MCV as a guide to phlebotomy therapy for hemochromatosis

Charles D. Bolan, Cathy Conry-Cantilena, Glorice Mason, Tracey A. Rouault, and Susan F. Leitman

BACKGROUND: A multitude of recommendations exist for laboratory assays to monitor the pace and endpoints of phlebotomy therapy for hemochromatosis. All of these recommendations rely on an assessment of storage iron to guide treatment, and none have been prospectively evaluated.

STUDY DESIGN AND METHODS: Nine consecutive patients underwent serial monitoring of Hb, MCV, transferrin saturation, and ferritin during weekly phlebotomy to deplete iron stores (induction therapy) and less frequent sessions to prevent iron reaccumulation (maintenance therapy). Changes in MCV and Hb were used to guide the pace of phlebotomy over a median of 7 years of follow-up.

RESULTS: During induction therapy, the MCV increased transiently because of reticulocytosis and then stabilized for a prolonged period before decreasing more sharply, which reflected iron-limited erythropoiesis. Iron depletion was achieved after a median of 38 phlebotomies and removal of 9.0 g of iron. Maintenance phlebotomy was targeted to maintain the MCV at 5 to 10 percent below prephlebotomy values and the Hb at >13 g per dL. Transferrin saturation fluctuated considerably during treatment, but remained below 35 percent during MCV-guided maintenance therapy. Ferritin values were not useful guides to the pace of phlebotomy. The median maintenance therapy phlebotomy interval was 7.5 weeks (range, 6-16), which corresponded to an average daily iron removal of 35 to 67 µg per kg. Most patients showed evidence of iron reaccumulation at phlebotomy intervals of 8 weeks or more.

CONCLUSION: The MCV is an inexpensive, precise, physiologic indicator of erythropoietic iron availability. When used in conjunction with the Hb, it is a clinically useful guide to the pace of phlebotomy therapy for hemochromatosis.

Hereditary hemochromatosis is present in 1 of 200 to 250 persons of Northern European descent and is the most frequently inherited disorder in this population.[1,2] Homozygosity for a tyrosine-to-cysteine substitution at position 282 (C282Y) of the recently discovered *HFE* gene is present in 83 to 100 percent of hemochromatosis patients in this ethnic group.[1-4] Disease manifestations are related to progressive iron loading of the liver, heart, pancreas, and other organs,[5,6] which is associated with inappropriately increased iron absorption by gastrointestinal enterocytes[7,8] and abnormal iron cycling by macrophages of the reticuloendothelial system.[9-11]

Phlebotomy therapy is the only effective therapy for iron overload in hemochromatosis.[12] During treatment, net removal of body iron occurs as a result of iron's mobilization from sites of excess storage, incorporation into Hb during erythropoiesis, and subsequent removal by phlebotomy. Despite recent advances in the understanding of the disease, however, the clinical practice of phlebotomy has changed little since its introduction 50 years ago.[12,13] Present recommendations for the management of phlebotomy are based largely on retrospective experience, and they provide little information about clinical values during therapy or the design of long-term maintenance programs.[12,14-20] In addi-

ABBREVIATION: CBC = complete blood count.

From the Department of Transfusion Medicine, Warren G. Magnuson Clinical Center, and the Cell Biology and Metabolism Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland.

Address reprint requests to: Susan F. Leitman, MD, Department of Transfusion Medicine, Building 10, Room 1C711, National Institutes of Health, Bethesda, MD 20892-1184; e-mail: sleitman@mail.nih.gov.

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tion, despite the intimate relationship between iron availability and RBC size, guidelines for laboratory monitoring during phlebotomy therapy generally do not utilize RBC values, but employ only the Hb concentration or Hct and serial assessments of iron stores. Most recent research priorities have focused on molecular studies or population-screening programs, and there has been much less emphasis on improving approaches to short- or long-term management.

This report describes the utility of the MCV as a simple, inexpensive, and physiologic guide to phlebotomy therapy for hemochromatosis. Serial routine changes in the MCV, a precise and directly measured indicator of erythropoietic iron availability,[21,22] accurately reflected the onset and maintenance of iron-limited erythropoiesis during phlebotomy. Measurement of the Hb and MCV provided a patient-specific guide for achieving iron depletion without symptomatic anemia and for performing maintenance phlebotomy without iron reaccumulation. The need for additional laboratory testing was obviated, and the pace of therapy could be simply and individually tailored for a clinically diverse cohort of patients.

MATERIALS AND METHODS

Study subjects

This report includes all patients with hemochromatosis who were referred to this transfusion medicine center for therapeutic phlebotomy between 1987 and 1997. Before 1997, a diagnosis of hemochromatosis was suggested by ferritin levels and at least two assessments of transferrin saturation, and it was confirmed by liver biopsy. Subsequently, all subjects underwent analysis for the C282Y and H63D mutations in the *HFE* gene.[1] Subjects gave informed consent for the use of their blood in laboratory research, and they were not assessed charges for phlebotomy.

