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Original Article

Kinetics of iron removal by phlebotomy in patients with iron overload after allogeneic hematopoietic cell transplantation

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Abstract: Excess body iron could persist for years after allogeneic hematopoietic cell transplantation (HCT) with possible deleterious sequels. An iron depletive therapy with phlebotomy seems rational. Kinetics of iron removal by phlebotomy without erythropoietin support in non-thalassemic adult patients with iron overload after HCT and the impact of pre- and post-HCT hemochromatosis (HFE) genotype on iron mobilization were investigated. Patients and methods: Phlebotomy was initiated in 61 recipients of allografts due to hematologic malignancies (median age 48 years) after a median of 18 months. The prephlebotomy median serum ferritin (SF) was 1697ng/ml and the median number of blood transfusions 28 units. Alanine aminotransferase (ALT)/aspartate aminotransferase (AST), alkaline phosphates (AP), and bilirubin were elevated in 55.7%, 64% and 11.5% patients respectively. HFE-genotype was elucidated by polymerase chain reaction using hybridization probes and melting curve analysis. Results: Phlebotomy was well-tolerated irrespective of age or conditioning. A negative iron balance in 80% of patients (median SF 1086 ng/ml) and a rise in hemoglobin were observed ($p<0.0001$). Higher transfusional burden and SF were associated with a greater iron mobilization per session ($p=0.02$). In 58% of patients, a plateau after an initial steady decline in SF was followed by a second decline under further phlebotomy. The improvement in ALT ($p=0.002$), AST ($p=0.03$), AP ($p=0.01$), and bilirubin ($p<0.0001$) did not correlate with the decline in SF. Mutant HFE-gene variants were detected in 14/55 (25%) pre-HCT and 22/55 (40%) patients post-HCT. Overall, dissimilar pre- and posttransplantational HFE-genotypes were detected in 20/55 (40%) patients. Posttransplantational mutant HFE variants correlated with a slower decline in SF ($p=0.007$). Conclusions: Phlebotomy is a convenient therapy of iron overload in survivors of HCT. A negative iron balance and a rise in hemoglobin were observed in the majority of patients. Liver dysfunction improved irrespective of SF reduction suggesting a probable rapid decline of the deleterious labile plasma iron. In recipients of grafts with mutant HFE variants a "mixed chimerism" of HFE in body tissues might be created with a change in the set point for iron regulation. The transient plateau in SF after an initial decline might reflect iron mobilization from various tissues.

Keywords: Iron overload, ferritin, phlebotomy, allogeneic HCT

Introduction

The negative impact of transfusional iron overload on overall survival and non-relapse mortality (NRM) after allogeneic hematopoietic cell transplantation (HCT) in both thalassemic and non-thalassemic patients is well recognized [1, 2]. Thus, management of iron overload in HCT recipients in the pre-, peri-, and posttransplantation phases to improve short and long-term outcome seems rational. Although data from controlled trials documenting a survival impact of managing iron overload in non-thalassemic adult patients are lacking, some data imply that treatment could be associated with improved survival and reduced NRM [3]. Initiating an iron depletive therapy in the posttransplantation phase emerges from the growing body of evidence suggesting that excess body iron could persist for many years in long-term survivors of HCT. Even in pediatric patients, utilization of iron for growth alone cannot normalize iron stores in moderate-to severely iron-overloaded patients [4-8]

Generally, effective treatment strategies for iron overload include iron chelatation therapy and venesection. Phlebotomy, the standard

treatment in patients with primary (hereditary) hemochromatosis (HH), is obviously not feasible in the pre-, and peritransplantation phases in transfusion dependent thalassemic and non-thalassemic patients. However, after successful treatment of the hematologic disease by HCT, a restored erythropoiesis capable of producing a hyperplastic response to phlebotomy might be anticipated to allow mobilization of iron from augmented iron stores. Although data on phlebotomy after HCT are limited, its safety and efficacy in reducing serum ferritin levels (SF) have been shown in a few series including small number of patients [9-16]. In the largest published post-HCT phlebotomy program including 41 ex-thalassemic patients at a mean age of 16 years, a significant decrease in SF, liver iron concentration evaluated on liver biopsy as well as liver enzyme values was achieved by venesection [15].

We present results on the kinetics of iron removal under phlebotomy without erythropoietin support after allogeneic HCT in 61 non-thalassemic adult patients with iron overload who were included in a phlebotomy program at the University of Leipzig. Since the prevalence of mutations in the hemochromatosis (HFE) gene in the European population is high [17-19], we investigated the causal role of the pre- and posttransplantation HFE genotype in augmenting body iron and on iron mobilization by phlebotomy. Additionally, the influence of venesection on hemoglobin (Hb) and liver enzymes is addressed.

