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Initial Serum Ferritin Predicts Number of Therapeutic Phlebotomies to Iron Depletion in Secondary Iron Overload

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Abstract

Background—Therapeutic phlebotomy is increasingly used in patients with transfusional siderosis to mitigate organ injury associated with iron overload (IO). Laboratory response parameters and therapy duration are not well characterized in such patients.

Methods—We retrospectively evaluated 99 consecutive patients undergoing therapeutic phlebotomy for either transfusional IO (TIO, n=88; 76% had undergone hematopoietic transplantation) or non-transfusional indications (hyperferritinemia or erythrocytosis) (n=11). CBC, serum ferritin (SF), transferrin saturation, and transaminases were measured serially. Phlebotomy goal was an SF < 300 mcg/L.

Results—Mean SF prior to phlebotomy among TIO and nontransfusional subjects was 3,093 and 396 mcg/L, respectively. Transfusion burden in the TIO group was 94 ± 108 (mean $\pm$ SD) RBC units; about half completed therapy with 24 ± 23 phlebotomies (range 1–103). One-third was lost to follow-up. Overall, 15% had mild adverse effects, including headache, nausea, and dizziness, mainly during first phlebotomy. Prior transfusion burden correlated poorly with initial ferritin and total number of phlebotomies to target (NPT) in the TIO group. However, NPT was strongly correlated with initial SF ($R^2=0.8$; $p<0.0001$) in both TIO and nontransfusional groups. ALT decreased significantly with serial phlebotomy in all groups (mean initial and final values, 61 and 39 U/L; $p = 0.03$).

Conclusions—Initial SF but not transfusion burden predicted number of phlebotomies to target in patients with TIO. Despite good treatment tolerance, significant losses to follow-up were noted. Providing patients with an estimated phlebotomy number and follow-up duration, and thus a finite endpoint, may improve compliance. Hepatic function improved with iron off-loading.

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Keywords

therapeutic phlebotomy; transfusional siderosis; secondary iron overload; ferritin; transplantation; aplastic anemia; sickle cell disease; myelodysplastic syndrome

INTRODUCTION

In the past three millennia, therapeutic phlebotomy has been applied, with generally grim and adverse outcomes, to conditions as varied as fever, apoplexy, pneumonia, cholera, and gonorrhea.^{1,2} More recently, principles of evidence-based medicine have defined two disorders for which phlebotomy is an established primary therapy: genetic hemochromatosis associated with homozygosity for the *HFE* p.C282Y allele,^{3,4} and polycythemia vera associated with the *JAK2* p.V617F mutation.^{5,6} In both conditions, therapeutic phlebotomy not only results in symptom improvement but may also offer a survival benefit.

Therapeutic phlebotomy is becoming increasingly used to reverse systemic iron overload in previously transfusion-dependent patients who achieve remission of their primary disorder with hematopoietic progenitor cell (HPC) transplantation or chemo/immunotherapy.^{7–12} Less common applications include the management of non-transfusional hyperferritinemia, wherein iron overload may or may not be present, including conditions such as porphyria cutanea tarda^{13–16} and non-alcoholic fatty liver disease,^{17–19} or to reduce the risk of thrombosis in secondary erythrocytosis (congenital cyanotic heart disease, erythropoietin-secreting tumors).^{20,21} Mini-phlebotomy (removal of 200–400 mL of blood per session) has also been shown to be beneficial in iron loading anemias with ineffective erythropoiesis,^{22–24} and may reduce incidence of stroke in sickle cell disease.^{25–28} Finally, bloodletting has been studied and lacks sufficient evidence for use in conditions such as peripheral arterial disease,^{29,30} African iron-overload^{31–33} and chronic hepatitis C^{34–36} (Table 1).

Our institution has accumulated substantial experience in the treatment of iron overload in p.C282Y-homozygous *HFE*-hemochromatosis, wherein targeted endpoints of treatment are well-defined.^{4,37} Whereas clinical outcomes and laboratory parameters to monitor iron mobilization kinetics have been studied extensively in patients with hemochromatosis,^{38,39} few studies have evaluated therapeutic goals in other iron overload conditions treated with phlebotomy. In the case of hemochromatosis, lifelong maintenance phlebotomy is often required to prevent iron re-accumulation. With transfusional siderosis, iron off-loading by phlebotomy is typically temporary. Therapeutic goals and clinical outcomes are reasonably well-characterized in thalassemia patients with hematopoietic recovery following HPC transplantation;¹⁰ however, most studies in individuals with secondary hemochromatosis and erythrocytosis, including those with hematologic malignancies and hematopoietic recovery following chemotherapy or transplantation, involved small numbers of subjects assessed using inconsistent or less practical methods for iron overload, variable follow-up parameters and poorly defined clinical end-points. Interventions often included a combination of chelation and phlebotomy therapy, confounding the objective assessment of therapeutic bloodletting alone^{10,40–42}. Better understanding of the kinetics and pace of iron

mobilization in acquired siderosis may allow prospective counseling of patients and provision of an estimated endpoint of therapy. In addition, assessing the clinical utility of phlebotomy in these situations is also warranted, given the paucity of such data⁴³. The objective of this study was to characterize the response to phlebotomy, the ability to achieve targeted endpoints of therapy, the evidence for clinical benefit, and the compliance rates and adverse effects of treatment in patients with acquired transfusional and nontransfusional iron overload undergoing phlebotomy therapy.