Phlebotomy therapy

The phlebotomies were performed by technologists and directed by physicians in the transfusion medicine department of a tertiary-care clinical research center.[23] Subjects were questioned at each visit with regard to their exercise tolerance, energy, and sense of well-being. The criterion for performing phlebotomy on the day of a patient's visit was the Hb concentration as measured by a fingerstick device (HemoCue, Angelholm, Sweden) immediately before phlebotomy. The initial threshold for phlebotomy was a Hb >11 g per dL and, more recently, a level >12.5 g per dL. After phlebotomy, the time to the next session was determined by the MCV and Hb of the prephlebotomy complete blood count (CBC), as described below.

Laboratory measurements

At each session, a CBC, including RBC indices, was performed by an automated electronic particle counter (Coulter STKS, Brea, CA [1987-96]; Cell Dyn 3500, Abbott, Santa Clara, CA [1996-99]) on a sample obtained before phlebotomy. Measures of ferritin, iron, and transferrin saturation were determined by standard assays in routine use in the clinical laboratories in our center.[24] The amount of iron removed during a phlebotomy session was calculated as 0.34 percent of the circulating Hb concentration multiplied by the volume of blood in the phlebotomy bag.[7] The presence of the C282Y and/or H63D mutations in the *HFE* gene was analyzed by PCR (Mayo Medical Labs, Rochester, MN, and research laboratories in this institution). Liver biopsy reports were reviewed before therapy.

Interventions

In this study, weekly phlebotomy to deplete excess iron stores is referred to as "induction therapy," and subsequent phlebotomy to prevent the reaccumulation of iron is referred to as "maintenance therapy." Prephlebotomy Hb, Hct, and MCV; the phlebotomy volume; and patient symptoms were recorded at each visit. The serum iron level and the percentage of transferrin saturation were assessed every 2 to 4 weeks and ferritin levels every 3 to 6 months. A ferritin level was also obtained at the time of transition from induction therapy to maintenance therapy, to confirm iron depletion.

The method for MCV-guided therapy was derived from observations made in the first three patients and applied prospectively to subsequent patients. The interval between consecutive (weekly) phlebotomies was lengthened when the MCV fell to approximately 5 percent below the prephlebotomy baseline (generally, to 86-90 μ^3) and the Hb and MCV were decreasing in concert. If the Hb in the CBC fell to <11 g per dL before the targeted MCV was reached, the interphlebotomy interval was lengthened to 2 weeks. When the targeted MCV was reached, the interphlebotomy interval was lengthened to 2 weeks for the initial patients in the series and, with further experience, to 4 weeks or longer at the onset of iron-limited erythropoiesis. The maintenance therapy phlebotomy interval was targeted to maintain the Hb at >13 g per dL and the MCV at approximately 5 to 10 percent below the prephlebotomy baseline. Laboratory values are given as mean (SD) or median (range) unless otherwise stated.

RESULTS

Study subjects

The clinical and laboratory characteristics of the nine consecutive patients in this study (7 men and 2 women) are listed in Table 1. The median age at diagnosis was 46 years (range, 31-75). Reasons for referral included elevated iron levels detected on routine screening tests (n = 3), evaluation of abnormal liver function tests (n = 3), arthritis (n = 2), and screening secondary to diagnosis of an affected fam-

TABLE 1. Baseline clinical and laboratory characteristics of hemochromatosis patients

Patient (sex)	Age (years)	Reason for evaluation	HFE C282Y	Liver biopsy	Hb (g/dL)	MCV (μ^3)	Transferrin saturation (%)	Ferritin (ng/mL)	Associated conditions
1 (F)	75	Abnormal LFTs*	–/–	Yes	12.6	98	79	5000	ETOH,† arthritis
2 (M)	31	Family screen	+/+	No	15.6	92	95	671	None
3 (M)	45	Laboratory screen	+/+	Yes	14.7	NA	95	2430	Arthritis
4 (M)	46	Laboratory screen	+/+	Yes	15.4	90	90	1818	None
5 (M)	44	Arthritis	+/+	Yes	15.5	96	95	1786	Arthritis
6 (F)	58	Abnormal LFTs	+/+	Yes	13.7	97	94	2468	ETOH, arthritis
7 (M)	72	Laboratory screen	+/+	No	14.5	90	86	819	HTN‡
8 (M)	46	Arthritis	+/+	No	15.9	90	95	1045	Arthritis
9 (M)	50	Abnormal LFTs	+/+	Yes	15.4	118	85	2024	HIV, HCV, ETOH
Totals or medians	46		8/9	6/9	15.4	94	94	1818	

* Liver function tests.
† Mild ethanol consumption.
‡ Hypertension.

ily member (n = 1). No subject had hyperpigmentation, diabetes, or congestive heart failure. Eight were homozygous for the C282Y mutation; one had no identifiable HFE mutation. Patients 2 and 3 were treated after the initiation of phlebotomy at another facility. Arthralgias were noted at diagnosis in five of the nine patients, with radiographic findings demonstrating periarticular bony proliferation, subcortical cysts, beak-like osteophytes, and other changes consistent with hemochromatosis.[25,26] One patient (Patient 9) was infected with both HIV and HCV and was kept on antiretroviral therapy throughout the study.

