Repeat whole blood donors with a ferritin level of 30 mu g/L or less show functional iron depletion

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Repeat whole blood donors with a ferritin level of 30 μg/L or less show functional iron depletion

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BACKGROUND: Whole blood donors are screened for iron depletion through hemoglobin measurement alone or in combination with ferritin. Ferritin measurement gives the advantage of earlier detection of iron depletion. In a previous study we identified a ferritin level of 30 μg/L or less as a possible indicator of suboptimal erythropoiesis. In this study, erythropoietic parameters were measured to determine if a ferritin level of 30 μg/L or less is indicative of iron-deficient erythropoiesis in repeat whole blood donors.

STUDY DESIGN AND METHODS: Twenty-one healthy male repeat whole blood donors were divided into two groups according to their predonation ferritin values: 30 μg/L or less (low-ferritin group) and greater than 30 μg/L (normal-ferritin group). Ferritin and erythropoietic parameters were measured before whole blood donation and weekly in the 8 weeks after donation.

RESULTS: A significantly lower value was found for hemoglobin, mean corpuscular volume (MCV), reticulocytes, and reticulocyte hemoglobin content on at least three of the nine time points in the low-ferritin group compared to the normal-ferritin group ($p < 0.05$). Of these parameters, MCV and reticulocyte hemoglobin content were significantly lower before donation as well as during all 8 weeks following donation ($p < 0.05$).

CONCLUSION: Based on the lower values of the erythropoietic parameters in the low-ferritin group, it can be concluded that repeat whole blood donors with a ferritin value of 30 μg/L or less have iron-deficient erythropoiesis and therefore require a longer donation interval than the current 56 days.

Iron depletion in repeat whole blood donors is an important adverse effect. Different degrees of iron deficiency (ID) have been established reflecting gradual loss of iron stores: functional iron depletion or iron-deficient erythropoiesis (IDE), iron-deficient anemia, and absent iron stores. Unsuccessful recovery from iron loss will eventually result in donor deferral because of low hemoglobin levels.[1,2] In a blood bank setting, ID is indirectly measured by the hemoglobin concentration, but hemoglobin shows a delayed response and does not reflect the true iron status.[1,3] The Dutch blood bank has recently started to measure ferritin as a marker for ID alongside hemoglobin. Ferritin is an indicator of intracellular iron stores and can be used to identify ID earlier.[3] Therefore, further iron loss can be halted and donor deferral prevented by giving the donor, for example, a longer recovery time between donations.

In a previous study, we identified a ferritin value of 30 μg/L or less as an indicator for a less responsive erythropoiesis based on the extent of reduction in glycated hemoglobin A_{1c} (HbA_{1c}) in the 8 weeks after whole blood donation, possibly indicating IDE. Whole blood donors with a significant reduction in HbA_{1c}, defined as a percentage reduction

ABBREVIATIONS: HbA_{1c} = hemoglobin A_{1c}; ID = iron depletion; IDE = iron-deficient erythropoiesis; MCV = mean corpuscular volume.

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from the baseline HbA_{1c} value (mmol/mol) of 4.28% or greater within 8 weeks after donation, had a ferritin value greater than 30 μg/L. Whole blood donors without a significant reduction in HbA_{1c} had a ferritin value of 30 μg/L or less.[4]

In this study, we measured several erythropoietic parameters to substantiate the occurrence of functional ID in repeat whole blood donors in relation to ferritin levels.

MATERIALS AND METHODS

This study (NL47160.075.13) was approved by the Medical Ethics Review Committee (Isala Hospital, Zwolle, The Netherlands). Written informed consent was obtained from all volunteers.

Twenty-one healthy male repeat whole blood donors participated.[4] All whole blood donors were eligible to donate with a hemoglobin value of 8.4 mmol/L (135 g/L) or higher based on a hemoglobin testing system (HemoCue, Radiometer Group, HemoCue Ab) measurement. Based on the aforementioned ferritin cutoff value, two groups were identified (Table 1): 10 repeat whole blood donors with a predonation ferritin value of 30 μg/L or less (low-ferritin group) and 11 repeat whole blood donors with a predonation ferritin value of greater than 30 μg/L (normal-ferritin group).

Parameters (ferritin, hemoglobin, hematocrit, mean corpuscular volume (MCV), reticulocytes, high-fluorescence reticulocytes, medium-fluorescence reticulocytes, low-fluorescence reticulocytes, reticulocyte hemoglobin content) were measured before donating approximately 475 mL of whole blood and weekly in the 8 weeks after donation. Erythropoietic parameters were measured using a hematology analyzer (XN-9000, Sysmex). Ferritin was measured using a modular analyzer (Cobas 8000, Roche Diagnostics).

