Prolonged iron depletion after allogeneic 2-unit RBC apheresis

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BACKGROUND: Allogeneic 2-unit RBC apheresis is a safe procedure offering many advantages for donors and blood banks. A controlled study was performed to determine whether the recommended minimum interval of 4 months between 2-unit RBC apheresis donations is appropriate in terms of the recovery of RBCs and the regeneration of iron stores.

STUDY DESIGN AND METHODS: Twenty male subjects each donated 2 units of RBCs by apheresis. The RBC count, reticulocyte count, EPO, and measures of iron status were analyzed before and during the 4 months after donation.

RESULTS: A significant decrease in Hb (15.89 ± 0.82 [mean ± SD] vs. 14.08 ± 0.97 mg/dL, baseline vs. Day 7; $p<0.001$) was equalized within 2 months. In contrast, ferritin values declined significantly from 54.2 ± 33.7 to 23.42 ± 21.94 µg per L (predonation vs. Day 30) and remained significantly below predonation values, but within the normal range, until the end of the study period.

CONCLUSION: A donation interval of 4 months is appropriate in terms of RBC recovery, but may not be appropriate in terms of iron store regeneration. The tendency to shorten the donation interval should be reconsidered in light of the measurements of iron storage. The use of ferritin levels is recommended as a preselection criterion for allogeneic 2-unit RBC apheresis.

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Modern apheresis technologies make it possible to collect 2 units of RBCs from one donor at a single donation. This procedure, widely used in autologous donations in preoperative settings, is also increasing in value and use for allogeneic donations because of its many advantages for donors and blood banks. RBC apheresis has been proven to be a safe procedure with very low risk of serious side effects[1-4] and high donor acceptance.[3] RBC units collected by apheresis are similar in storage and quality measures to RBC units prepared from conventionally collected whole blood.[5-7] The latter procedure produces units with greatly varying absolute RBC volumes, because of physiologic variations in donor Hct. The ability to collect a specified absolute RBC volume represents a great advantage of RBC apheresis over traditional whole-blood collection. Furthermore, 2-unit RBC apheresis offers the potential to adapt component collection to inventory needs,[8] by facilitating the collection of a sufficient number of phenotypically matched RBC units to meet specific requirements, while reducing the number of random donors from whom blood is collected.

Regular blood donation is well known to involve the risk of iron deficiency by continually depleting iron stores in a frequency-dependent manner.[9-11] As double-dose RBC donation removes approximately twice the amount of RBCs as does whole-blood donation, recommendations for the donation interval double the minimum time span to 4 months.[12] In addition, FDA donor recruitment criteria are more stringent for Hct, Hb, and the weight and height of donors. But, as for whole-blood donors, recruitment criteria do not include actual storage iron values.

Recently, it was proposed that the donation interval for repeated 2-unit RBC apheresis could be substantially shortened.[15,16] Therefore, it was the aim of our study to determine whether a donation interval of 120 days (3 months, rather than 4) is appropriate for donors in terms of hematologic recovery and iron balance, whether the interval can safely be shortened, or whether additional donor-selection criteria, such as serum ferritin, should be applied.

MATERIALS AND METHODS

Donor recruitment

Twenty experienced male volunteer blood donors (mean age, 40.45 ± 12.92 years), selected for weight, height, and Hb or Hct, were recruited from our regular plasma or platelet donor pool. Thirteen of this group had also donated whole blood in the past 2 years, but not within the 3 months preceding 2-unit RBC apheresis. Eight and three donors had donated 1 and 2 units of whole blood, respectively; one had donated 3 units, and one 4 units. All donors gave informed consent to the study protocol, were in good health, and met the FDA criteria for 2-unit RBC apheresis.[13] Their weight (83.15 ± 11.3 kg) and height (181.3 ± 7.28 cm) were well above the recommended minimum levels. No additional blood donation was made by any of the donors during the study period of 120 days, and none took supplemental iron medication.