Baseline hematologic and iron studies

The Hb was 15.4 (range, 12.6-15.9) g per dL and MCV was 94 (90-118) μ^3 before the initiation of phlebotomy (Table 1). Initial transferrin saturation and ferritin levels were 94 (79-96) percent and 1818 (671-5000) ng per mL, respectively. Liver function tests were mildly elevated (2× to 3× the upper limit of normal) in Patients 1, 3, 5, 6, and 9. Liver biopsy revealed marked to massive parenchymal iron overload in six patients who underwent biopsy. Patient 4 had no fibrosis or cirrhosis on biopsy, while Patients 1, 3, 5, 6, and 9 had portal, nodular, or bridging fibrosis with early or incomplete cirrhosis. The hepatic iron concentration was markedly elevated in biopsies for which this value was reported (Patients 5 [38,158], 6 [11,800], and 9 [8,615]; normal, 400-2200 µg/g dry weight).

Clinical and laboratory values during phlebotomy therapy

The initial phlebotomy volume was 400 mL in Patient 1 and 450 to 490 mL in the others. The amount of iron removed was 217 (154-254) mg, or 2.5 (1.9-3.2) mg per kg per phlebotomy during induction therapy. Patient 2, a long-distance runner, noted exercise intolerance after the last induction phlebotomy, in association with a Hb nadir of 10.5 g per dL at the next session and MCV nadir of 72 μ^3 two sessions later. Phlebotomy was otherwise well tolerated, without complaints, in other patients, and the interphlebotomy interval did not need to be adjusted because of symptoms.

During induction therapy phlebotomy, the MCV increased transiently during initial reticulocytosis[27] and then stabilized for a period of 8 to 50 weeks. Subsequently, a rapid decrease in the MCV with each successive bleed heralded the onset of iron-limited erythropoiesis, and this measure was used to initiate the transition from weekly to maintenance phlebotomy (Fig. 1A). Hb levels decreased in a similar fashion, slightly before the onset of the decrease in the MCV (Fig. 1B). The transition to maintenance therapy phlebotomy occurred at a median Hb of 11.7 g per dL and MCV of 89 μ^3 (Table 2). Hb and MCV values continued to fall for several phlebotomy sessions after transition, with nadirs of 11.6 (9.9-13.3) g per dL and 80 (72-88) μ^3, respectively (Fig. 1A/1B). Serial transferrin saturation (Fig. 1C) and ferritin levels decreased steadily without a plateau or sudden drop. At the point of iron depletion and transition to maintenance therapy, 38 (25-67) weekly phlebotomies had been performed, removing a cumulative total of 9.0 (4.9-16.0) g of iron.

The transition period from the end of induction therapy to the onset of maintenance therapy (interphlebotomy interval ≥6 weeks) occurred after an additional five (range, 3-25) biweekly to monthly phlebotomies. During a median of 7.0 years of follow-up, subjects underwent maintenance phlebotomy for 144 (61-535) weeks, with a median stable interphlebotomy interval during maintenance phlebotomy of 7.5 (6-16) weeks. Most patients had increasing MCV and transferrin saturation levels as evidence of increasing iron availability at phlebotomy intervals of >8 weeks. The median of the average amount of iron removed to maintain a stable MCV and Hb in each patient over the course of maintenance phlebotomy was 4.6 (1.7-5.9) mg per day. Average daily iron removal requirements during maintenance were more uniform when adjusted for patient weight, with a median removal of 48 (35-67) µg per kg per day. During the period of stable, MCV-targeted mainte-