MATERIALS and METHODS

Study subjects

The medical records of all patients referred to our transfusion medicine department for a course of therapeutic phlebotomy during the 14 year period from 1998 to 2012 were retrospectively reviewed. Persons with genetic hemochromatosis were excluded, as they had been previously reported.^{4,37} All other consecutive subjects who underwent at least two therapeutic phlebotomies were included. Subjects were categorized as having transfusional iron overload (TIO) or nontransfusional indications for phlebotomy, including but not limited to iron overload. Those with TIO had achieved disease remission and transfusion independence following either HPC transplantation (75%) or chemo- or immunotherapy (25%). Transfusion burden was calculated from our facility's records, from calls made to referring facilities, and from patients' responses to health history questions. All subjects gave informed consent according to the Declaration of Helsinki and per institutional treatment protocols.

Phlebotomy therapy

Phlebotomy therapy was initiated following consultation with a transfusion medicine physician. A hemoglobin of 10 g/dL was recommended as the threshold value to initiate treatment in patients with transfusional siderosis whose blood counts were recovering following definitive therapy. Venous adequacy was ascertained at the initial visit. Patients with inadequate venous access were referred to our facility's vascular access device service to undergo peripheral venous line placement using an ultrasound device (SonoSite, Bothell, Washington) prior to each bleed. Subjects were questioned at each visit with regard to their exercise tolerance, energy, and sense of well-being. Serial phlebotomies were performed by apheresis nursing staff and directed by transfusion medicine physicians. Initial treatment interval ranged from 1 to 6 weeks. The volume of blood removed at each visit was determined by subject weight, not to exceed either 500 mL or 10.5 mL/kg, whichever was smaller, and by a sliding scale based on fingerstick hemoglobin concentration (HemoCue, Angelholm, Sweden) obtained immediately before phlebotomy. If fingerstick hemoglobin was > 12.5 g/dL, 400–500 mL was removed; if it was 11.0–11.9 g/dL, 350–400 mL was removed; for 10.0–10.9 g/dL, 200–300 mL was removed. Volume removed thus ranged from 200–500 mL per session. Recombinant erythropoietin in the form of either darbepoetin (Aranesp, Amgen Inc., Thousand Oaks, CA) 200 µg subcutaneously every 2 weeks or epoetin alfa (Procrit, Ortho Biotech Products, Raritan, NJ) 40,000 IU subcutaneously weekly was used to facilitate planned phlebotomy. Erythropoietin therapy was initiated for hemoglobin levels < 11 g/dL and was discontinued at 12 g/dL. It was also used to enable

optimum volumes (>350 mL) to be removed at each phlebotomy session so as to shorten total treatment course.

Phlebotomy goal was a serum ferritin < 300 mcg/L, regardless of gender, based on data in patients with thalassemia and transfusional siderosis.¹⁰ In some patients with chronic GVHD, infection, or steatosis, where ferritin elevation also reflected systemic inflammation or hepatic injury and could not be used as a reliable endpoint indicator, an MCV decrease of 3–5% from baseline and/or a transferrin saturation < 30% were used as endpoint goals.⁴ Among patients undergoing phlebotomy for non-transfusional iron loading states, the endpoint goal was a serum ferritin < 50 mcg/L.¹⁵ If the indication for phlebotomy was an absolute or relative elevation in red cell mass, a hematocrit cut-off of < 45% was used, as previously described.^{5,6, 20} Pre- and post-phlebotomy vital signs were monitored and, at nursing discretion, infusions of 100–200 mL of normal saline were administered immediately after phlebotomy for symptoms such as dizziness or nausea, or for a greater than 15% decrease in systolic blood pressure. The need for adequate oral hydration during the 6 hours prior to phlebotomy was reinforced by the nursing staff.

