Effects of repeated blood donations on iron status and hematologic variables of canine blood donors

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Objective—To evaluate the bone marrow regenerative response and iron status of canine blood donors subjected to repeated blood collections for 1 year.

Design—Prospective cohort study.

Animals—57 blood donor dogs.

Procedures—Hematologic variables, including reticulocyte percentage, were evaluated before and 10 days after each blood collection in 16 dogs donating 13% of total blood volume (TBV) every 2 months (group 1), 16 dogs donating 13% of TBV every 3 months (group 2), and 25 dogs donating 15% of TBV every 3 months (group 3) for 1 year. Serum concentrations of iron, transferrin, and ferritin were analyzed before inclusion in the study and 10 days after the last donation.

Results—Significant increases in RBC distribution width, platelet count, WBC count, and reticulocyte percentage were detected after blood donation in all groups. Dogs of group 2 had a significantly higher serum ferritin concentration than did dogs of group 1; dogs of group 1 had a significant decrease in serum ferritin concentration. A positive correlation between the number of blood donations and both RBC distribution width and reticulocyte percentage was found for all groups.

Conclusions and Clinical Relevance—All blood donation regimens induced a bone marrow regenerative response, which was able to restore depleted blood cells within 10 days after blood donation while maintaining iron status within the calculated reference range. However, dogs donating 13% of TBV every 2 months had a significant decrease in iron stores, which suggested that iron-related variables must be monitored during prolonged blood donor programs. (*J Am Vet Med Assoc* 2014;244:1298–1303)

An increase in the demand for blood components associated with better emergency and critical care treatments has led to the creation of several animal blood banks as well as an increase in the number of animals that provide frequent blood donations. As a consequence, the safety and bioethics for frequent blood donations with regard to donor well-being deserve special attention. Although many transfusion-related issues (eg, blood compatibility and transfusion efficacy) have been evaluated, there is a paucity of reports on donor care, and to the authors' knowledge, none have addressed the safety of animals providing frequent blood donations.

In humans, a large study[1] on transfusion medicine revealed that among frequent donors, there was iron-deficient erythropoiesis in 66.1% of women and 48.7% of men and no iron stores (serum ferritin concentration < 12 ng/mL) in 27.1% of women and 16.4% of men. In another study[2] performed in healthy human blood donors in Norway, repeated donations without iron supplementation led to significant reductions in hemoglobin concentration in women and serum ferritin concentrations in women and men. Guidelines for human blood banks recommend a maximum of 4 donations/y for men and 3 donations/y for women, with intervals of ≥ 3 months between donations.[3,4] Furthermore, each procedure must result in a depletion of ≤ 13% of TBV.[3,4] Several protocols have been reported in the veterinary literature, with blood collections ranging from 10 to 22 mL/kg (4.5 to 10 mL/lb) every 21 to 28 days without nutritional iron supplementation[5–9] or every 10 to 21 days with nutritional iron supplementation.[10] However, the lack of long-term studies of dogs that are frequent blood donors limits knowledge of the cumulative effects of such frequent blood collections.

Abbreviations

MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
RDW	RBC distribution width
TBV	Total blood volume

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Iron status is the major limiting factor for frequent human blood donations,[11–14] and iron depletion is the most important medical reason for deferral of blood collections from humans who are frequent blood donors.[12] Dogs have approximately 20 to 50 mg of iron/kg (9.1 to 22.7 mg/lb), most of which is in the hemoglobin of RBCs at concentrations of 0.5 mg/mL of blood.[15] For a typical blood donation of 450 mL, approximately 225 mg of iron are depleted, which can lead to iron deficiency in dogs that are frequent blood donors.[16] Although hemoglobin represents the largest pool of iron, other pools of iron, such as ferritin (an intracellular protein that stores iron[17]) and serum iron, are preferentially mobilized in iron-deficient states.[18] Thus, although iron-deficiency anemia may be easily identified by a low Hct, low hemoglobin concentration, and decreased MCHC and MCV, these variables cannot be used to identify the development of iron depletion from tissues, which always precedes iron-deficiency anemia.[19] Therefore, prevention of iron-deficiency anemia in frequent blood donors should rely on iron biochemical variables, which are more sensitive for the identification of preclinical iron-deficient states.[1,2,11,12,18,20]