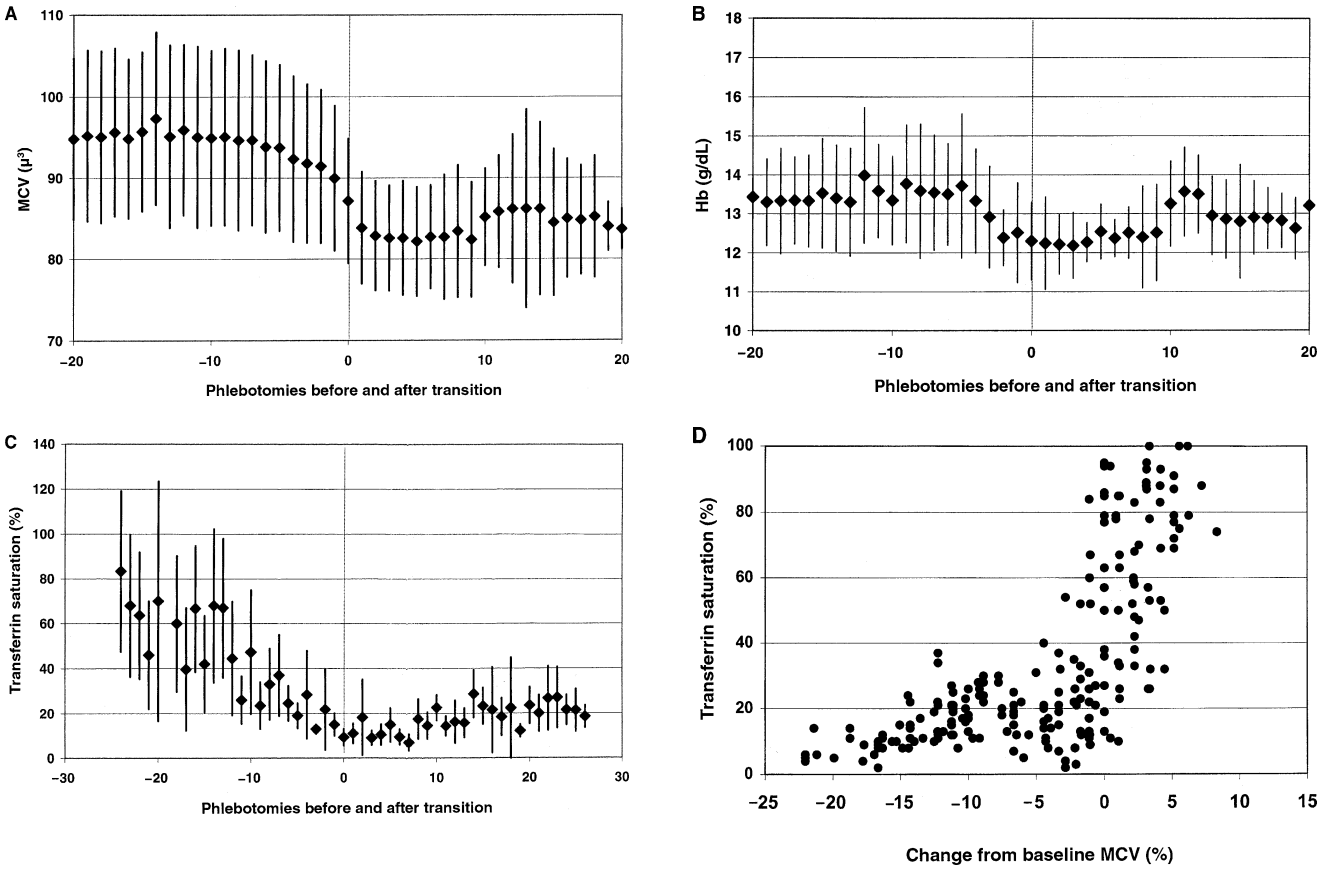

Fig. 1. Mean MCV (A), Hb (B), and transferrin saturation (C) plotted against the number of phlebotomies before or after transition (the change from induction therapy to maintenance therapy phlebotomy); and D) transferrin saturation plotted against the percentage of change in MCV. The decrease in Hb preceded the decrease in MCV. Decreases in transferrin saturation preceded and were more gradual than changes in the MCV and Hb, but changes in the saturation were less consistent than the patterns seen for the MCV and Hb. When the MCV was maintained at 5 to 10 percent below baseline, transferrin saturation was almost always less than 35 percent. Error bars are ± 1 SD.

nance phlebotomy, median Hb and MCV values were 13.3 g per dL and 87 μ^3, and median ferritin levels and percentages of iron saturation were 39 ng per mL and 19 percent, respectively. When the MCV was maintained at 5 to 10 percent below baseline, transferrin saturation was nearly always <35 percent (Fig. 1D).