Patients and methods

The phlebotomy program

Phlebotomy was started after HCT in all transfusion independent patients with iron overload who were in complete remission with a complete donor chimerism when Hb value was $\geq$ 8 g/dl. Iron overload was defined by a SF level $\geq$ 1000 ng/ml with a concomitant c-reactive protein (CRP) < 10 mg/l on two occasions at least two weeks apart. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphates (AP), and bilirubin were measured prior to phlebotomy and at two weeks interval thereafter. Patients with alcoholic, drug induced, viral or autoimmune liver disease were excluded. Informed consent was obtained prior to venesection.

Phlebotomy was started at a median of 18 (range 3-52) months after HCT every two weeks in an outpatient setting. Erythropoietin administration was not allowed. A median of 200 (150-300) ml blood per session was removed. Under phlebotomy, SF was assessed monthly.

Patients characteristics

Sixty-one (31 males and 30 females) patients with a median SF of 1697 (range 1000-6832) ng/ml treated consecutively with phlebotomy at the University of Leipzig were included. Median age was 48 (range 21-66) years. Diagnosis were acute leukemia and myelodysplastic syndrome (MDS) in 37 (61%), chronic leukaemia in 15 (24%), and others in 9 (15%) patients. Conditioning consisted of 12 Gy total body irradiation (TBI) and cyclophosphamid 120 mg/kg in 33 (54%) patients while 28 (46%) patients received allografts following reduced intensity conditioning (RIC) with fludarabin 30 mg/m^2/day for 3 days and 2 Gy TBI applied once. Donors were matched related donors (MRD) in 21 (34.4%) patients and matched unrelated donors (MUD) in 40 (65.6%). Acute GVHD $\geq$ grade II was diagnosed in 25 (41%) with 5 (8.2%) patients having hepatic manifestations. Chronic GVHD was diagnosed in 19 (31%) with GVHD of the liver in 7 (11.5%) patients. The median number of blood transfusions was 28 (range 4-102) units. ALT or AST were above the upper limit of normal (ULN) in 34 (55.7%) patients. Both enzymes were elevated in 30 (88%) patients including those with chronic GVHD of the liver. AP and bilirubin were above ULN in 39 (64%) and 7 (11.5%) patients respectively. AP values were above ULN in all patients with chronic GVHD of the liver (**Table 1**).

Donor chimerism and HFE genotype

Marrow and blood donor chimerism was regularly monitored by fluorescence in situ hybridisation (FISH) for the XY chromosome in gender mismatched or PCR-based analysis of polymorphic microsatellite regions in gender-matched HCT.

Patients were screened for their HFE genotype prior to and after HCT. Mutations in DNA extracted from peripheral blood were detected by polymerase chain reaction (PCR) using hybridization probes and melting curve analysis as described previously [20].

Table 1. Characteristics of patients treated by phlebotomy for iron overload after hematopoietic cell transplantation

	n (%)
Gender	
Male	31 (50.8)
Female	30 (49.2)
Diagnosis	
Acute leukemia & MDS	37 (61.0)
Chronic leukemia	15 (24.0)
Others	9 (15.0)
Type of donor	
Matched related	21 (34.4)
Matched unrelated	40 (65.6)
Conditioning regimens	
Reduced intensity conditioning	28 (46.0)
Conventional	33 (54.0)
Acute GVHD	25 (41.0)
liver	5 (8.2)
Chronic GVHD	19 (31.0)
liver	7 (11.5)

Abb. MDS, myelodysplastic syndrome; GVHD, graft- versus- host disease.

Statistical analysis

To assess the kinetics of iron removal by phlebotomy a quotient of SF prior to phlebotomy to last SF (Quot-F) was generated. The amount of SF reduction under phlebotomy divided by the number of sessions was used to evaluate the mean decline in SF per session. To address whether the decline in SF under venesection occurred in a linear pattern, each 500 ml blood removed by phlebotomy was paired with the concomitant SF value at that point and the Wilcoxon-Test used. Univariate analysis was performed using the Chi^2- test and Fisher´s exact-test for categorical and Mann-Whitney-Test for continuous variables. Cox-Regression was used for multivariate analysis. For non-parametric correlations, we used the Spearman-Rho-Test and the Pearson-correlation. Data were analyzed using SPSS15 software. A P value of < 0.05 was considered statistically significant.