Erythropoiesis is regulated by tissue oxygenation, which depends on the number of circulating erythrocytes, respiratory and cardiovascular function, and atmospheric oxygen concentration.[21] After blood depletion, the induced tissue hypoxia stimulates erythrocyte production in the bone marrow,[22] which can be identified by increases in the Hct and hemoglobin concentration within 2 to 4 days as well as by increases in reticulocyte counts and RDW. Splenic contraction also contributes to postdepletion increases of the Hct and hemoglobin concentration, but this is an acute and transitory phenomenon that lasts for only approximately 1 hour.[23,24]

Humans who are frequent blood donors have a low circulating hemoglobin concentration, Hct, MCHC, serum iron concentration, total iron binding capacity, serum ferritin concentration, and transferrin saturation, with a gradual but significant decrease in iron status with each successive blood donation.[20] Investigators in another study[25] found, by the use of repeated ferritin measurements, that iron deficiency is widespread among women, with a prevalence ranging from 21% for 1-time donors to 46% for those who donate 4 times during a 1-year period. Iron deficiency is evident in 14% of men who donate blood ≥ 4 times during a 1-year period.[25]

The objective of the study reported here was to evaluate the bone marrow regenerative response and iron status of dogs that provided frequent blood donations during 1 year of blood collections to assess the safety of repeated donations.

Materials and Methods

Animals—A group of 57 healthy mixed-breed dogs that provided frequent blood donations were included in the study. All dogs were client-owned animals. Owners provided consent for participation of their dogs in the study. The study protocol was approved by the University of Porto Institute for Biomedical Sciences of Abel Salazar Ethics Committee (project No. 008/2012).

Body weight of the dogs ranged from 21 to 45 kg (46.2 to 99.0 lb). All dogs were 1 to 6 years old, had been vaccinated and dewormed, and had serum alkaline phosphatase and alanine aminotransferase activities and creatinine, total protein, and glucose concentrations within the respective reference ranges. Furthermore, they had negative results of PCR analysis for *Anaplasma* spp, *Ehrlichia* spp, *Babesia canis*, *Leishmania infantum*, and *Dirofilaria immitis*. All dogs were fed a complete and balanced commercial food, and none of them had a history of previous blood donations. During the study, all donors received preventative treatments for fleas, ticks, and heartworms. Dogs with an initial PCV < 38% were excluded from the study.

Study design—Dogs were allocated into 3 groups. Sixteen dogs (13 females and 3 males) were used for blood collection of 13% of TBV (11.4 mL/kg [5.2 mL/lb]) every 2 months (group 1), 16 dogs (10 females and 6 males) were used for blood collection of 13% of TBV every 3 months (group 2), and 25 dogs (11 females and 14 males) were used for blood collection of 15% of TBV (13.2 mL/kg [6.0 mL/lb]) every 3 months (group 3). Thus, during the 1-year period of the study, there were 7 blood collections/dog for group 1 and 5 blood collections/dog for groups 2 and 3. Rationale for the choice of the 3 regimens was to ensure that the blood collections would not result in clinically apparent deleterious effects for the dogs. Therefore, the minimum values described in the veterinary literature[5–10] (groups 1 and 3) and the maximum values recommended in human standards[3,4] (group 2) were selected. The preference for a percentage of TBV instead of a fixed blood volume of 450 mL allowed for the collection of specific blood volumes from each dog and thus safe use of donors with a wide range of body weights.

Blood donations—All blood collections were performed by the same investigator (RRFF). A complete physical examination was performed on each donor dog. Dogs then were placed in lateral recumbency, and the puncture area over a jugular vein was clipped of hair and aseptically prepared (chlorhexidine and alcohol). Jugular venipuncture was performed, and blood was allowed to flow by gravity into a collection bag. The total volume collected was calculated on the basis of each dog's body weight, with the assumption that the TBV of dogs is 85 mL/kg (38.6 mL/lb)[26] and that 1 mL of whole blood weighs 1.053 g.[27]

Sample collection and analysis—An initial blood sample was collected from each dog before the blood donation program was started, and a final blood sample was collected from each dog 10 days after the last blood donation. Initial and final blood samples (5 mL/sample) were collected from a cephalic vein and used for analysis of concentrations of serum iron, transferrin, and serum ferritin.