RESULTS

After whole blood donation, the normal-ferritin group had a maximum ferritin drop of 28.3 μg/L (±12.3) in the 8 weeks after donation. After the minimum time interval between donations, 56 days, this group had a mean ferritin value of 21.6 μg/L (±10.9) below their predonation value ($p = 0.0003$). In comparison, the low-ferritin group did not show a significant reduction of ferritin in the weeks after donation (Fig. 1A).

A significant difference was found between both groups for hemoglobin throughout all 8 weeks, before and after donation. After 56 days, both groups had a significantly lower hemoglobin compared to their predonation value (normal-ferritin group: $p = 0.014$; low-ferritin group: $p = 0.002$). No significant difference was found between both groups before donation (Fig. 1B).

Hematocrit was lower for repeat whole blood donors in the low-ferritin group after donation but not significant compared to the normal-ferritin group. However, the low-ferritin group did not return to their predonation value 8 weeks after donation ($p = 0.006$; Fig. 1C).

The MCV was significantly lower before donation and throughout all 8 weeks after donation for the low-ferritin group compared to the normal-ferritin group (Fig. 1D).

Reticulocytes, immature red blood cells (RBCs), are an important indicator of the rate of erythropoiesis. One week after whole blood donation, a peak was observed in the number of reticulocytes in both groups. The normal-ferritin group showed a significantly larger peak at week 1, suggesting a more active erythropoiesis ($p = 0.002$). Also in the 8 weeks after donation, the normal ferritin group had a higher reticulocyte fraction overall (Fig. 1E).

Reticulocytes can be divided into different maturity stages according to their ribonucleic acid (RNA) content, with the youngest reticulocytes having the highest RNA content, and are high fluorescent, while mature reticulocytes have the lowest content of RNA and therefore are low fluorescent.[5,6] Both high- and medium-fluorescence reticulocytes were more numerous in the low-ferritin group and had a lower fraction of low-fluorescence reticulocytes (Fig. 1F-H).

The hemoglobin content of reticulocytes was measured as an indicator of the iron availability for the biosynthesis of

TABLE 1. Baseline characteristics of the low- and normal-ferritin groups*

Characteristics	Low-ferritin group	Normal-ferritin group	p value‡
n	10 men (white)	11 men (white)	
Ferritin (μg/L)	19.1 (4.3)	59.6 (26.2)	<0.0001
Hemoglobin (mmol/L)	8.8 (0.6)	9.2 (0.6)	0.12
Hemoglobin (g/L)	142 (9.7)	148 (9.7)	0.12
Age (years)	61.1 (5.9)	60.9 (6.6)	0.93
Number of donations in the previous year	4.0 (0.5)	3.6† (0.8)	0.35
Days since previous donation	99 (26.3)	110† (16.0)	0.24
Total number of donations in the previous 5 years	18.6 (4.0)	16.3 (5.6)	0.43
Body mass index	25.5 (2.3)	28.4 (3.3)	0.05
Number of donors with low hemoglobin deferrals (n) in the previous 5 years	1 (1)	1 (1)	

* Values are given in mean (SD).
† Excluding one donor who did not donate blood 1.5 years before the start of the study.
‡ p values were calculated by the Mann-Whitney U test.