Laboratory measurements and clinical data

At each session, a fingerstick hemoglobin was performed and a CBC, including RBC indices, was assayed by an electronic cell counter on a sample obtained before phlebotomy. Iron studies (serum ferritin, iron, and transferrin saturation), and liver and thyroid function tests were determined by standard assays in routine clinical use in our hospital laboratory. Cardiac function assessed by serial 2-D echocardiograms, liver biopsy and MRI data were reviewed if available. The amount of iron removed during a phlebotomy session was calculated using the formula, iron removed in grams = $(0.34\% \times \text{circulating hemoglobin concentration}) \times (\text{blood volume in each phlebotomy bag})$ ⁴⁴. Ancillary clinical data on incidental *HFE* mutations among Caucasian subjects, presence of chronic GVHD, viral hepatitis, infections, hepatic steatosis, and alcohol use were obtained by medical record review.

Statistical analysis

Summary statistics were calculated for all numerical data. Graphics and standard data analysis were performed with a spreadsheet application (Excel, Microsoft, Redmond, WA). Two-tailed paired and unpaired Student t-tests were used to compare groups of two with a normal distribution which was ascertained by histograms. Analysis of variance was used to compare more than 2 groups. Categorical variables were compared using a 2-tailed Fisher exact test. Pearson correlation coefficients were used to estimate relation of serum ferritin, transfusion burden, total number of phlebotomies and total iron removed. Data are provided as mean $\pm$ SD or median (range) unless otherwise stated. A p-value of < 0.05 was considered significant.

RESULTS

Subject demographics

During the 14 year period from 1998 to 2012, 99 patients met criteria for having undergone at least 2 phlebotomy treatments for iron overload or erythrocytosis. Overall, 89% (n=88) of subjects had transfusional iron overload (TIO) and were able to undergo phlebotomy therapy following hematopoietic transplantation (76%, n=67), immunomodulatory therapy (19%, n=17), or chemotherapy (5%, n=4). Severe aplastic anemia, sickle cell disease, and myelodysplastic syndrome were the three most common diagnoses in both the transplanted and non-transplanted TIO groups (Figure 1). Among the 11% (n=11) of patients with nontransfusional indications for phlebotomy, 6 had chronic hepatitis C infection and hyperferritinemia, 3 of whom also had porphyria cutanea tarda and 4 of whom carried a single variant HFE allele. The nontransfusional group also included 4 patients with secondary erythrocytosis and one with hereditary sideroblastic anemia. Due to the nature of the referral pattern to our institution, 66 of 99 patients (67%) lived at a great distance from our center, either in another state or another country. Many patients remained in a locally provided residence for 3–6 months after discharge.

Significant heterogeneity in demographic characteristics and laboratory parameters was present between the transfusional and nontransfusional hyperferritinemia/erythrocytosis groups at time of referral (Table 2). Mean age of the group overall was 34 ± 15 with a male predominance (73%). Hemoglobin was lower and transferrin saturation higher in the group with transfusional siderosis. Serum ferritin was markedly higher among the TIO patients versus the nontransfusional group (mean ferritin 3,093 vs 396 mcg/L, respectively). Persons with nontransfusional conditions were treated with higher phlebotomy volumes than those with TIO (mean 6.5 vs 6.3 g/kg of whole blood at each visit), due to higher initial hemoglobin levels. Among those completing a course of phlebotomy therapy and reaching the targeted endpoints defined above, the median number of phlebotomies to endpoint was 12, spanning a median of 14 months. The TIO group, as expected from initial ferritin levels, required a significantly greater number of phlebotomies (median 13), spanning a 15 month period, for a faster pace of bleeds than that used in the nontransfusional group, where the median number of bleeds was 5 over a period of 12 months. Among those with TIO, median transfusion burden was 65 units (range 3–800). Median time to referral following transfusion independence in the TIO patients was 6 months (range 1–67). Additional demographic details comparing those who underwent transplant or chemo-immunotherapy for transfusional siderosis are provided in the supplementary appendix (S.A. Table 1).

Contributors to hyperferritinemia other than TIO at the time of phlebotomy initiation are detailed in Table 3. Hepatic iron overload was documented in 9 of 16 patients undergoing liver biopsy. However, timing of biopsies was inconsistent in relation to phlebotomy initiation, and no further analysis was performed on this subset. Among patients who underwent HPC transplantation, 41% (n=27) had clinically significant chronic GVHD involving the liver, gut, lung and/or skin. Eighteen percent of the overall cohort had active infections during the period of phlebotomy therapy. No patient was homozygous for the HFE p.C282Y mutation, but 5 patients were found to be simple heterozygotes for variant

HFE alleles (C282Y/WT in three and H63D/WT in two subjects). Non-alcoholic steatohepatitis, hepatitis C (less commonly hepatitis B) and chronic alcohol use likely contributed to hyperferritinemia in 14%, 12% and 5% of the subjects, respectively (Table 3).