Individual responses in patients with both normal and abnormal baseline laboratory values, atypical responses to phlebotomy, or features of hemochromatosis that respond uncharacteristically to ferritin-based therapy further highlight the utility of MCV-guided therapy. Figure 2 illustrates the value of the MCV in simplifying and guiding the course of phlebotomy, by identifying the onset of iron-limited erythropoiesis and defining a stable maintenance interval in a 46-year-old man (Patient 4). The MCV and Hb increased at phlebotomy intervals longer than 6 to 7 weeks and decreased at shorter intervals, which is consistent with a requirement for daily iron removal of 5.1 mg (48 μg/kg/day). In another patient (Patient 9), who had a baseline MCV of 118 μ^3 as a result of antiretroviral therapy, MCV-targeted phlebotomy accurately guided the transition to iron-limited erythropoiesis at an MCV of 111 μ^3 and defined a stable maintenance therapy phlebotomy interval of every 10 weeks. In an elderly patient (Patient 1) without a detectable *HFE* mutation, decreasing Hb, MCV, and transferrin saturation levels were observed when ferritin levels were still markedly elevated. Prolongation of the phlebotomy interval to 2 weeks, while the Hb was maintained at >11 g per dL and the MCV was allowed to decrease to 15 percent below baseline, resulted in iron depletion after an additional 35 phlebotomies. Similar atypical responses in elderly patients were described in prior reports, wherein liver iron stores appeared to be released more slowly during phlebotomy.[28-31] Finally, the limitations of ferritin-based therapy were illustrated in Patient 2, who was transferred to this center after undergoing maintenance therapy every 3 to 4 months at another facility. Although the patient's ferritin level was <50 ng per mL upon referral to our center, transferrin saturation remained consistently above 80 percent, and the MCV was unchanged from the prephlebotomy baseline. The pace of

TABLE 2. Clinical and laboratory values during phlebotomy therapy

	At transition*						During maintenance therapy†							
Patient	Hb (g/dL)	MCV (μ^3)	Transferrin saturation (%)	Ferritin (ng/mL)	Phlebotomies‡	Iron§ (g)	Hb (g/dL)	MCV (μ^3)	Transferrin saturation (%)	Ferritin (ng/mL)	Interval‖ (weeks)	Iron removal¶ mg/day	Iron removal¶ µg/kg/day	Duration** (weeks)
1	10.6	89	13	1900	31	4.9	14.0	87	22	47	16-18	1.7	35	535
2	11.5	89	4	20	41	10.6	13.3	89	27	14	7-8	4.6	67	144
3	NA	NA	NA	NA	NA	NA	14.2	87	14	39	6	5.8	48	243
4	12.2	85	19	35	58	11.4	13.6	88	26	16	6-7	5.1	48	278
5	11.4	95	9	26	67	16.0	12.6	81	10	6	8-9	3.7	38	106
6	13.4	98	45	50	66	13.0	13.1	98	47	40	5-6	5.9	60	89
7	11.9	89	10	38	35	7.3	13.1	86	13	NT	8	3.5	41	92
8	11.4	84	7	NT	31	7.4	14.0	85	22	10	6-7	5.2	59	170
9	12.5	111	5	34	25	6.5	12.4	92	5	12	7-10	3.5	40	61
Median	11.7	89	9.5	35	38	9.0	13.3	87	19	39	7.5	4.6	48	144

* Values at the time of the transition to maintenance therapy.
† Average values during maintenance therapy phlebotomy as determined by Hb and MCV.
‡ Numbers of phlebotomies performed at the time of transition.
§ Total amount of iron removed by phlebotomy at the time of transition.
‖ Maintenance therapy interphlebotomy interval.
¶ Phlebotomy-induced iron removal during maintenance therapy phlebotomy.
** Entire time course of maintenance therapy phlebotomy.