Study design

With a cell separator (MCS+, Haemonetics, Braintree, MA), 2 units of RBCs (200 mL each) were collected from each donor by the RBC donation (SDR) protocol. CPD-50 in a ratio of 1:16 was used for anticoagulation, and 400 mL of saline was used as replacement fluid for the donor. During donation, phlebotomists recorded any adverse reactions or symptoms experienced by the donors. Their age, weight, and height were recorded, and blood pressure was measured before and immediately after donation.

RBC count, reticulocyte count, serum iron, transferrin, transferrin saturation, ferritin, and endogenous EPO were measured before donation (Day 0); on Days 1 and 7; and 1, 2, 3, and 4 months after donation. Standard laboratory methods were used for RBC count values and measures of iron metabolism; endogenous EPO was measured by ELISA technique (DRG Instruments, Marburg, Germany).

Statistics

Wilcoxon's signed-rank test was used to compare predonation and postdonation blood pressure. A repeated-measures ANOVA evaluated the dependence of all hematologic measures on time. Changes at a level of $p<0.05$ were considered significant. Data were expressed as mean ± 1 SD. Statistical analysis was performed with statistical software (SPSS version 9.0, SPSS, Chicago, IL).

RESULTS

All 20 donors completed the study. No side effects were noted by the phlebotomists during or after apheresis donation. Systolic blood pressure before donation differed significantly from that after donation (130.79 ± 12.72 mmHg vs. 123.68 ± 12.45 mmHg). However, no clinical symptoms related to hypovolemia were reported.

Hematologic measures

Hb concentration averaged 15.89 ± 0.82 mg per dL before donation, fell to a nadir of 14.08 ± 0.97 mg per dL 7 days after donation, and remained significantly below predonation values up to and including Day 30 ($p\leq0.001$). Hematologic recovery after donation-induced blood loss was evident by subsequent significant increments in endogenous EPO and reticulocytes, with peak values on Days 1 and 7, respectively ($p<0.001$). Platelet values were significantly elevated ($p\leq0.005$) from baseline levels throughout the study period (Table 1).

Iron balance measurements

Mean ferritin values declined from 54.2 ± 33.78 µg per L to a nadir of 23.42 ± 21.94 µg per L (predonation vs. Day 30; $p<0.001$) and remained significantly below baseline levels until the end of the study period, whereas changes in serum iron values were modest, with a significant decrease only on Day 7 (Fig. 1). The number of donors with complete depletion of their iron stores (ferritin values ≤12 µg/L) increased from two to seven (predonation vs. Day 30; p = NS [chi-square]). At the end of the study period, five donors still had complete depletion of iron stores.

Transferrin levels increased significantly on Day 7, peak values occurred on Day 60, and the levels remained significantly elevated 4 months after donation ($p\leq0.001$). Accordingly, transferrin saturation at first declined significantly (28.8 ± 11.75% to 19.55 ± 6.28%, predonation vs. Day 7; $p<0.05$), but then slowly and intermittently increased (Table 2).

DISCUSSION

As in previous studies,[1,2,4] which revealed that serious side effects of 2-unit RBC apheresis donation are rare, no major donor symptoms were noted during or after donation, and no side effects were recorded in our study. Only a few studies have evaluated hematologic recovery and iron metabo-

TABLE 1. Measures of hematologic recovery

Day	Hb (13.3-17.7 mg/dL)*	RBCs (4.4-5.9 × 10^6/µL)*	Platelets (140-400 × 10^3/µL)*	EPO (6-25 U/mL)*	Reticulocytes (0.4-2.5%)*
0	15.89 ± 0.82	5.19 ± 0.26	218.8 ± 41.6	7.11 ± 2.92	1.21 ± 0.48
1	14.16 ± 1.22†	4.62 ± 0.39†	248.25 ± 39.6†	19.64 ± 12.75†	1.45 ± 0.47‡
7	14.08 ± 0.97†	4.61 ± 0.32†	261.9 ± 42.55†	14.03 ± 6.26†	2.31 ± 0.77†
30	15.08 ± 1.01†	4.95 ± 0.34†	238.26 ± 44.04‡	8.65 ± 4.08‡	1.61 ± 0.42‡
60	15.82 ± 1.04	5.19 ± 0.34	238.7 ± 45.04‡	9.12 ± 3.82‡	1.21 ± 0.34
90	15.71 ± 0.91	5.24 ± 0.34	247.35 ± 47.16†	9.09 ± 3.67‡	1.13 ± 0.40
120	15.74 ± 0.78	5.25 ± 0.3	242.79 ± 46.07‡	10.14 ± 6.05‡	1.04 ± 0.40