Efficacy of parameters used to guide therapy and determine endpoints

Significant decreases in serum ferritin and transferrin saturation were observed in all patients who underwent 5 or more phlebotomy sessions, whether or not they achieved the targeted endpoints of therapy (Table 4). Forty-eight patients completed the planned course of therapy, including 31/67 (46%) with TIO who underwent transplant, 8/21 (38%) with TIO who did not undergo transplant, and 9/11 (82%) with non-transfusional indications for phlebotomy. In the 48 patients completing phlebotomy and achieving targeted endpoints, MCV values remained stable in 57% of patients, with a less than 5% change from baseline over time, and decreased in 43% of patients. Hemoglobin values remained stable in 31% of patients, with a less than 5% change from baseline, improved in 15% (all of whom were undergoing continued hematopoietic recovery), and decreased in 54% of patients (S.A. Figure 1).

Among these 48 patients who completed phlebotomy, mean ferritin decreased from 1,758 to below 227 mcg/L in 42 of 48 subjects with mild (grade I) or no chronic GVHD, and transferrin saturation decreased from 52% to 19% (S.A. Figure 1). In 6 patients with clinically documented grade II or greater chronic GVHD, mean ferritin decreased from 3,345 to 1,910 mcg/L but remained elevated above 1,000 mcg/L despite continued phlebotomy. In these cases, a decline in MCV of greater than 5% from baseline, and on some occasions, a decline in the transferrin saturation to < 30% were used to guide phlebotomy cessation (Figure 2).

Among patients with TIO, initial serum ferritin levels and total iron removed by phlebotomy correlated poorly with estimated transfusion burden when plotted as continuous variables (Figures 3A and 3B). When subjects were divided into 3 ordered groups based on number of RBC units received (< 50, 50–100, and >100 red cell units), a significant trend toward increasing ferritin levels and total iron removed by phlebotomy was seen among the 3 groups (mean initial ferritin 954, 2631 and 3,906 mcg/L, respectively, $p < 0.001$). Serum ferritin at the time of treatment initiation was strongly correlated with the total amount of iron to be removed ($R^2 = 0.7$; $p < 0.0001$) and could be used to predict the number of phlebotomies to treatment completion using the formula $y = 0.01x + 3.6$, where y = number of phlebotomies needed to reach the targeted endpoint and x = initial ferritin level (Figures 3C and 3D). In all categories of TIO patients, the median number of phlebotomy sessions required to reach the targeted endpoint of therapy was 36% of the estimated number of prior red cell transfusions (mean 43%, range 2–124%). In the 48% of our cohort who reached target and discontinued phlebotomy, the serum ferritin remained stable for up to 2 years of follow-up.

Effect of phlebotomy on clinical symptoms and organ function

In the TIO group, it was not possible to determine the effect of phlebotomy on clinical symptoms, as these were mainly due to sequelae of their primary disorders or treatment. In

the 48 patients who achieved targeted endpoints and completed the planned course of phlebotomy therapy, mean ALT declined significantly, from 61 ± 60 to 39 ± 27 ($p = 0.03$), but remained elevated in 15 of 48 (31%) of patients. AST showed a similar but less pronounced trend (Table 4). Cardiac function as assessed by echocardiography improved in 2 of 5 transplanted patients with sickle cell disease following hematopoietic recovery and phlebotomy therapy. No significant changes were noted in thyroid function tests (TSH, FT4) with phlebotomy therapy.

Among the 11 patients with nontransfusional indications for phlebotomy, nine of whom completed the planned therapeutic course, cutaneous lesions improved or resolved in all 3 patients with porphyria cutanea tarda, fatigue decreased by subjective report in 2 of 4 patients with chronic HCV, and episodes of blurred vision or dizziness decreased and plethoric appearance improved in 3 of 5 patients with secondary erythrocytosis who reached a target hematocrit below 45%. ALT was elevated in 5 of 10 patients, and normalized in all. In the one subject with hereditary sideroblastic anemia phlebotomy, a marked decrease in ferritin was accompanied by an equally marked paradoxical increase in hemoglobin and MCV during long-term phlebotomy therapy (Figure 4).

Compliance with and adverse effects of phlebotomy

Forty-eight percent of the patients completed phlebotomy therapy and attained the targeted endpoint of therapy; 18% were still undergoing treatment at the time of this analysis, and 2% had disease relapse or death. One third of the subjects did not complete therapy and were lost to follow-up, including 3% of the total group who had procedure intolerance. Among the entire group, 15% had mild adverse effects including headache, nausea, and dizziness, most of which occurred during or immediately after the first phlebotomy. Fifteen percent of subjects received 100–200 mL of normal saline following each bleed to mitigate symptoms of dizziness and nausea. The sliding scale method to determine phlebotomy volume was used in 35% of patients at one or more visits, based on fingerstick hemoglobin less than 12.5 g/dL. Erythropoietin or darbepoetin was used at some time during phlebotomy in 30% and 7% of subjects, respectively, and when given, was generally given 1–2 weeks prior to the next bleed. Parenteral or oral chelation with deferoxamine or deferasirox had been attempted in 7 and 5 subjects, respectively, prior to initiating phlebotomy therapy. However, no subject received chelation therapy while receiving phlebotomy treatment. One subject was given deferasirox after the development of phlebotomy intolerance. Compared to phlebotomy, mild to moderate chelation-related adverse effects and/or inconvenience were documented in 50% of patients who had received either chelating agent. Two subjects who discontinued chelation therapy due to intolerance did well with phlebotomy therapy.