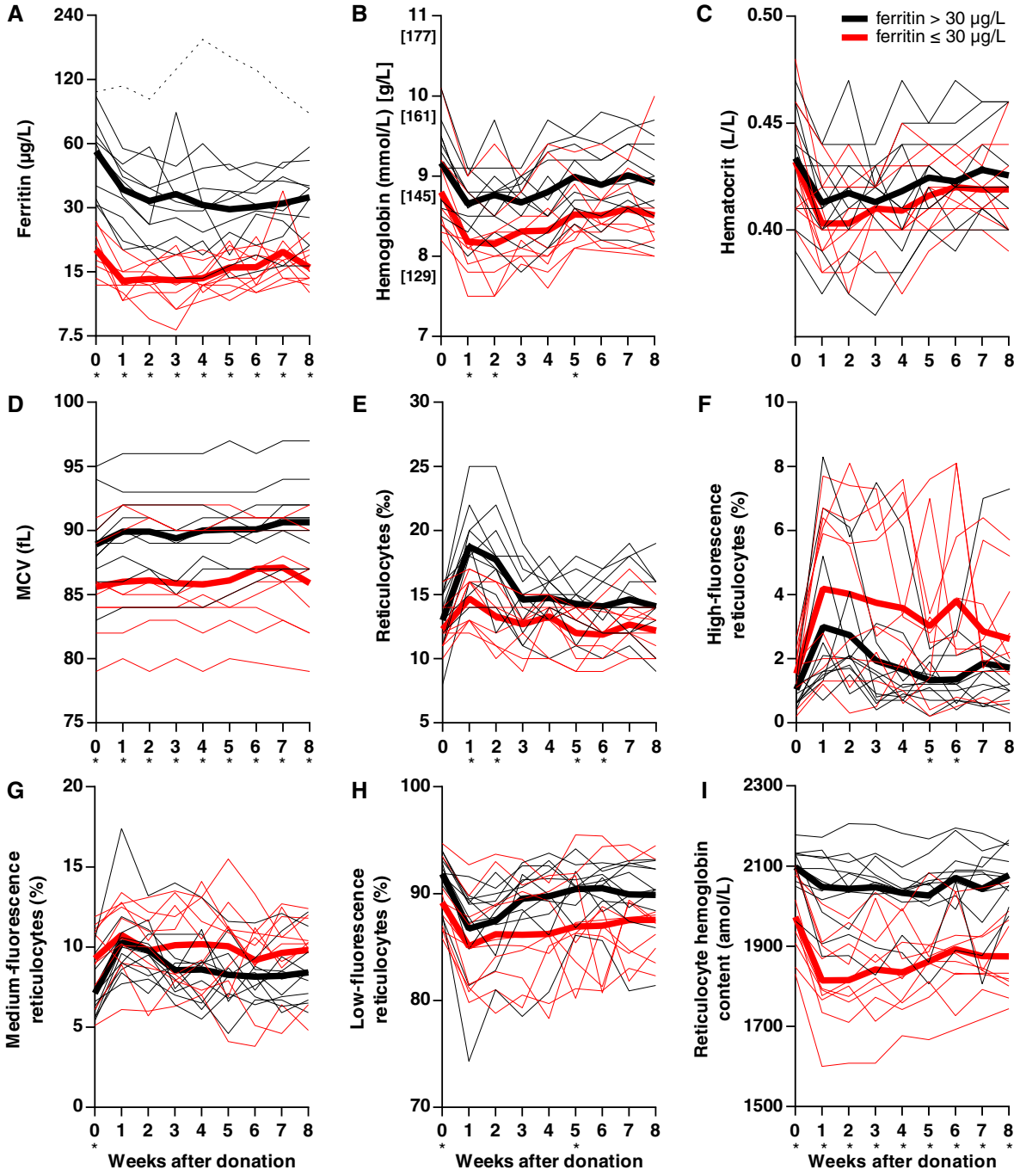

Fig. 1. Parameters of erythropoiesis before donation and in the 8 weeks after whole blood donation. Red lines represent individual repeat whole blood donors in the low-ferritin (≤30 µg/L) group; black lines represent individual repeat whole blood donors in the normal-ferritin (>30 µg/L) group. Bold lines indicate the mean of each group calculated for the following parameters: A, ferritin, dashed line indicates donor omitted from calculated mean (Weeks 1–8 were identified as outliers); B, hemoglobin; C, hematocrit; D, MCV; E, reticulocytes; F, high-fluorescence reticulocytes; G, medium-fluorescence reticulocytes; H, low-fluorescence reticulocytes; I, reticulocyte hemoglobin content. Asterisk indicates a significant difference ($p < 0.05$) between both groups at the indicated time point (two-tailed unpaired t test).

hemoglobin and allows earlier detection of ID compared to the hemoglobin content of mature RBCs.[7,8] The low-ferritin group had a significantly lower reticulocyte hemoglobin content before and after donation compared to the normal-ferritin group ($p \leq 0.002$; Fig. 1I). Interestingly, in the low-ferritin group, the reticulocyte hemoglobin content was

significantly lower 8 weeks after donation compared to before donation ($p = 0.002$).