* Normal range.
† $p\leq0.001$.
‡ $p<0.05$.

lism after 2-unit RBC donation. Sherman et al.[17] manually collected 2 units of RBCs from eight donors and found Hb, serum iron, and ferritin levels still significantly lower than baseline levels at 16 weeks after donation. In our study, Hb had recovered by 2 months and serum iron was not significantly decreased, except on Day 7 after donation, whereas the course of ferritin was similar to the results of Sherman et al.[17] The reasons for the later Hb and serum iron recovery in their study might be found in the donation method, the lower weight of donors, or in the presence of female participants, half of whom were still iron-deficient 16 weeks after donation.

As a result of RBC loss, endogenous EPO increased significantly, with a peak value on Day 1 after donation; this was followed by a rise in reticulocyte count to a peak on Day 7, which coincided with the nadirs of Hb and serum iron. The increase in EPO seen in our study is in accordance with the findings of Smith et al.,[3] who performed a postdonation study on sham, 1-unit, and 2-unit RBC donors for 14 days after donation. They found a maximum increase in endogenous EPO 2 days after donation, with the greatest changes occurring in 2-unit donors, which corresponded to the larger amount of blood loss.

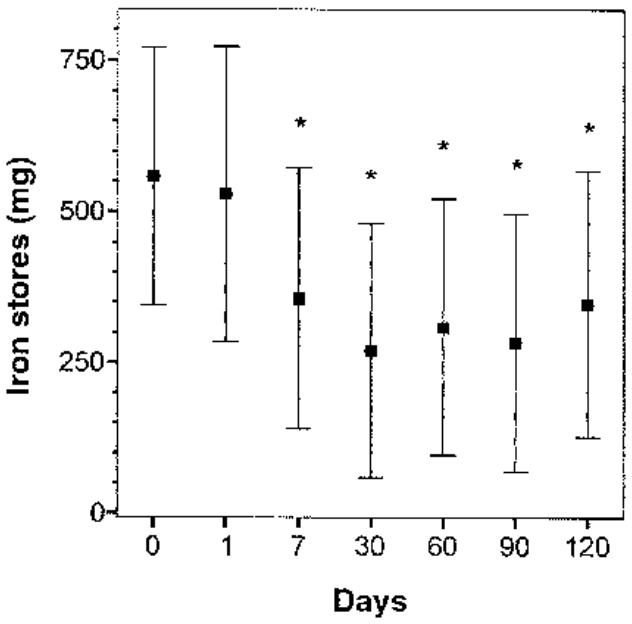

Fig. 1. The course of iron stores over the study period of 4 months as calculated by the method of Cook et al.[19] and modified by Garry et al.[20] At 1 month, iron stores were less than half of predonation levels (269.01 ± 209.66 mg vs. 557.87 ± 213.57 mg). *p<0.001.

Meyer et al.[18] compared 2-unit RBC apheresis donors and 1-unit whole-blood donors after 1 year of maximal donation frequency of 4 and 2 months, respectively. Neither a significant decrease in ferritin levels nor any intergroup difference in ferritin levels was noted. This can be explained by the fact that half of the donors took an iron supplement and that donors were recruited from among repeat whole-blood donors with ferritin levels already at a very low normal value, so that the observed decrease did not reach significance.