Results

HFE genotypes pre- and post-HCT

Data were available for 55 patients. Wild type genotype prior to and after HCT were detected in 28 (51%) patients. Prior to HCT, mutations were found in 14 (25.5%) patients (11 females, 3 males). Nine patients received allografts from a MUD. Mutations were heterozygosity for H63D (n=10), heterozygosity for C282Y (n=3), and homozygosity for H63D (n=1) (**Table 2**).

Posttransplantation, 22 (40%) mutations in the HFE gene were found (heterozygosity for H63D (n=12), for C282Y (n=4), for S65C (n=4), and homozygosity for the H63D in 2 patients). Seven patients showed the same mutational status pre- and post-HCT.

Overall, dissimilar pre- and posttransplantational HFE-genotypes were detected in 20/55 (40%) patients. The pre-HCT mutational status in two patients (one homozygote for H63D and one heterozygote for H63D) changed to heterozygote for H63D and C282Y genotype respectively after HCT. Five of the patients with a mutation prior to HCT demonstrated a wild type HFE genotype after HCT. On the other hand, a mutated HFE gene variant was found after HCT in 13 patients with wild type genes prior to HCT.

Interestingly, our cohort included 5 patients with HFE gene mutations prior to or after HCT with SF > 1000 ng/ml despite a median of only 12 (4-18) units of blood (**table 3**).

Phlebotomy

Venesection was well tolerated irrespective of age or type of conditioning. After a median of 9 (range 3-23) phlebotomy sessions, a median of 1700 (range 500-6000) ml blood was removed.

Table 2. HFE gene mutations before and after hematopoietic cell transplantation

Number	Gender	Donor	HFE genotype prior to HCT	HFE genotype after HCT
1	m	MUD	hetH36D	wild type
2	f	MUD	hetH63D	wild type
3	f	MUD	hetC282Y	wild type
4	f	MRD	hetH63D	hetH63D
5	f	MUD	homoH63D	hetH63D
6	m	MUD	hetH63D	hetC282Y
7	f	MRD	hetH63D	hetH63D
8	f	MUD	hetC282Y	wild type
9	f	MUD	hetH63D	hetH63D
10	f	MUD	hetH63D	wild type
11	f	MRD	hetH63D	hetH63D
12	m	MUD	hetH63D	hetH63D
13	f	MRD	hetC282Y	hetC282Y
14	f	MRD	hetH63D	hetH63D
15	m	MUD	wild type	hetS65C
16	m	MUD	wild type	hetH63D
17	m	MUD	wild type	hetHd3D
18	f	MUD	wild type	hetH63D
19	M	MUD	wild type	hetH63D
20	m	MUD	wild type	homoH63D
21	m	MUD	wild type	hetC282Y
22	m	MRD	wild type	hetS65C
23	f	MUD	wild type	hetS65C
24	f	MUD	wild type	homoH63D
25	f	MUD	wild type	hetH63D
26	f	MUD	wild type	hetS65C
27	f	MUD	wild type	hetH63D

Abb. M, male; f, female; MRD, matched related donor; MUD, matched unrelated donor; het, heterozygote; homo, homozygote.

Table 3. Patients with HFE gene mutations, serum ferritin > 1000 ng/ml and less than 20 units of blood transfusions (n=5)

Number	Gender	Donor	serum ferritin ng/ml	blood transfusions n	HFE genotype before HCT	HFE genotype after HCT
1	m	MUD	1229	18	wild type	hetS65C
2	m	MUD	1263	18	wild type	hetH63D
3	f	MUD	3431	4	wild type	hetH63D
4	f	MUD	1254	12	hetH63D	wild type
5	f	MUD	1950	12	wild type	homoH63D

Abb. MRD, matched related donor; MUD, matched unrelated donor; het, heterozygote; homo, homozygote.

A significant decline in SF under phlebotomy occurred in 49 (80%) patients. SF decreased from a prephlebotomy median of 1697 (range 1000-6832) ng/ml to a median of 1086 (range 161-4280) ng/ml at the time of data collection ($p<0.0001$).

Overall, each millilitre blood removed by phlebotomy corresponded to a median decline in SF of 0.2 (range 0-6.5) ng/ml.

Under phlebotomy a significant improvement in ALT ($p=0.002$), AST ($p=0.03$), AP ($p=0.01$), and bilirubin ($p<0.0001$) was observed. Interestingly, improvement in liver enzymes under phlebotomy did not correlate with the decline in SF. Actually, 11 of the 12 patients (92%) with no change in SF under phlebotomy had at least one elevated liver parameter with improvement occurring in 8 (73%) patients. **Table 4** summarises the effect of phlebotomy on liver function tests.

