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Evaluation of low red blood cell mean corpuscular volume in an apheresis donor population

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Abstract

BACKGROUND—Apheresis donors are routinely evaluated with a complete blood count (CBC). Low red blood cell mean corpuscular volume (MCV) values (<80 fL) in the presence of an acceptable hemoglobin (Hb; ≥12.5 g/dL) could be due to iron deficiency or hemoglobinopathy. The etiology of a low MCV in a healthy apheresis donor population was assessed.

METHODS—Predonation samples for CBC were obtained from 1162 consecutive apheresis donors. Donors with a MCV of less than 80 fL were evaluated by CBC, iron studies (ferritin, serum iron, transferrin, percentage of transferrin saturation), and hemoglobin (Hb) electrophoresis. Iron deficiency was defined as a ferritin value below the reference range. Beta chain Hb variants were determined by Hb electrophoresis. Alpha thalassemia trait was presumed if the red blood cell (RBC) count was elevated, no variant Hbs were detected, and the iron studies were within normal ranges.

RESULTS—In a 19-month period, 33 of 1162 apheresis donors had low MCV values. Iron deficiency was present in 64%; 49% had isolated iron deficiency and 15% had iron deficiency plus hemoglobinopathy. Hemoglobinopathy without concomitant iron deficiency was found in the remaining 36%.

CONCLUSION—Iron deficiency is present in the majority of apheresis donors with repeatedly low MCV values and Hb levels of 12.5 g/dL or more. Hemoglobinopathy is also commonly present but may not be easily recognized in the setting of iron deficiency. The MCV is a useful screening tool to detect iron deficiency and hemoglobinopathy. Low MCV values should be investigated to determine if iron replacement therapy is indicated.

Donors of apheresis blood components are routinely assessed with a complete blood count (CBC) at the time of each donation at most blood centers. In order to qualify for the current or subsequent apheresis collections, the donor's CBC must meet a defined minimum value for the intended cellular product being collected (e.g., platelets [PLTs], granulocytes, or lymphocytes). Other than the hemoglobin (Hb) or hematocrit levels, the results of the other red blood cell (RBC) parameters are often not evaluated.

In otherwise healthy donors, low mean corpuscular volume (MCV) values of less than 80 fL, in the presence of an acceptable Hb level of greater than or equal to 12.5 g/dL, could indicate iron deficiency or a hemoglobinopathy, such as alpha thalassemia or beta chain variant. Iron deficiency in blood donors may warrant treatment with oral iron, whereas donors with hemoglobinopathies in the absence of iron deficiency do not need iron

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CONFLICT OF INTEREST
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replacement therapy. If a donor has a low MCV value due to a hemoglobinopathy, individualized lower normal limits for their MCV values should be established to accommodate the microcytic effects of the hemoglobinopathy but not mask the potential development of iron deficiency. In this study, the etiology of low MCV values in a healthy apheresis donor population was assessed.

MATERIALS AND METHODS

Donors

The apheresis donor population at our facility consists of two groups of donors: volunteer apheresis donors who donate for transfusion purposes and research apheresis donors who are financially compensated for donations which are used for in vitro laboratory research at our institution. During a 19-month period, routine predonation samples for CBC were obtained from 1162 consecutive healthy volunteer and research apheresis donors donating PLTs, plasma, granulocytes, lymphocytes, and monocytes. All of these donors successfully completed the predonation screening process and had acceptable Hb levels (≥12.5 g/dL). Apheresis donors with Hb screening values of less than 12.5 g/dL were not included in this study and were evaluated in a separate protocol for donors with low Hb levels.

MCV values of less than 80 fL were flagged electronically via a computerized donor database application (Blood Bank Computer System, Inc., Auburn, WA) for review by medical staff. Donors with MCV values of less than 80 fL were evaluated for iron deficiency and the presence of hemoglobinopathies at the time of a subsequent donation visit. This prospective study was conducted as an institutional review board–approved apheresis donor research protocol.

Health history screening

Donors with low MCV values noted on a previous donation were asked additional questions as part of a health history screening questionnaire to determine blood donation and deferral frequencies; current or past iron therapy; history of gastrointestinal or genitourinary blood loss; obstetric and gynecologic history; medications; diet; and personal or family history of anemia, clotting or bleeding disorders, hemoglobinopathy, or cancer.