Transferrin concentration was measured with an ELISA kit[a] with canine anti-transferrin antibodies, in accordance with the manufacturer's protocol. Serum iron and ferritin concentrations were measured with a biochemical analyzer.[b] Serum iron concentration was determined by releasing iron from transferrin, which was followed by a reduction reaction and measurement of the change in absorbance. Ferritin concentration was analyzed by immunologic agglutination with

human anti-ferritin polyclonal antibodies; antigen-antibody complex precipitates were determined turbidimetrically. Although it has been suggested that anti-ferritin antibodies are species specific,[18] canine ferritin has been accurately measured with human anti-ferritin polyclonal antibodies.[c]

The bone marrow regenerative response to depletion was assessed through evaluation of the PCV, RBC count, hemoglobin concentration, MCHC, MCV, RDW, platelet count, WBC count, and reticulocyte count in samples obtained before and 10 days after each blood donation. The PCV was obtained with a micro-Hct centrifuge in accordance with standard methods,[28] and the remaining variables were measured with an automatic analyzer[d] used in accordance with the manufacturer's protocol. For reticulocyte staining, 2 drops of new methylene blue[e] and 1 drop of thoroughly mixed blood were added to a conventional 1.5-mL microtube. The mixture was gently stirred for 10 minutes and then allowed to sit undisturbed at room temperature (22°C [72°F]) for another 10 minutes. Blood films then were prepared and examined by means of light microscopy. Any RBC with ≥ 2 blue-stained particles was counted as a reticulocyte. Counting of 1,000 RBCs was performed with a 100× oil immersion objective and 10× ocular in randomly selected areas of the film where RBCs were close to each other but did not touch or overlap. The reticulocyte number was recorded, and values were reported as the reticulocyte percentage.[29]

Statistical analysis—Results were analyzed with statistical software.[f] Normal distribution of data was assessed with the Kolmogorov-Smirnov test. A 1-way ANOVA was used to compare differences of the post-donation hematologic variables and iron status biochemical variables among groups. Significant differences between groups were identified by use of a post hoc Bonferroni test. The same tests allowed comparison of hematologic variables for groups 1, 2, and 3 in samples obtained 10 days after each blood collection. A paired-sample Student *t* test was used to compare initial and final values for iron status as well as differences in hemogram variables before and after blood donations. The Pearson correlation coefficient was used to assess a possible linear relationship between hematologic variables and time of evaluation. Values were considered significant at $P \leq 0.05$.

Reference limits (with a 95% confidence interval) of iron status variables were calculated to identify possible extreme values. The following equation was used: reference limits = mean ± (1.96 × SD).

Results

A total of 317 blood collections were performed (112 for group 1, 80 for group 2, and 125 for group 3). Data for all variables were normally distributed. Initial values of the biochemical variables for iron status (n = 57 dogs) were calculated (**Table 1**). Initial and final values of the biochemical variables for iron status of each group were determined (**Table 2**). A significant ($P = 0.011$) decrease of serum ferritin concentration was found between the initial and final sample for group 1. Dogs of group 2 had a significantly ($P = 0.019$) higher mean final serum ferritin concentration than did dogs of group 1.

In samples obtained 10 days after each blood collection, there were significant increases in RDW, platelet count, WBC count, and reticulocyte percentages for all groups, compared with values before blood collection (**Table 3**). Furthermore, dogs of group 1 had significant increases in PCV and MCV and decreases in MCHC at 10 days after each blood collection. Comparison between initial and final hematologic variables revealed significant increases in RDW and reticulocyte percentage for all groups ($P < 0.001$), WBC count for group 1 ($P = 0.009$), and RBC count for group 3 ($P < 0.001$).

Comparison of final hematologic variables measured 10 days after the last blood donation revealed that dogs of group 1 had a significantly higher mean PCV ($P = 0.035$) and hemoglobin concentration ($P = 0.009$) than did dogs of group 2, and dogs of groups 1 ($P = 0.001$) and 2 ($P < 0.001$) had a significantly lower mean RDW than did dogs of group 3 (**Table 4**). Furthermore, dogs of group 1 also had a significantly ($P = 0.03$) higher mean WBC count than did dogs of group 3.

