74.6)	72.6 (70.4, 79.8)	.14
CH_m (pg) ^a	23.7 (22.3, 24.5)	24.0 (22.7, 25.8)	.17
%Hypo _m (%) ^a	0.3 (0.1, 1.8)	0.3 (0.1, 1.3)	.76
Reticulocyte count (/ μ L)	16,000 (9,000, 38,000)	36,000 (12,000, 74,000)	<.01*
RI: 13,000-102,000			
MCV_r (fL) ^a	88.8 (83.4, 95.5)	90.8 (87.2, 97.5)	.03*
CH_r (pg) ^a	24.6 (23.1, 26.6)	25.9 (24.4, 27.3)	<.01*
%Hypo _r (%) ^a	52.1 (28.2, 78.4)	43.0 (31.3, 68.9)	.31
Serum iron (μ g/dL)	198 (124, 407)	212 (80, 321)	.22
RI: 88-238			
Serum ferritin (ng/mL)	308 (143, 692)	362.5 (183, 763)	.37
RI: 80-800			
Total iron-binding capacity (μ g/dL)	379 (318, 408)	403 (225, 493)	.02*
RI: 246-450			

^aRI have not been established.

*Denotes a significant difference ($P < .05$) between groups.

donating a single time compared to ≥ 6 times annually. Importantly, assessment of hematocrit or PCV alone using population-based reference intervals before blood donation would fail to detect iron deficiency or IDE.

The absolute automated reticulocyte count was also significantly lower in the donor group compared to the control group. Given the recent loss of blood through donation, one would anticipate a similar or potentially increased reticulocyte count in the donor dogs. The automated reticulocyte count in our study was evaluated immediately before a donation, or approximately 7 weeks after a donation, therefore the reticulocyte count might have been increased above the control group in the days immediately after donation. Ten days after donation, dogs had a mean manual reticulocyte count of $91,203/\mu\text{L}$ after donating 13% TBV every 2 months for 1 year.¹⁵ Although the precise volume of blood donated was not recorded for the purposes of this study, our donation goal was 400–450 mL. If this goal were achieved, dogs in our study would donate a median of 15–17% TBV with a donation interval less than every 2 months (median of 8 blood donations in the preceding 12 months). In people with IDE, administration of intravenous iron leads to an increase in the reticulocyte count within 14 days.¹⁹ It is possible that with an increased percentage of blood volume donated and a decreased interval between donations, the donor dogs in our study exhibited IDE leading to a suppressed absolute reticulocyte count.

An unexpected finding was the significantly lower TIBC in the dogs in the donor group. Although increased TIBC is a feature of iron deficiency in some species, this response does not appear to be consistent in dogs.^{20,21} Our finding might simply be the result a smaller sample size and further study assessing a greater number of dogs grouped according to the number of donations over a defined interval would be required to evaluate this finding further.

The differences detected in this study might initially seem to be of minimal clinical importance. However, vague but potentially serious clinical signs are associated with iron deficiency, including fatigue, impaired physical and cognitive abilities, and impaired immune function.^{22–24} Given the evidence supporting IDE in blood donor dogs, combined with negative effects of iron deficiency, further evaluation is warranted. The health of blood donors has become an important consideration in human medicine,²⁵ and should be more strongly considered and evaluated in veterinary medicine.

Further study should evaluate larger groups of dogs stratified based on their donation frequency. In addition, blood donor dogs should be evaluated on the basis of receiving or not receiving iron supplementation. Finally, the RBC and reticulocyte indices evaluated in this study should be measured more frequently between donations to evaluate their biologic variation and over the course of a year to determine if progressive changes are noted.

One weakness in our study is the low number of blood donors. However, a power analysis performed before initiation of the study using previously reported

data,¹³ found that enrollment of ≥ 12 dogs would permit detection of decreased MCV_r , CH_r , and serum ferritin with $>80\%$ power. Inclusion of a greater number of dogs might have strengthened our data; however, only 13 blood donor dogs, all of which were included, fulfilled the inclusion criteria.

Another potential weakness is the omission of bone marrow aspiration to assess for stainable iron. However, serum ferritin levels correlate with body iron stores assessed via bone marrow sampling in dogs,²⁶ and MCV and CH might decrease before serum ferritin in dogs fed an iron-deficient diet, and therefore might be more sensitive than serum ferritin for the diagnosis of iron deficiency.¹³ With the advent of newer generation hematology analyzers, RBC and reticulocyte indices have been demonstrated in both people^{9–11} and dogs^{12,13} to be sensitive markers of iron deficiency. Because of the documented correlation of serum ferritin and body iron stores, as well as the perceived difficulty in recruiting both donor dogs and control dogs if bone marrow sampling were required, we elected not to evaluate iron stores in the bone marrow.

In conclusion, the results reported in this study suggest that dogs donating blood ≥ 6 times annually are at risk of developing of iron deficiency and IDE. Further study is necessary to determine an accepted frequency of donation and whether or not oral iron supplementation is beneficial in this population of dogs.

Footnote

^a ADVIA 120 Hematology System, Siemens Medical Solutions USA, Malvern PA

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Conflict of Interest Declaration: Authors disclose no conflict of interest.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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