DISCUSSION

Phlebotomy therapy is the standard of care for patients with hereditary hemochromatosis and polycythemia vera, in whom it may prevent progressive iron-mediated tissue and organ injury,³ avert vascular complications,^{5,6} and prolong survival. Phlebotomy therapy has been less frequently applied in transfusional iron overload, since long term survival with normal hematopoiesis has only recently been achieved in patients with beta thalassemia, sickle cell

disease, marrow failure syndromes and hematopoietic malignancies following allogeneic hematopoietic stem cell transplantation.^{7,45} Guidelines for therapeutic phlebotomy and expected outcomes following treatment in these settings are not well characterized. In this retrospective study, we found that a targeted ferritin endpoint of less than 300 mcg/L in patients without chronic GVHD or systemic inflammatory conditions was easily achievable with phlebotomy intervals of two weeks to two months. In patients in whom the ferritin level could not be used as an indicator of iron depletion, the combination of a 3–5% decline in the MCV and a transferrin saturation of less than 30% appeared suitable as surrogate markers for iron depletion. In both groups, the total number of phlebotomy sessions necessary to achieve targeted endpoints varied over a large range, comprising a median of 36% of the estimated red cell transfusion burden. The large difference between the units of blood transfused and the units removed is likely due to the large volume of blood removed for diagnostic purposes in critically ill patients undergoing HPC transplantation, chemo- or immunotherapy at our institution. We estimate this volume to be large as 100 mL of blood daily. As has been noted previously in patients with beta thalassemia major, the ability to remove blood units at regular intervals while maintaining normal hemoglobin levels in patients “cured” of severe aplastic anemia, myelodysplasia or sickle cell disease, who had experienced months to years of frequent prior red cell transfusion requirements, was quite remarkable.¹⁰

Therapy cessation due to procedure intolerance and side-effects was minimal in our study population. Those patients who had undergone a prior trial of chelation therapy reported a higher incidence of adverse reactions to chelation than to phlebotomy, greater dosing inconvenience, and significantly greater expense.⁴⁰ Additionally, most adverse effects associated with phlebotomy improved over time or were mitigated by adequate hydration before or after the procedure and by varying the amount phlebotomized based on pre-procedure hemoglobin levels. However, one-third of subjects with transfusional iron overload did not complete the planned course of therapy. This high rate of non-compliance was likely related to the fact that many of our patients were clinical trial enrollees who lived overseas and returned to their home countries for long-term follow-up care. It is also likely that the lack of a predicted duration of treatment affected compliance. The ability to inform patients of the estimated number of bleeds at a given pace of therapy that would achieve a targeted endpoint may help to improve long term compliance. Further, a recent randomized trial demonstrated that erythropoietin therapy hastens erythroid recovery when started one month after allogeneic transplant⁴⁶. In our cohort, the timing of phlebotomy initiation following curative therapy and the use of erythropoietin to facilitate phlebotomy were variable and were performed at the primary transplant team’s discretion. We hypothesize that early, consistent use of erythropoietin to hasten time to phlebotomy initiation, and use during the treatment course to enable optimum volumes removed at each visit; would likely expedite treatment end-points.

Serum ferritin levels and red cell indices are practical, easily available, and inexpensive clinical tools with which to monitor response to serial phlebotomy therapy.⁴⁵ These assays are most helpful in cases where information on transfusion burden may be inaccurate. In our study, transfusion burden correlated poorly with the total amount of iron removed at completion of phlebotomy, likely due to unquantifiable but considerable losses during

intermittent hemorrhagic episodes (due to thrombocytopenia in the aplastic patients) and/or phlebotomy for diagnostic purposes. In patients without concomitant inflammatory conditions such as chronic GVHD, initial serum ferritin was strongly correlated with total iron removed. The regression equation for the relationship between initial ferritin and iron removed remained useful as a predictor of the number of phlebotomies necessary to achieve targeted endpoints over a wide range of ferritin levels in our patients. MCV and transferrin saturation proved useful in patients with hyperferritinemia due to inflammatory states, where phlebotomy endpoints were determined by an MCV decrease of 3–5% and the achievement of normal transferrin saturation levels. Interestingly, the amount of iron removed was accurately predicted by the regression equation based on initial ferritin in these patients as well. Hemoglobin levels increased in 15% of patients despite ongoing phlebotomy, as the effect of increasingly robust erythropoiesis outweighed the losses caused by phlebotomy.