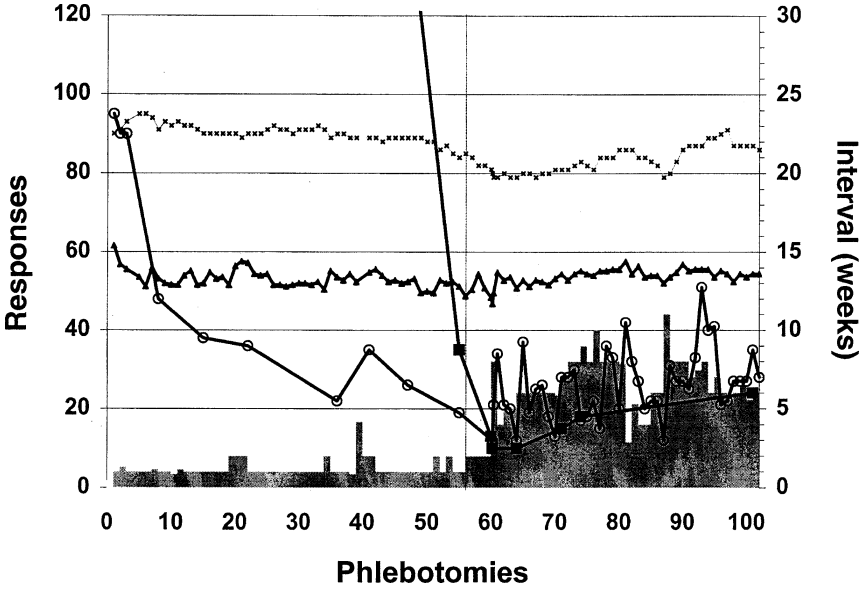

Fig. 2. Responses during 350 weeks of phlebotomy therapy in a 46-year-old man (Patient 4) plotted against the numbers of phlebotomies. Approximately 11.4 g of iron had been removed at the onset of iron-limited erythropoiesis (dotted vertical line), when the transferrin saturation was 19 percent and the ferritin level was 35 ng per mL. Phlebotomy was performed every 8 weeks for the next 2 years and every 6 to 7 weeks for the subsequent 5 years. During this period, the transferrin saturation (○) averaged 26 percent, the ferritin level (■) 16 ng per mL, and iron removal 5.1 mg (48 µg/kg) per day. At intervals (shading) longer than every 7 weeks, the MCV rose back toward baseline, and transferrin saturation increased. At intervals shorter than 6 weeks, progressive decreases were observed in the Hb (▲) and MCV (×).

phlebotomy was then briefly accelerated, with five phlebotomies performed over 6 weeks, and the MCV decreased from 92 to 86 μ^3. This patient was subsequently maintained at a stable interval of 7 to 8 weeks, with a mean transferrin saturation of 27 percent and a ferritin level of 14 ng per mL.

All subjects remain alive and well at the time of this report, without development of new signs or symptoms related to hemochromatosis. Rheumatologic symptoms were the only consistently reported complaints referable to hemochromatosis. Arthritis, present in five patients at diagnosis, was subjectively improved in two, was progressive in two, and was unchanged in one during phlebotomy therapy.

DISCUSSION

Despite advances in the understanding of the molecular pathophysiology and the genetics of iron metabolism,[1,3,13] the practice of phlebotomy for hemochromatosis has evolved little over the past 50 years.[12,13] Guidelines are based largely on retrospective analyses of aggressive iron-unloading regimens for severely iron-overloaded patients, vary widely in recommendations for induction therapy, and place little emphasis on the design of long-term maintenance programs (Table 3).[14,15,17-20,32-40] Recommendations for maintenance therapy phlebotomy range from intervals of every 6 to 8 weeks[36] to every 3 to 6 months[20] to none at all[39] and are based on semiannual to annual assessment of ferritin levels.

Because an accumulating body of evidence suggests that excess iron is toxic to tissues,[41-43] we feel it is advisable to maintain tight, long-term control over iron deposition in

TABLE 3. Guidelines for induction therapy, transition, and maintenance therapy phlebotomy for hemochromatosis

Reference (year of publication)	Induction therapy phlebotomy*	Transition guidelines†	Maintenance therapy phlebotomy	
			Interval (months)	Values
Finch[32] (1955)	Monthly SI	↓ SI → bi- or tri-weekly phlebotomy	1-3	Not stated
MacGregor[34] (1957)	Weekly SI, TIBC	Hb <11-12, ↑ TIBC, "lasting" ↓↓SI	3-6	↓ SI, ↑ TIBC
Bomford[35] (1976)	Chelatable iron	Hb <10, iron <10	≥3	Chelatable iron, ferritin
Milder[36] (1980)	SI, TIBC, ferritin	↓SI, ↓TS, ↓Hb	2-6	Annual ferritin <200
Edwards[14] (1980)	SI, TS, ferritin	"Precipitous" ↓Hb, SI, and TS‡	1-3	TS near 50
Halliday[38] (1982)	Ferritin	Hb <11, ferritin "approximately" 10	3-4	Low normal ferritin
Edwards[37] (1993)	Mild anemia*	MCV <75, TS <15, ferritin <20	2-6	Annual ferritin within normal range
Adams[39] (1993)	NA	Ferritin <50	Variable; in some, never	Annual ferritin, reinstitute weekly phlebotomy if >normal range
Witte[15] (1996)	Mild anemia	Absolute iron deficiency	2-6	Semi-annual ferritin <100
Bacon[17] (1997)	Periodic TS, ferritin	Ferritin <20-50 and TS <50§	2-3	Not stated
Bothwell[33] (1998)	Hb	↓ Hb (TS low, ferritin generally <20)	3	Low normal TS and ferritin
Barton[18] (1998)	Ferritin every 4-8 to 1-2 weeks	Ferritin 10-20 or Hb <11 × 3 weeks	3-4	Normal Hb, ferritin ≤50
Olynyk[20] (1999)	Ferritin every 1-2 months	Ferritin <50	3-6	Annual or bi-annual ferritin
Worwood[19] (1999)	Ferritin, "not anemic"	Ferritin <20 and TS <16	Several	TS <60, ferritin <100
Brissot[40] (2000)	Ferritin, TS, Hb >11	Ferritin <50 and TS <20	As needed	TS <35, ferritin <50
Present study (2001)	MCV, Hb >12.5‖	MCV 5-10% below baseline, ferritin <50	1.5-4	Hb >13, MCV 5-10% below baseline