Our results show a decrease by half in the mean ferritin values, with a nadir at 1 month after donation, and a slow subsequent increase, which corresponded to a refilling of iron stores. Although mean values were not below the range of normal, at 4 months after donation, ferritin was still significantly below baseline levels, which means that another donation at that time would inappropriately deplete iron stores. We calculated iron stores for each individual by the method of Cook et al.,[19] as modified by Garry et al.[20] This method uses a combination of biochemical measures of iron status to estimate body iron, with different degrees of iron status requiring separate calculations; we found a significant reduction in iron stores, similar to the course of ferritin for the whole study period (Fig. 1). The number of donors with completely depleted iron stores (indicated as ferritin values <12 µg/L) increased from two before donation to five after 4 months.

Frequent whole-blood donation is known to involve the risk of iron deficiency. Plasma ferritin levels steadily decrease with each blood donation until a plateau is reached at a mean concentration of approximately 20 µg per L.[9,20] As there is a delayed response in the increase in iron absorption, the first donation mainly leads to a mobilization of iron stores. With each successive donation, there is less dependency on iron mobilization from iron stores and much more dependency on increases in physiologic iron absorption, which can rise to a limited maximum of about 4 mg per day in men, regardless of iron intake.[20-22]

Iron loss from donating 2 units of RBCs by apheresis on a single occasion is about 320 to 420 mg.[15] Although maximal iron absorption of 4 mg per day in men, given a normal daily iron loss of 1

TABLE 2. Measures of iron balance

Day	Serum iron (7-35 µmol/L)*	Ferritin (12-307 µg/L)*	Transferrin (200-360 mg/dL)*	Transferrin saturation (16-45%)*
0	18.48 ± 4.66	54.2 ± 33.78	275.35 ± 31.57	28.8 ± 11.75
1	20.54 ± 10.56	52.95 ± 31.83	275.4 ± 31.98	30.7 ± 16.91
7	14.36 ± 4.45†	31.5 ± 27.83‡	296.0 ± 28.8‡	19.55 ± 6.28†
30	16.66 ± 11.24	23.42 ± 21.94‡	310.74 ± 36.77†	21.0 ± 11.77†
60	18.1 ± 9.71	28.4 ± 24.91‡	325.2 ± 35.83‡	21.95 ± 10.67
90	16.92 ± 5.79	27.2 ± 26.37‡	317.3 ± 27.11‡	21.5 ± 8.15†
120	18.79 ± 7.33	30.32 ± 29.89‡	306.89 ± 39.32‡	24.68 ± 10.89

* Normal range.
† $p<0.05$.
‡ $p\leq0.001$.

mg per day (13 µg/kg),[20,22] theoretically could nearly equalize this iron loss, it is evident from our data that iron loss in our male donor group was not compensated for within 4 months. Thus, repeat 2-unit RBC apheresis donation at a minimum interval would likely lead to iron deficiency or iron store depletion, particularly in women. This calculation might also apply to whole-blood donors donating at maximal frequency. Recommendations for the use of oral iron supplementation[17] in volunteer donors must be viewed with skepticism, as iron absorption is limited, its effect on frequent whole-blood donors following a normal diet seems to be minimal,[21] and the potential side effects of the medication are not inconsiderable.

The fact that many donors are able to sustain multiple blood donations per year for several years[20,21] without becoming anemic is probably not a sufficient criterion for donor safety, as pre-existing or latent iron deficiency without anemia might cause relevant, though unspecific, clinical symptoms.[23] One must consider that the absolute iron loss per whole-blood donation slowly decreases with each donation as RBC volume and Hb levels also decline. By contrast, RBC apheresis removes absolute RBC volumes, regardless of donor Hct.

We therefore conclude that a minimum donation interval of 4 months between 2-unit RBC apheresis donations, as currently recommended,[12-14] is certainly adequate in terms of RBC recovery, but not in terms of iron store regeneration. Recommendations to shorten the donation interval[15,16] without a determination of predonation ferritin values should be reconsidered. We recommend that ferritin measurement be added to preselection criteria, at least for first-time allogeneic 2-unit RBC donors, and we also suggest that a yearly ferritin screening be implemented for frequent 2-unit RBC apheresis donors.

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