Laboratory testing

Hb screening values were obtained by the capillary finger-stick method using a portable Hb screening device (HemoCue Hb 201+, HemoCue, Angelholm, Sweden). Samples for CBC were collected by venipuncture and analyzed by an automated hematology instrument (Cell-Dyn 4000, Abbott Laboratories, Abbott Park, IL). Serum iron determinations were performed on an automated chemistry analyzer (LX20, Beckman-Coulter, Fullerton, CA) as were ferritin and transferrin levels (Immulite 2500, Diagnostics Product Corporation, Los Angeles, CA). Hb electrophoresis was performed using ion-exchange high-performance liquid chromatography (Varian HPLC system, Bio-Rad Diagnostics Group, Hercules, CA). Chain analyses to confirm alpha thalassemia were not performed.

Iron deficiency was defined as a ferritin below the normal range of 9 μmg/L in females and 18 μmg/L in males or a transferrin saturation level of less than 15%. Beta and specific alpha Hb chain variants were determined by the findings on Hb electrophoresis. Alpha thalassemia trait was presumed if the RBC count was elevated; no variant Hbs were detected; and the ferritin, serum iron, and transferrin saturation were all within normal range.

Oral iron replacement therapy

Donors found to be iron deficient were given 60 tablets of ferrous sulfate 325 mg (65 mg of elemental iron). Tablets were dispensed in child-resistant blister packs. Donors were instructed to take one tablet by mouth daily, 30 minutes before bedtime, with a half glass of water. Donors with a history of sensitivity to ferrous sulfate, or who developed intolerance to the tablets, were given 325-mg ferrous gluconate tablets (38 mg of elemental iron). Subjects were instructed to notify the blood bank physician promptly if adverse effects occurred. Repeat laboratory testing was performed at subsequent donor visits to assess the effectiveness of the iron replacement therapy and to evaluate the presence of hemoglobinopathy after the correction of the iron deficiency.

Donor counseling

Donors with confirmed hemoglobinopathies were counseled regarding the impact and genetics of possessing a variant Hb trait.

Referral to primary care physicians

Donors whose responses to health history screening questions and/or laboratory test results indicated a potentially serious health concern were referred to their primary care physician for follow-up.

Statistical analyses

Group outcomes were compared using Student's t test. When appropriate, paired t tests were used, with statistical significance defined as a p value of less than 0.05.

RESULTS

In a 19-month period, 1162 apheresis donors presented for donation. These subjects consisted of 795 volunteer donors (53% male; 87% Caucasian, 4% African American, 3% Asian, 2% Hispanic) and 367 paid research donors (68% male; 54% Caucasian, 36% African American, 4% Asian, 3% Hispanic). Thirty-three of these donors (2.8%) had acceptable Hb levels of 12.5 g/dL or more with low MCV values of less than 80 fL. These donors were more likely to be African American (15 of 33, 45%) and Asian (3 of 33, 9%) compared to the overall apheresis donor population (Table 1).

Iron deficiency was present in 64% of donors with low MCV values and acceptable Hb levels; 49% had isolated iron deficiency and 15% had iron deficiency plus hemoglobinopathy (Table 2). The mean ferritin and percentage of transferrin saturation values in the apheresis donors with iron deficiency were significantly lower than those with isolated hemoglobinopathies. MCV values in the iron-deficient groups were slightly higher than those of the hemoglobinopathy group without iron deficiency (Table 3).

Hemoglobinopathy without concomitant iron deficiency was found in the remaining 36% of donors with low MCV values and acceptable Hb levels (Table 4). Specific hemoglobinopathies identified in this group included one case of a single beta chain variant (Hb Lepore), nine cases of alpha chain variants (including one example of the alpha chain variant HbG Philadelphia coexisting with an additional alpha thalassemia trait and one example of a probable double deletion alpha thalassemia trait), and two cases of beta chain variants coexisting with alpha thalassemia traits (one HbS trait with a single deletion alpha thalassemia trait and one HbS trait with double deletion alpha thalassemia trait). In the five donors with both hemoglobinopathy and iron deficiency, one had a beta chain variant (HbC trait) and the other four had alpha thalassemia trait which became more evident upon normalization of their iron studies after successful iron replacement therapy.

Donor health history screening questionnaires and results of laboratory testing did not reveal any potentially serious health concerns requiring referral to primary care physicians. Upon request, donors were given copies of their laboratory results to share with their physicians.

Among the 21 donors with iron deficiency, 19 received and responded appropriately to iron replacement therapy with normalization of their ferritin and transferrin saturation levels and resolution of fatigue and pica, when present. Two donors refused iron replacement therapy because they did not like to take pills. Overall compliance with daily oral iron therapy was 82 ± 27.2% (range, 20%-100%) in the group that took the iron tablets. Intolerance to ferrous sulfate was not reported by any donor before treatment but gastrointestinal symptoms developed in 2 of the 19 donors (10.5%) who took ferrous sulfate. These donors were given ferrous gluconate and none reported intolerance to this formulation.