A positive correlation was detected between the number of blood collections and RDW values for group 1 ($r = 0.42$; $P < 0.001$), group 2 ($r = 0.28$; $P = 0.016$), and group 3 ($r = 0.42$; $P < 0.001$). There was a positive correlation between the number of blood collections and reticulocyte percentages for group 1 ($r = 0.23$; $P < 0.05$), group 2 ($r = 0.33$; $P = 0.002$), and group 3 ($r = 0.42$; $P < 0.001$). These results indicated adaptation of the bone marrow to blood depletion in all groups during the study period.

Table 1—Analysis of iron status biochemical variables for 57 canine blood donors prior to inclusion in a blood donation program.

Variable	Mean ± SD	Range	95% confidence interval
Iron (µg/dL)	144.12 ± 42.41	54.00–253.00	61.00–227.24
Ferritin (ng/mL)	171.47 ± 74.29	68.00–439.00	25.86–317.08
Transferrin (mg/L)	374.24 ± 76.42	188.86–590.20	224.46–524.02

Table 2—Mean ± SD values for iron status biochemical variables for dogs before inclusion in a blood donor program (initial) and after the final blood donation in 16 dogs donating 13% of TBV every 2 months (group 1), 16 dogs donating 13% of TBV every 3 months (group 2), and 25 dogs donating 15% of TBV every 3 months (group 3) for 1 year.

Group	Variable	Initial	Final
1	Iron (µg/dL)	167.50 ± 40.77	147.19 ± 32.23
	Ferritin (ng/mL)	218.81 ± 113.61	147.31 ± 43.59*
	Transferrin (mg/L)	458.61 ± 143.55	452.62 ± 194.60
2	Iron (µg/dL)	134.80 ± 27.47	136.60 ± 32.70
	Ferritin (ng/mL)	163.13 ± 57.69	195.73 ± 64.95†
	Transferrin (mg/L)	430.06 ± 210.48	525.87 ± 95.86
3	Iron (µg/dL)	137.52 ± 41.88	148.08 ± 30.83
	Ferritin (ng/mL)	143.68 ± 48.39	162.84 ± 36.26
	Transferrin (mg/L)	411.42 ± 175.89	486.59 ± 148.49

*Value differs significantly ($P \leq 0.05$) from the initial value for group 1. †Value differs significantly ($P \leq 0.05$; post hoc Bonferroni test) from the corresponding final value for group 1.

Table 3—Mean ± SD values of hematologic variables before and 10 days after each blood collection in 16 dogs donating 13% of TBV every 2 months (group 1), 16 dogs donating 13% of TBV every 3 months (group 2), and 25 dogs donating 15% of TBV every 3 months (group 3) for 1 year.

Group	Variable	Before collection	After collection
1	PCV (%)	49.82 ± 4.79	51.54 ± 5.10*
	Hemoglobin (g/L)	174.11 ± 23.31	176.81 ± 19.70
	RBC count (× 10^6 cells/µL)	7.04 ± 0.64	7.07 ± 0.79
	MCHC (g/L)	345.58 ± 16.40	337.42 ± 19.39*
	MCV (fL)	72.38 ± 3.21	74.57 ± 4.53*
	RDW (%)	12.84 ± 0.98	13.26 ± 1.07*
	Platelet count (× 10^3 platelets/µL)	233.43 ± 70.84	293.18 ± 83.18*
	WBC count (× 10^3 cells/µL)	8.31 ± 2.67	10.39 ± 2.53*
	Reticulocytes (%)	0.36 ± 0.20	1.29 ± 0.73*
2	PCV (%)	46.81 ± 4.81	46.05 ± 3.96
	Hemoglobin (g/L)	164.13 ± 18.23	161.99 ± 15.17
	RBC count (× 10^6 cells/µL)	6.62 ± 0.75	6.57 ± 0.66
	MCHC (g/L)	341.37 ± 21.66	341.21 ± 19.26
	MCV (fL)	72.89 ± 4.13	72.57 ± 4.10
	RDW (%)	12.95 ± 1.49	13.84 ± 1.65*
	Platelet count (× 10^3 platelets/µL)	247.23 ± 81.77	290.96 ± 92.51*
	WBC count (× 10^3 cells/µL)	9.14 ± 2.77	9.83 ± 2.02†
	Reticulocytes (%)	0.32 ± 0.24	1.05 ± 0.57*
3	PCV (%)	48.23 ± 4.34	47.81 ± 4.02
	Hemoglobin (g/L)	172.08 ± 22.42	169.96 ± 15.40
	RBC count (× 10^6 cells/µL)	7.31 ± 0.74	7.15 ± 0.82
	MCHC (g/L)	336.11 ± 35.81	337.80 ± 18.23
	MCV (fL)	70.36 ± 3.65	70.82 ± 3.93
	RDW (%)	15.74 ± 2.45	16.23 ± 2.51*
	Platelet count (× 10^3 platelets/µL)	213.18 ± 61.01	246.48 ± 59.54*
	WBC count (× 10^3 cells/µL)	9.15 ± 2.44	10.06 ± 2.61*
	Reticulocytes (%)	0.23 ± 0.14	1.29 ± 0.56*