* Values in addition to Hb: SI = serum iron; TIBC = total iron-binding capacity; TS = transferrin percent saturation.
† Transition from induction to maintenance therapy phlebotomy: Hb in g per dL, ferritin in ng per mL, MCV in μ^3.
‡ Induction therapy continued until iron-limited erythropoiesis occurred; values indicate iron depletion.
§ Induction therapy continued until iron-limited erythropoiesis occurred, as indicated by falling Hb that did not recover by the next session.
‖ Assessed by a prephlebotomy fingerstick.

patients with hemochromatosis. This is especially relevant in the current practice environment, where liver biopsies are performed less frequently because of the availability of a genetic test.[44] Because patients with normal liver function tests may still have fibrosis,[45] or even cirrhosis if they are over 40 years old,[46] we feel it is especially prudent to treat all patients, not only those with known cirrhosis, with a maintenance program that prevents iron reaccumulation. However, the conventional tests used to assess iron status, such as the ferritin level and transferrin saturation, have theoretical and practical limitations when used to attain this goal.[47] Ferritin levels may be normal at diagnosis in some patients with hemochromatosis, despite significant excess iron stores.[48] Ferritin levels may also remain deceptively depressed and fail to reflect substantial iron reaccumulation, as assessed by significant increases in chelatable, hepatic, and phlebotomy-mobilized iron stores after initial iron depletion in hemochromatosis patients.[36,49] Furthermore, because the major role of the ferritin molecule is to detoxify increasing intracellular iron stores,[47] maintenance therapy phlebotomy guided by the detection of increasing ferritin levels might not produce optimal physiologic protection from iron-mediated tissue damage. Finally, the relationship between iron stores assessed by ferritin levels and transferrin saturation values is markedly different in hemochromatosis patients from that in normal subjects, with the former exhibiting consistently elevated transferrin saturation levels despite ferritin values in the normal range.[50] In fact, transferrin saturation levels rebound rapidly after initial iron depletion,[49,50] and transferrin-mediated parenchymal iron deposition may continue despite ferritin levels that remain in the normal range.[51-53]

Although an assessment of transferrin saturation thus might be a more physiologic guide to phlebotomy than ferritin levels, the utility of this value is limited by a lack of interlaboratory standardization in its measurement[24] and by significant fluctuations due to diet and diurnal variation.[54,55] The assay is most accurate when performed with the patient in a fasting state, but patients and blood donors are instructed not to fast before phlebotomy, which limits the reliability of transferrin saturation as a guide to phlebotomy therapy.[23] Other measurements of body iron stores, such as those for the chelatable iron pool[49] or serum nontransferrin-bound iron,[43,56] may more closely reflect the potential for iron-related tissue damage. However, such tests are not widely available, and the optimal laboratory guides for monitoring and preventing iron-mediated cellular damage in hemochromatosis are not established.

In this study, tandem changes in the MCV and Hb were highly reliable guides to the development of an iron-limited state and to the subsequent maintenance of a state just verging on iron-limited erythropoiesis. Initial iron depletion was achieved when the MCV first decreased by 5 percent from prephlebotomy baseline values, to a value of about 90 μ^3. The abrupt decrease in the MCV at this point provided the signal for initiating the transition from induction therapy to maintenance therapy phlebotomy. Subjects were subsequently maintained at a very mild level of iron-limited erythropoiesis by targeting the MCV to a value that was within the normal range (85-90 μ^3), but still 5 to 10 percent below the individual subject's baseline. The median frequency of phlebotomy necessary to maintain this mildly iron-limited state was every 7.5 weeks, which corresponded to a median iron removal of 48 (35-67) µg per kg daily. Hb

levels were also kept slightly below baseline prephlebotomy values, but they remained in the normal range, >13 g per dL, throughout maintenance therapy. Maintenance of the MCV within the target range was associated with normal or low normal ferritin levels (Table 2) and a transferrin level below 35 percent at nearly all times, levels similar to the targets recommended in a recent review of long-term maintenance therapy.[40] Ferritin levels were helpful at diagnosis and to confirm iron depletion, but they were not useful in guiding the pace of induction or maintenance therapy. These data suggest that RBC indices, such as the MCV and Hb alone, may be sufficient and possibly superior to standard tests in monitoring phlebotomy therapy for hemochromatosis.