DISCUSSION

This study focused on changes in erythropoietic parameters in the 8 weeks after whole blood donation. We used a ferritin cutoff value of 30 μg/L based on a previous study to discriminate between optimal and suboptimal erythropoiesis. Herein, the cutoff value was determined by a significant reduction in HbA_{1c} in the 8 weeks after whole blood donation. The physiologic mechanism behind this reduction is that blood donors lose approximately 10% of their RBCs containing HbA_{1c} when donating whole blood. The bone marrow will compensate this by releasing newly formed RBCs still devoid of HbA_{1c}. When erythropoiesis is optimal (ferritin >30 μg/L), a fast influx of newly formed RBCs will lead to a significant dilution, and hence reduction, of the predonation HbA_{1c}. When erythropoiesis is suboptimal (ferritin ≤30 μg/L), the slower influx of newly formed RBCs does not alter the predonation glycated hemoglobin level.[4] Previous studies have identified a similar cutoff value (approx. 30 μg/L and 26.7 μg/L) for IDE based on either reduced hemoglobin, MCV, and mean cellular hemoglobin levels or on the log ratio of the soluble transferrin receptor to ferritin.[9–11] Thus, a ferritin cutoff value of approximately 30 μg/L seems to be a good predictor of IDE based on several studies using different research strategies.

Detailed insight into changes in erythropoietic parameters was provided in the 8 weeks after whole blood donation for repeat whole blood donors with low versus normal predonation ferritin levels. Distinct differences were found between the low- and normal-ferritin group. First of all, our data showed that the use of hemoglobin is limited in IDE detection for individual repeat whole blood donors. Eight weeks after whole blood donation, the hematocrit in the low-ferritin group was significantly lower compared to before donation, which strongly indicates a less effective erythropoiesis in the low-ferritin group. Nonoptimal erythropoiesis in the low-ferritin group was also evident based on a lower fraction of reticulocytes, with more reticulocytes in the immature stage. This indicates that the process of reticulocyte maturation is slower in the low-ferritin group, most probably due to reduced availability of iron.[12] Furthermore, the reduced reticulocyte hemoglobin content indicated there was insufficient iron available for optimal erythropoiesis, highly indicative for IDE. Also the MCV was significantly lower, which suggests a possibly long-standing reduced iron availability for hemoglobin biosynthesis in the low-ferritin group. Other causes of suboptimal erythropoiesis were ruled out. All donors had normal liver and kidney function and no hemoglobinopathies.

Repeat whole blood donors were unable to adequately return to their predonation ferritin within 8 weeks. Schotten and colleagues[13] showed on average that repeat whole blood donors returned to their predonation ferritin level after 180 days. However, donors with a ferritin value of 30 μg/L or less remained below 30 μg/L 180 days after donation and thus require a longer recovery time. For this group we suggest a recovery time of more than 180 days to adequately replenish their iron stores and reduce the chance of future deferral. In addition, in case of long-standing ID, despite a reduction in donation frequency, other causes of iron loss should be investigated.

Implementing the ferritin cutoff value of 30 μg/L in blood donation centers and extending the donation interval of donors with a ferritin value of 30 μg/L or less, to allow for recovery of lost iron stores, will lead to an increase in costs concerning material use, testing, processing the data, and executing the new strategy. However, it will lower the cost related to donor deferral. In addition, the total number of blood donors who can donate will decrease due to the extended donation interval requiring a larger blood donor population to adequately fulfill the demand for blood products.[14,15] Implementing the above strategy will eventually result in an optimized donation process. We expect that donors who are invited to donate will have adequate iron stores and therefore have adequate hemoglobin levels and will not be deferred. Indeed, O'Meara and colleagues[14] and Goldman and colleagues[15] showed benefits from routinely measuring ferritin in whole blood donors. An increase in hemoglobin was observed and donor deferral rates dropped, but return rates decreased. Even so, these results were obtained not only by donation interval extension but also partly through iron supplementation. So far, several studies have shown benefit from daily iron supplementation, with a positive effect on hemoglobin recovery and ferritin.[16–19] As such, iron supplementation in donors with ID might reduce the required extension of the donation interval, maintaining individual donation frequency and preventing the need for a larger blood donor population. Yet, long-term daily iron supplementation required for maintaining iron stores and thus positively influencing the donation frequency could be accompanied by an increase in adverse effects of oral iron intake and can result in medicalization of the donor.

A limitation of this study is the use of a cohort of only elderly men. Previous studies have used different cohorts, for example, 38-year-old women or a cross-sectional sample of the male blood donor population, and found a similar cutoff value, albeit based on different parameters.[9,11] This suggests that the ferritin cutoff value for IDE is independent of age and sex. Nevertheless, it would be of interest to confirm the cutoff value in young, menstruating females, especially considering their increased representation in the total blood-donating population in, for example, the United States.[20]

In conclusion, a ferritin cutoff level of 30 μg/L allows for a clear distinction between optimal and suboptimal erythropoiesis in repeat whole blood donors and can be used to identify IDE.

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CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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