†Within a row, values differ significantly ($P \leq 0.05$; †$P < 0.001$).

Table 4—Mean ± SD values of hematologic variables measured 10 days after the final blood donation in 16 dogs donating 13% of TBV every 2 months (group 1), 16 dogs donating 13% of TBV every 3 months (group 2), and 25 dogs donating 15% of TBV every 3 months (group 3) for 1 year.

Variable	Group 1	Group 2	Group 3
PCV (%)	51.38 ± 5.18[a]	47.19 ± 3.73[b]	48.00 ± 4.57[a,b]
Hemoglobin (g/L)	183.69 ± 21.76[a]	162.79 ± 16.53[b]	171.59 ± 16.77[a,b]
RBC count (× 10^6 cells/µL)	7.39 ± 0.69	6.84 ± 0.78	7.27 ± 0.62
MCHC (g/L)	342.94 ± 14.48	337.43 ± 12.89	334.09 ± 20.27
MCV (fL)	72.41 ± 3.37	70.70 ± 2.82	70.72 ± 3.23
RDW (%)	14.44 ± 1.20[c]	15.39 ± 2.07[c]	17.47 ± 1.40[d]
Platelet count (× 10^3 platelets/µL)	271.80 ± 93.67	277.79 ± 74.13	225.95 ± 53.01
WBC count (× 10^3 cells/µL)	11.26 ± 3.13[a]	9.42 ± 2.01[b]	9.09 ± 2.17[a,b]
Reticulocytes (%)	1.66 ± 0.86	1.34 ± 0.52	1.68 ± 0.58

[a–d]Within a row, values with different superscript letters differ significantly ([a,b]$P \leq 0.05$; [c,d]$P \leq 0.001$; post hoc Bonferroni test).

Discussion

Although iron depletion has been evaluated on the basis of biochemical variables related to iron bioavailability in a number of human studies,[1,2,11–14,19,20,25,30–33] to our knowledge, the subject has not been addressed in canine blood donors. However, some authors have suggested[16,34] that iron-deficiency anemia can be a consequence of excessive phlebotomies in such animals.

Iron is distributed into 3 main compartments: transport (serum iron and transferrin), storage (ferritin and hemosiderin), and functional (hemoglobin, myoglobin, and several enzymes).[34] Serum iron concentration is typically extremely low in animals with iron-deficiency anemia,[35,36] but it is within the reference range and is not correlated with iron stores in preclinical iron-deficiency states.[36] Ferritin is an intracellular iron storage protein[34,36] that can also be detected in serum; thus, it provides a convenient and relatively noninvasive means of estimating iron stores in dogs.[37] Measurement of ferritin concentrations may be used to identify nonanemic iron-deficiency stages, and it is extremely sensitive for evaluating the effects of repeated collections from blood donors. However, ferritin is also an acute-phase protein, and the ferritin concentration can increase as a result of inflammatory states, neoplasms, liver disease, or hemolysis.[15,18] Transferrin is a protein produced by the liver that is able to bind 1 or 2 iron atoms, which allows for plasma transport.[15,34] Bone marrow aspirates stained with Prussian blue enable the

detection of hemosiderin deposits and a qualitative assessment of body iron stores. However, although iron deficiency can be ruled out by visual inspection for hemosiderin, lack of hemosiderin deposits is not necessarily predictive of iron deficiency.[35,36]