The diagnosis of transfusional iron overload remains controversial. Liver biopsy is the gold standard, but associated discomforts and risks preclude its routine use. Magnetic susceptometry accurately assesses liver iron burden, but is available in only a few centers.⁹ In patients with thalassemia major and sickle cell disease, serum ferritin correlates well with liver iron content in patients with a lifetime transfusion burden of <100 units (< 400 mg/kg).⁴⁵ However, at lower levels of transfusion burden (< 250 mg/kg), significant inter-individual variability in SF levels is seen.⁴⁷ Recent studies support the use of MRI and CT techniques in the initial assessment of cardiac and hepatic iron overload.^{9,48,49} Phlebotomy referrals in our study were based on clinician assessment of a combination of parameters including transfusion burden, laboratory testing, MRI or CT imaging, and more rarely, liver biopsy.

It was not possible to determine the effect of phlebotomy therapy on long term organ function or survival in our patients. In thalassemic patients, persistent iron overload following successful transplantation is associated with progressive liver disease, endocrine failure, and heart disease, all of which are mitigated by phlebotomy treatment.^{45,48,50} However, some thalassemic patients with more modest transfusion burdens have been observed to spontaneously although slowly mobilize excess iron and reduce their ferritin levels without specific intervention following transplantation. The long-term efficacy of phlebotomy therapy in transfusional iron overload in non-thalassemic patients remains unknown. In our cohort, hepatic inflammation improved, as reflected in a decreased ALT in nearly all patients; however, the cause of the improvement was likely multifactorial and included resolution of hepatic GVHD in many cases. Although we were unable to assess the contribution of phlebotomy to these improvements in TIO subjects, our data demonstrate that patients with persistently elevated ALT following completion of phlebotomy therapy were highly likely to have non-IO as the cause. Improvements in endocrine or cardiac function were either not evident or not documented. Subjective improvement was noted in patients undergoing phlebotomy for non-transfusional indications, as previously reported¹². The six patients who underwent phlebotomy for HCV infection all had concomitant processes (PCT, alcohol excess, and/or variant HFE alleles) that may have contributed to elevated ferritin levels. With the recent availability of highly active antiviral agents for HCV, phlebotomy is no longer performed in an attempt to increase the rate of sustained viral response to drug therapy. In the single patient with hereditary sideroblastic anemia, serial

phlebotomy reduced cellular iron content, decreasing iron-mediated toxicity in marrow red cell precursors and improving erythropoietic efficacy.²⁴

In summary, phlebotomy therapy is a safe and effective means to manage hyperferritinemia in patients with a diverse group of disorders. Our study uniquely demonstrates that serum ferritin levels at referral are a practical guide to assess response to phlebotomy therapy and may be useful as a predictor of iron removed and duration of treatment necessary to reach targeted endpoints. In the presence of concomitant inflammatory conditions, ferritin levels may be combined with parameters such as MCV and transferrin saturation as indicators for therapy cessation. While transfusion burden history offers a ballpark estimate of phlebotomy target, it is less useful than the serum ferritin in predicting the amount of iron removed. Phlebotomy therapy may be a less toxic and more cost-effective means to off-load excess iron compared to chelation therapy in patients with transfusional iron overload and hematopoietic recovery following definitive therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Distribution of patients among the three diagnostic categories. SAA = severe aplastic anemia, SCD = sickle cell disease, MDS = myelodysplastic syndrome, LGL = large granular lymphocytic leukemia, HCV = hepatitis C virus infection, PCT = porphyria cutanea tarda, Sid anemia = hereditary sideroblastic anemia. "Other" diagnoses included pure red cell aplasia (3), renal cell carcinoma (2), chronic myelocytic leukemia (2), acute myeloid leukemia (1), chronic lymphocytic leukemia (1), beta thalassemia (1), paroxysmal nocturnal hemoglobinuria (1), and Blackfan-Diamond syndrome (1). All patients with erythrocytosis had secondary polycythemia; no patient had polycythemia vera.

Figure 2.

Utility of the MCV and transferrin percent saturation as an indicators of iron depletion by phlebotomy in 6 patients in whom serum ferritin was not a valid endpoint indicator, due to concomitant presence of chronic GVHD. Iron depletion was achieved when the MCV declined by 5% from baseline and/or the transferrin saturation declined to less than 30%, at a time when the ferritin was still > 1,000 mcg/L or steadily above our phlebotomy cessation cut-off levels (Panels A-C). Serial ferritin and MCV levels in a single patient undergoing phlebotomy therapy demonstrate relatively unchanged ferritin levels beyond phlebotomy number 42; however, MCV declined significantly (>5%) suggesting depletion of iron stores, necessitating phlebotomy cessation (Panel D).