Median Hb prior to phlebotomy was 12 (range 8.2-15.8) g/dl. Interestingly, phlebotomy was accompanied by a significant increase in Hb values with a median value of 12.6 (range 9.1-15.3) g/dl at the time of data collection com-

Table 4. Effect of phlebotomy on liver values (N=61)

Parameter	patients with values > ULN prior to phlebotomy		patients with improvement under phlebotomy		p
	n	%	n	%	
ALT	34	55.7	30/34	88	0.002
AST	34	55.7	30/34	88	0.03
AP	39	64	31/39	79.5	0.01
Bilirubin	7	11.5	777	100	<0.0001

Abb. ALT, Alanine aminotransferase; AST, aspartate aminotransferase; AP, alkaline phosphatase.

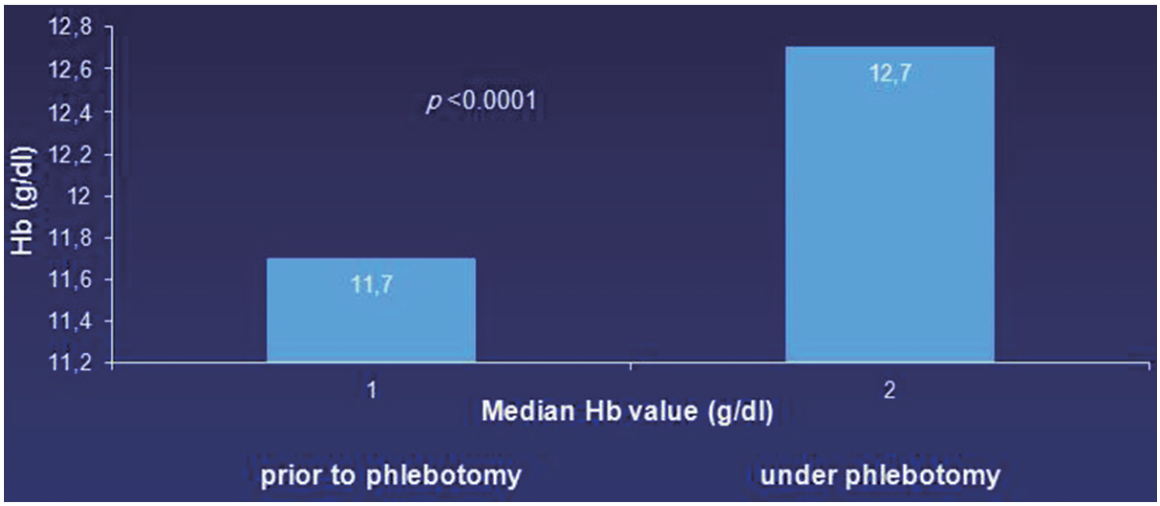

Figure 1. Hemoglobin levels prior to and under phlebotomy. Phlebotomy was accompanied by a significant increase in Hb values with a median value of 12.6 (range 9.1-15.3) g/dl at the time of data collection compared to pre-treatment values [median 12 (range 8.2-15.8) g/dl] (p<0.0001).

pared to pre-treatment values (p<0.0001). A median increase of 1 (range 0.2-6.6) g/dl was observed in 26 of 29 (90%) patients with a Hb < 12 g/dl prior to phlebotomy (**Figure 1**).

Pattern of iron removal by phlebotomy

Prephlebotomy SF values correlated with the number of previous blood transfusions (p<0.0001) but not with the HFE genotype prior to or after HCT.

In univariate analysis, the Quot-F and the mean decline in SF per session was not influenced by gender, age, type of donor or conditioning, acute or chronic GVHD, and HFE genotype prior to HCT. On the other hand, transfusional burden and the initial SF level correlated with the efficacy of iron mobilization expressed as the reduction in SF per phlebotomy session. In multivariate analysis, only the prephlebotomy SF value showed a significant association with the SF reduction per session. The higher the initial SF, the more the degree of decline in SF per session (r=0.6, p=0.02) (**Figure 2**).