Acceptable lower limits of MCV values were established in donors with hemoglobinopathies to compensate for their specific Hb variant(s) and to reflect a level at which the potential development of iron deficiency would need to be considered. Iron deficiency subsequently developed and was identified in 4 of the 12 donors (25%) initially identified as having a hemoglobinopathy without concomitant iron deficiency. These donors were placed on iron replacement therapy and demonstrated 100% compliance with treatment, no reported intolerance to ferrous sulfate, and normalization of their iron studies.

DISCUSSION

In this study of healthy nonanemic apheresis blood donors, the evaluation of low MCV values was useful in the detection of iron deficiency and hemoglobinopathy. Of the 2.8% of apheresis donors (33 of 1162) with low MCV values and acceptable Hb levels, 49% had isolated iron deficiency, 36% had isolated hemoglobinopathy, and 15% had concomitant iron deficiency and hemoglobinopathy. Overall, iron deficiency was present in 64% of apheresis donors with low MCV values and Hb levels of 12.5 g/dL or more. Iron replacement therapy was beneficial in the iron-deficient donors taking iron supplements, with resolution of symptoms of iron deficiency, when present, and few unfavorable side effects, all of which resolved with a change in iron formulation. In the donors found to have hemoglobinopathies, counseling was provided to explain the significance of their Hb variant trait, thereby providing a community service. Identification of these donors allowed the donor center to establish reasonable lower MCV limits for those with hemoglobinopathies. This practice maintained a safety net to identify those who subsequently became iron deficient (25%, 4 of 12 donors).

Previous studies have debated the use of RBC indices and various formulas (some of which are cumbersome) to differentiate the presence of iron deficiency and hemoglobinopathy traits, especially thalassemia (Table 5).[1-20] Each of these parameters or “discriminant functions” may be advantageous in given patient and geographical populations, but several authors concur that they may not be useful as screening tools or mechanisms to definitively distinguish between iron deficiency and thalassemia.[21-24] Many authors feel that the MCV alone is an effective, reliable, and easily available laboratory test for the differentiation of microcytosis caused by iron deficiency and hemoglobinopathies (especially thalassemias) and that there is no advantage in using more complex formulas.[12-13] Wonke and colleagues[25] reported that when microcytosis was encountered in a multiethnic community, further analysis of ferritin levels and Hb electrophoresis provided an efficient and cost-effective method for the accurate diagnosis of iron deficiency and hemoglobinopathy.

Low MCV values in a healthy apheresis donor population with Hb values of 12.5 g/dL or more are usually due to iron deficiency and/or hemoglobinopathy. Determination of the

etiology of low MCV values is important, since donors with iron deficiency can benefit from iron replacement therapy, thus preventing further declines in iron stores and possibly avoiding deferral for low Hb values. Identification of donors with hemoglobinopathies provides valuable information to the donors and allows the blood center to reevaluate an acceptable range for MCV levels in these donors. Choice of a customized MCV alert value in patients with hemoglobinopathies avoids unnecessary deferrals from donation and also provides an early indication of the subsequent development of iron deficiency. However, the coexistence of iron deficiency and some hemoglobinopathies can present a diagnostic dilemma, since iron deficiency must often be corrected before the laboratory diagnosis of a thalassemic trait. Alpha thalassemia trait can be presumed if the donor has microcytosis and an elevated RBC count with normal iron stores. However, beta thalassemia trait can often be masked on Hb electrophoresis since iron deficiency may suppress the Hb A2 level to the normal range.

For hospital-based blood centers with limited resources to perform a thorough laboratory workup for low MCV values as described in this study, a simple ferritin level would identify donors with iron deficiency. Iron replacement therapy could be provided or recommended by the medical staff with reevaluation of MCV levels at future donations. In blood centers without resources for a ferritin level, a 60-day trial of iron replacement therapy could be recommended, since iron deficiency is the most common cause of low MCV values in apheresis donors with Hb levels of 12.5 g/dL or more. If iron deficiency is partially or completely responsible for the low MCV value, improvement in this RBC parameter would be expected after treatment. If an increase in MCV is not achieved, a hemoglobinopathy is more likely.