Figure 3.

Although there was a statistically significant relationship between the red cell transfusion burden and the initial ferritin (A) and the total iron removed by phlebotomy (B), the correlation between these values and the red cell transfusion was poor. In contrast, the initial ferritin was strongly correlated with the total iron removed by phlebotomy, and could be used to predict the total number of phlebotomies required to reach the targeted endpoint (C, D).

Figure 4.

Effect of serial phlebotomy on laboratory parameters in a patient with hereditary sideroblastic anemia. WB = whole blood.

Table 1

Indications for Therapeutic Phlebotomy

Indication	Pathophysiology	Diagnosis	Phlebotomy Target*	Clinical outcomes	Comments	Ref.
Primary Iron Overload (IO)						
Hereditary hemochromatosis	Homozygous <i>HFE</i> (p.C282Y) mutation [†] → decreased hepcidin synthesis → excess iron absorption → end organ damage	SF>300 in M; >200 in F TS>45%; HFE gene studies	Weekly bleeds until SF<50, then maintenance bleeds every 3–4 months to keep SF<50–100	Prevention of liver, heart, endocrine injury; reversal of liver damage; survival benefit related to prevention of advanced liver disease	Linear SF decrease with serial phlebotomy	3,4
Secondary Iron Overload						
Hematologic malignancies (leukemia, MDS)	Parenteral iron loading due to transfusions	SF>1000 [‡] ; LIC by biopsy, T2-MRI, SQUID	SF<200–300 (in the absence of GVHD/infection)	Improved transaminitis; survival benefit unclear	Indicated if transfusion-independent after primary therapy	7–9
Sickle cell disease	Parenteral iron loading due to transfusions	SF>1000	SF <200–300 (bleed only if Hb>7g/dL and transfusion independent)	Resolution of IO; improved liver histology	Indicated if transfusion-independent after HSCT	25–28
Thalassemia	Ineffective erythropoiesis → enteral iron loading + transfusional siderosis	SF>1000 [‡] ; LIC by biopsy, T2-MRI, SQUID	SF<200–300 (in the absence of GVHD/infection)	Reversal of liver damage [§] , improved cardiac function	Indicated if transfusion-independent after HSCT	10–12
Dyserythropoietic anemias (HSA, CDA)	Ineffective erythropoiesis → enteral iron loading ± transfusional siderosis	Hyperferritinemia, molecular studies	SF in normal range, normalization of MCV	Paradoxical improvement of anemia	Initial Hb decrease, then increase	22–24
Iron Toxicity ± Overload						
Porphyria cutanea tarda	Enzyme defect in heme synthetic pathway → photoactive porphyrin accumulation, mild IO; worsened by HCV, HH, or excess alcohol	Skin lesions; elevated porphyrins in urine, feces; SF normal or elevated	SF < 50; typically 4–5 bleeds total	Improved skin lesions; normalized porphyrin levels		13–16
Chronic HCV infection	Hepcidin suppression by HCV-associated ROS	HCV PCR; SF>200	Phlebotomy not indicated	Treatment by newer highly effective antiviral agents		34–36
African IO	Diet, genetic	SF >300	SF <300	Theoretical benefit	No clinical trials	31–33
Non-alcoholic fatty liver disease (NAFLD)	Hepatic inflammation → hepcidin suppression	Clinical diagnosis of metabolic syndrome; SF>300; ↑ ALT steatosis on ultrasound or liver biopsy	Phlebotomy not indicated	Treatment directed at diet, exercise, weight loss	Elevated LIC generally not present on liver biopsy	17–19
Peripheral Arterial Disease (PAD)	Elevated iron stores may lead to ROS-mediated cardiovascular insult	Advanced PAD and SF>400	Investigational use only; SF=25–60, per study	May decrease risk of stroke, MI in subjects	Generally not performed outside of a clinical trial	29–30
Increased RBC mass						
Polycythemia rubra vera	Neoplastic clone of red cell progenitors	Erythrocytosis, suppressed EPO levels, JAK2/ other mutations	HCT<45%; maintenance bleeds may be needed	Decreased risk of CVA, DVT, improved survival	Concomitant aspirin and hydroxyurea in most studies	5,6
Secondary erythrocytosis	Excess EPO production (gene mutations in O2 sensing pathway, EPO-secreting tumors, COPD, high altitude)	Erythrocytosis, increased EPO, clinical history of associated conditions	HCT<55% and/or symptom improvement	Decreased thrombotic events, symptomatic relief	Cytoreductive therapy contraindicated	20–21