There are additional reasons why the MCV may be particularly suitable for monitoring phlebotomy therapy. Erythropoiesis and RBC iron utilization are normal in hemochromatosis,[30] in contrast to the observed abnormalities in gastrointestinal iron absorption and iron cycling by macrophages. Before the initiation of phlebotomy, the MCV of hemochromatosis patients is greater than that of controls, which suggests a constant access to the greater amount of iron available during RBC growth.[44,57] In normal patients and those with hemochromatosis, the erythron utilizes approximately 20 times more iron daily than is derived from dietary absorption.[51] Thus, when a patient is maintained on the verge of iron-limited erythropoiesis, the erythron would have the capacity to function as a reservoir for iron uptake, preventing further parenchymal deposition of excess iron.

In population-based studies, average measurements of RBC protoporphyrin or serum transferrin receptor have been reported to be superior to RBC indices in detecting the prevalence of early iron-limited erythropoiesis.[54,58] In this study, serial MCV measurements were highly reliable guides to iron depletion and subsequent maintenance therapy phlebotomy requirements, without the need for additional laboratory testing. Furthermore, because iron is not incorporated into RBCs after the reticulocyte stage,[59] the measured MCV reflects the aggregate contributions of all RBCs manufactured over the previous 120 days. The MCV might therefore be an ideal measurement of average iron status during maintenance therapy. During the transition from induction to maintenance therapy phlebotomy at the onset of iron-limited erythropoiesis, decreases in the MCV lagged slightly behind decreases in Hb (Fig. 1A/B), which reflects the average contribution of all RBCs, rather than only newly formed RBCs, to measurement of the MCV. Assessment of the reticulocyte MCV or other indices might provide more immediate information concerning the time to this transition.[60] Finally, the MCV is directly, precisely, and inexpensively measured by laboratory devices in widespread clinical use, and, as part of the automated CBC, it provides rapidly available results without additional blood sampling or analysis.

Other studies frequently do not report data on patient symptoms or laboratory values obtained during the course of phlebotomy therapy, despite quite stringent recommendations to attain absolute iron deficiency or an MCV <75 μ^3 during iron depletion (Table 3). In this study, phlebotomy was well tolerated, except in one competitive athlete, who developed symptomatic anemia at the time of iron depletion. During this patient's treatment, the criterion for proceeding with phlebotomy was a preprocedure fingerstick Hb of >11 g per dL. The present threshold for performing phlebotomy at our center has been increased to a Hb level of >12.5 g per dL, which allows a more gradual attainment of MCV-guided iron depletion, without symptoms. This guideline also serves to avoid significant iron deficiency anemia due to excessive phlebotomy, as described in a recent study, wherein MCV and Hb levels were as low as 59 μ^3 and 8.3 g per dL, respectively.[61]

The individually determined, stable maintenance therapy intervals shown in this study are shorter than those recommended in most prior reports. It is possible that maintenance of a mildly iron-limited state could directly lead to an increased need for phlebotomy, as an erythropoietic stimulus to increased gastrointestinal iron absorption is superimposed on a preexisting disease-mediated stimulus.[62] However, all prior studies of daily iron absorption in hemochromatosis have revealed uptake values similar to those in this study, even up to 10 years after the initial achievement of iron depletion in patients who did not undergo additional maintenance therapy (Table 4).[7,31,35,36,63] This close agreement with prior reports supports the hypothesis that maintenance therapy phlebotomy requirements in this study were not substantially influenced by erythropoiesis, but rather by the underlying state of pathologic iron absorption due to hemochromatosis.[62]

With the recent availability of genetic testing and renewed attention to and interest in broad-based population screening to detect hemochromatosis, it is likely that phle-

TABLE 4. Estimates of quantitative iron absorption in hemochromatosis after iron depletion

Reference (year of publication)	Number of subjects	Iron absorption (mg/day)	Months without maintenance therapy phlebotomy after iron depletion	Method
Chodos[63] (1957)	1	1.9-22	Not stated (iron-replete)	Radioisotope
Crosby[7] (1963)	3	1.7-5.75	21-40	Quantitative phlebotomy
Smith[31] (1969)	5	4.4	60	Differential chelation
Bomford[35] (1976)	12	1.4-4.9	48-120	Quantitative phlebotomy
Milder[36] (1980)	3	2-4	4-12	Quantitative phlebotomy
Present study (2001)	9	1.7-5.9	2-3	Quantitative phlebotomy*

* As assessed during stable, MCV-guided maintenance therapy.

botomy therapy will be increasingly used in patients with earlier diagnosis and smaller iron burdens. Recent FDA guidelines allow increased flexibility for allogeneic transfusion of blood obtained from hemochromatosis patients.[64] Therefore, the ability to guide phlebotomy by values available on a routine CBC would be of great practical importance to blood centers in simplifying and possibly improving the management of allogeneic blood donors who have hemochromatosis.[64,65] Whether tighter control of iron reaccumulation will prevent complications of iron-mediated cell damage during long-term therapy for hemochromatosis is an important consideration that is being addressed in active studies at the NIH.

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