In both of the above scenarios and as shown in our study, it is important to assess the possibility of an underlying condition for which iron deficiency is a marker. Although this cohort of apheresis donors has Hb levels acceptable for blood donation, signs and symptoms of gastrointestinal or genitourinary bleeding must be elicited from the donor. This information can easily be obtained by skilled health professionals stationed in the apheresis donor room. Any donor reporting a potentially serious health condition should be referred to their primary care physician for follow-up.

Iron replacement therapy is a cheap, safe, and efficacious treatment for iron deficiency, which often develops in blood donors. Parallel studies in progress by the authors have demonstrated the safety, feasibility, and practicality of iron replacement therapy in the routine management of blood donors. In these long-term studies, routine iron replacement therapy was not found to be harmful in donors regardless of whether they had iron depletion or deficiency, and the practice of routinely replacing the iron lost by blood donation prevented the development of iron depletion in a substantial fraction of donors.

MCV values are part of the routine CBC test obtained by most blood centers to qualify apheresis donors for donation. Evaluation of low MCV levels in the setting of acceptable Hb values provides helpful information to the blood center medical staff and also provides a community service to blood donors. Identification and treatment of iron deficiency in donors prevents further decline in iron stores and Hb values thus protecting donor health and preserving the blood supply.

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ABBREVIATION

CBC complete blood count

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TABLE 1

Demographics of apheresis donors and study population*

Demographic	Apheresis donors (n = 1162)	Apheresis donors with low MCV (n = 33)
Gender		
Male	671 (57.7)	26 (78.8)
Female	491 (42.3)	7 (21.2)
Race		
Caucasian	888 (76.4)	15 (45.5)
African American	165 (14.2)	15 (45.5)
Asian	36 (3.1)	3 (9.0)
Hispanic	26 (2.2)	
Native American	1 (0.1)	
Other	46 (4.0)	

* Data are reported as number (%).

TABLE 2

Categories of apheresis donors with low MCV values and acceptable Hb levels*

	Apheresis donors with low MCV values and acceptable Hb levels, n = 33		
	Isolated iron deficiency, n = 16 (49%)	**Iron deficiency plus hemoglobinopathy, n = 5 (15%)**	**Isolated hemoglobinopathy, n = 12 (36%)**
Caucasian	9 (56)	1 (20)	5 (42)
African American	6 (38)	3 (60)	6 (50)
Asian	1 (6)	1 (20)	1 (8)

*Data are reported as number (%).

TABLE 3

Iron study results in apheresis donors with low MCV values and acceptable Hb levels*

	MCV fL		Ferritin mcg/dL		
Low MCV donors with	**Males (nl = 79-100)**	**Females (nl = 82-97)**	**Males (nl = 18-370)**	**Females (nl = 9-120)**	**% Transferrin saturation (nl = 15-62)**
Iron deficiency	78.4 ± 1.8	78.4 ± 1.0	15.4 ± 12.5	6.0 ± 1.0	11.8 ± 8.2
Hemoglobinopathy without iron deficiency	75.3 ± 4.5	73.0 ± 7.1	70.3 ± 35.5	48.5 ± 19.1	23.3 ± 7.2
Iron deficiency and hemoglobinopathy	78.0 ± 0.0	78.5 ± 0.7	38.3 ± 25.2	15.0 ± 2.8	17.6 ± 8.7

nl = normal range for laboratory test.

*Data are reported as mean ± SD.

TABLE 4

Low MCV values in apheresis blood donors

Causes	Number (n = 33)	Percentage
Iron deficiency	16	49
Hemoglobinopathy	12	36
Alpha thalassemia trait	10	
HbG Philadelphia trait and alpha thalassemia trait	1	
Hb Lepore trait	1	
Iron deficiency and hemoglobinopathy	5	15
Alpha thalassemia trait and iron deficiency	4	
HbC trait and iron deficiency	1	

TABLE 5

Discriminant functions to differentiate between iron deficiency and thalassemia trait

Formula or parameter name	Equation	References
Mentzer	MCV/RBC	1
Green and King index	$[(MCV)^2 \times RDW]/(Hb \times 100)$	2
RDW index	MCV × RDW/RBC	3
England and Fraser index	MCV – RBC – (5 × Hb)–k	4
Bessman index	RDW alone	5,6
Shine and Lal index	$(MCV)^2 \times (MCH/100)$	7
Srivastava index	MCH/RBC	8
Ricerca index	RDW/RBC	9
MCV/RBC ratio	MCV/RBC	10
M/H ratio	% Microcytosis/% hypochromia	11
MCV	MCV alone	12-15
RBC and MCV	RBC and MCV	16
RBC, MCHC, and RDW	RBC and MCHC and RDW	17

k = constant determined by the method used to calibrate the Coulter Counter.