Key: SF, serum ferritin (mcg/L); TS, transferrin saturation (%); MDS, myelodysplastic syndrome; LIC, liver iron concentration; Hb, hemoglobin; HSCT, hematopoietic stem cell transplant; SQUID, liver iron quantitation by magnetic susceptibility; HSA, hereditary sideroblastic anemia; CDA, congenital dyserythropoietic anemia; MCV, mean corpuscular volume; ROS, reactive oxygen species; EPO, erythropoietin; HCT=hematocrit (%)

Unless otherwise specified, weekly or bimonthly therapeutic phlebotomy of 5–10 mL/kg until target reached
*
‡ Other rare causes of genetic hemochromatosis are not considered
‡ Little evidence for cut-off but used in most clinical studies to define transfusional iron overload
§ Only documented in thalassemia with extreme iron loading; hepatic damage reversible if not progressed to cirrhosis

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Table 2

Subject demographics

Demographics*	Total	Transfusional siderosis	Non-transfusional conditions	P Value
N	99	88	11	
Age	34 ± 15	32 ± 14	45 ± 9	0.004
Sex, male, %	74	74	73	0.9
Race: Caucasian (%)	25	17	91	<0.0001
Black (%)	27	31	0	
Hispanic (%)	29	32	9	
Asian (%)	9	10	0	
Unknown/Other (%)	9	10	0	
Pre-phlebotomy BMI	25 ± 4.8	25 ± 4.8	25 ± 4.6	0.8
Initial hemoglobin (g/dL)	13.4 ± 2.1	13.1 ± 1.8	15.8 ± 3.1	<0.0001
Initial serum ferritin (mcg/L) <i>mean, median (range)</i>	2793 2100 (8–26,465)	3093 2294 (55–26,465)	396 240 (8–1446)	0.008
Initial transferrin saturation (%)	56 ± 28	58 ± 28	41 ± 28	0.06
Initial MCV (fL)	98 ± 10	99 ± 10	90 ± 12	0.01
Transfusion burden (PRBC units) <i>mean, median (range)</i>	83 54 (0–800)	94 65 (3–800)	0	0.005
Phlebotomy initiation after transfusion independence, months <i>median (range)</i>	6.3 (1–67)	6.3 (1–67)	—	—
Whole blood removed per visit (g/kg)	6.3 ± 1.3	6.3 ± 1.3	6.5 ± 1.4	0.6
Total no. phlebotomies [†] <i>median (range)</i>	12 (1–103)	13 (1–89)	5 (1–103)	0.6
Months of phlebotomy [†] <i>median (range)</i>	14 (1–165)	15 (1–65)	12 (1–165)	0.06

* Data given as mean ± SD unless otherwise stated.

[†] Calculated for patients attaining pre-treatment specified phlebotomy endpoints (see Methods). Reference ranges: hemoglobin 13.7–17.5 g/dL (males), 11.2–15.7 g/dL (females); ferritin 18–370 mcg/L (males) and 9–120 mcg/L (females); transferrin saturation 15–62%; MCV 79.0–92.2 fL. None of the ranges changed substantially during the study period.

Table 3
Factors contributing to hyperferritinemia and AST/ALT elevation in patients undergoing phlebotomy therapy

Categories	Iron overload on liver biopsy*	cGVHD n, (%)	Active Infection n, (%)	NAFLD n, (%) [†]	HCV n, (%) [‡]	HFE mutations n [‡]	Alcohol excess n, (%)
Transfusional siderosis (transplanted) (n=67)	5/6	27 (41)	17 (25)	8 (12)	6 (9)	0/1	—
Transfusional siderosis (nontransplanted) (n=21)	3/5	—	1 (4)	5 (25)	—	1/2	1 (5)
Non-transfusional conditions (n=11)	1/3	—	—	1 (10)	6 (50)	4/6	4 (40)
Overall (n=99)	9/14	27 (28)	18 (18)	14 (14)	12 (12)	5/9	5 (5)

* Liver biopsies were performed for indications other than iron overload. Iron overload was defined as a hepatic iron index > 1.9, or by the pathologist's descriptive assessment of hepatocellular iron.

[†] NAFLD = nonalcoholic fatty liver disease, based on clinical phenotype, liver biopsy or characteristic ultrasound, CT or MRI appearance. HCV = hepatitis C virus infection.

[‡] One patient in the transfusional siderosis group (of two tested) and four in the nontransfusional group (of six tested) were found to be heterozygous for variant *HFE* alleles (C282Y/WT (3) and H63D/WT (2)).

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[†] Refers to patients who underwent at least 5 phlebectomies, but did not complete a full planned course. Includes 23/67 patients with TIO who underwent transplant, 8/21 patients with TIO who did not undergo transplant, and 2/11 patients with non-transfusional indications for phlebotomy.