In the present study, 3 biochemical variables were used to investigate the influence of repeated blood collections on the iron status of dogs used for frequent blood donations. Initial concentrations of serum iron and transferrin measured immediately before the first blood donation were similar to those previously reported.[16] It was difficult to compare the reference range for the serum ferritin concentration of the present study with values reported in the literature because of variation in the reported results.[18,37] The range for the transferrin concentration (224.46 to 524.02 mg/L) in the dogs of the present study was comparable to that for clinically normal humans.[38]

At the end of the study, there was a significant decrease in the serum ferritin concentration of dogs subjected to the most frequent blood collections (group 1), with half of those dogs having a reduction > 30%. Nonsignificant differences in the other groups suggested that regardless of the collected blood volume, the 3-month interval appeared to be sufficient for restoration of iron reserves, provided that the dogs remained healthy and were fed a nutritionally balanced diet. Blood donation programs based on more frequent blood collections may constitute a risk for iron depletion of donors. Final concentrations of serum iron and transferrin were not significantly different from initial concentrations, which suggested that iron in the transport compartment remained stable regardless of the volume or frequency of blood collection.

Repeated blood donation in humans leads to iron-deficient erythropoiesis and reduced iron stores.[1,2] In contrast, dogs appeared to be less susceptible to iron deficiencies in similar circumstances. Such a phenomenon is more frequent in women, probably because of an increase in iron depletion as a result of blood loss during the menstrual cycle,[38] which is nonexistent in bitches. In humans, a low-meat diet, which often is associated with lower incomes or is found in vegetarian diets without iron supplementation, is frequently related to iron deficiency.[38] For ethical reasons, we did not collect blood from dogs fed unbalanced diets; however, it appeared that balanced commercial pet foods had adequate iron for frequent canine blood donors, with no need for further supplementation.[39] Furthermore, dogs are able to absorb both ferrous (Fe^{2+}) and ferric (Fe^{3+}) nonheme iron, whereas humans have impaired absorption of Fe^{3+}.[15]

In deficient states, iron is preferentially shunted from stores to ensure the amount needed for the functional compartment (hemoglobin, myoglobin, and various enzymes).[34] This was the reason that none of the dogs in the present study developed iron-deficiency anemia.

Differences in hematologic variables before and 10 days after each blood collection indicated that the bone marrow response was adequate in all groups, with a significant increase of reticulocyte percentage and RDW in all groups and an absence of significant decreases of the remaining hematologic values at the end of the study. This response was also apparent for each blood collection, as indicated by the comparison between the predonation and postdonation PCV, hemoglobin concentration, RBC count, platelet count, and WBC count. Group 1 had the greatest differences between hematologic variables before and 10 days after each blood collection, with increases in PCV, MCV, platelet count, and WBC count for 71 (63%), 93 (83%), 95 (85%), and 96 (86%) of the 112 blood donations, respectively. Therefore, it can be concluded that as long as iron stores are adequate, the bone marrow regenerative capacity can compensate for frequent blood donation. The positive correlation between the number of blood collections and the gradual increases of RDW and reticulocyte percentages reinforced this concept.

The small but significant increase in the numbers of platelets and WBCs 10 days after each blood collection was probably related to their depletion during blood collections and a consequent release of specific cytokines (eg, thrombopoietin or interleukin-6) that can upregulate myelopoiesis and megakaryocytopoiesis.[40] Furthermore, during stressful events, the release of erythropoiesis growth factors (eg, stem cell factor) markedly enhances the activity of erythropoietin, which stimulates the proliferation of RBC precursors[41] and leads to production of erythrocytes as well as platelets and leukocytes. Increase in WBC count may also have been related to local inflammation induced by needle puncture. Stress-related increases in WBC or platelet counts was considered unlikely because this is usually a transient event that resolves within 24 hours[42] and should not be evident at 10 days after a blood collection.

The present study revealed that the 3 donation protocols (13% of TBV every 2 months for 1 year, 13% of TBV every 3 months for 1 year, and 15% of TBV every 3 months for 1 year) induced bone marrow regenerative responses that were able to restore depleted blood cells within 10 days after blood collection while maintaining iron status values within the calculated reference ranges. However, the significant decrease of iron stores for dogs subjected to blood donation of 13% of TBV every 2 months suggested that such variables must be monitored during prolonged blood donation programs.

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e. Reticulocyte stain solution, Sigma-Aldrich Corp, Steinheim, Germany.
f. SPSS, version 22.0.0, IBM Corp, Chicago, Ill.

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