Surprisingly, in both uni- and multivariate analysis, HFE genotype after HCT correlated with both (Quot-F) (p=0.007) and median reduction of SF per phlebotomy session (p=0.03). Patients with wild type HFE genotype after HCT had a median Quot-F of 0.6 (range 0.2-1.6), and a median of 74.4 (range 71.3-264) ng/ml SF reduction per session compared to a median Quot-F of 0.8 (range 0.2-1.7) (p=0.002), and a median SF reduction per phlebotomy of only 42.4 (range 84.8-91) ng/ml (p=0.02) in patients with mutated HFE genotype after HCT irrespective of the type of mutation (**Figure 3**).

Removal of the first 1000 ml blood resulted in a steady decline in SF. In 19/33 (58%) patients requiring further phlebotomy, a transient plateau in SF levels at a median of 1500 ml blood removed was observed followed by a second linear decline under continued phlebotomy

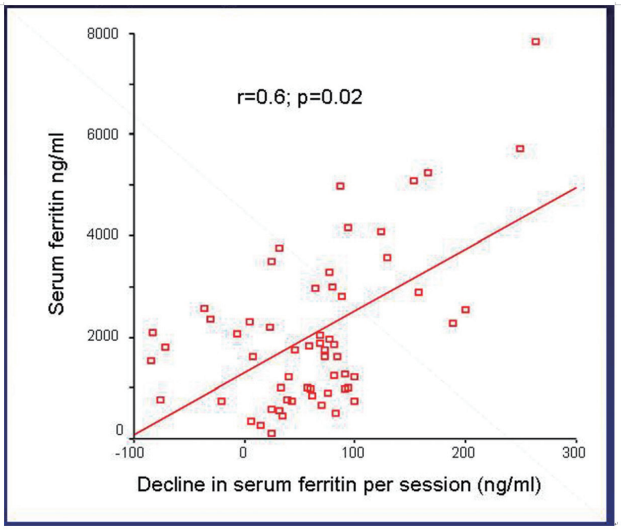

Figure 2. The decline in serum ferritin per phlebotomy session correlated with the prephlebotomy serum ferritin. The prephlebotomy serum ferritin level correlated with the efficacy of iron mobilization expressed as the reduction in serum ferritin per phlebotomy session. The higher the initial serum ferritin, the more the degree of decline in SF per session.

(**Figure 4**). In univariate analysis, gender, age, type of donor or conditioning, acute or chronic GVHD, HFE genotype prior to or after HCT, number of previous blood transfusions and the initial SF had no influence on the dynamics of SF changes under phlebotomy.

Discussion

Our data confirm that phlebetomy without erythropoietin support after HCT is a treatment option in non-thalassemic adult patients with iron overload. A negative iron balance could be achieved in the majority of patients. Elderly patients after RIC tolerated phlebotomy equally well compared to younger patients. The small amount of blood removed per session and the two weeks interval instead of the usual once weekly regimens used in patients with HH might have contributed to this excellent tolerability.

The limitations of serum ferritin as a surrogate marker for iron overload are well known. To differentiate hyperferritinemia due to iron-overload from elevated values due to other "non-iron overload" causes such as infection, hepatitis, inflammation, and GVHD, only serially elevated serum ferritin values with a concomitant low CRP were considered. Patients with alcoholic, drug induced, viral or autoimmune liver disease were excluded.

Assessment of liver iron concentration (LIC) by noninvasive magnetic resonance imaging techniques might provide a more reliable measure of whole-body iron levels [21]. Thresholds for LIC, however, have been determined primarily from thalassemia populations. Data on threshold levels specific to non-thalassemic recipients of allogeneic HCT are limited and require further study. Furthermore, the reported correlation between LIC and serum ferritin levels is rather variable and needs to be established [22-25].

In addition to serum ferritin, measurement of hepcidin concentrations which is investigational at the present time might be highly informative in understanding iron homeostasis in the setting of HCT. It is known that hepcidin expression, the key regulator of iron homeostasis, is modulated by systemic iron requirements, infectious, and inflammatory stimuli [26-30]. Usually, hepcidin and serum ferritin respond similarly to inflammation and changes in iron stores. Indeed, both pre- and post-HCT hepcidin levels were found to be highly elevated in a cohort of patients with AML and a high transfusional burden [31]. Yet, the correlation with serum ferritin was week. This implies that hepcidin might represent a compensatory mechanism counteracting transfusional iron input by preventing an increased ferroportin-mediated iron export from macrophages thereby reducing the severity of parenchymal iron loading and damage [32].

Transfusional burden is considered as the main cause of iron overload in patients with hematologic diseases [33, 34]. Thus, it is not surprising that serum ferritin in the study cohort correlated with the number of blood transfusions. Yet, other factors might also contribute to excess body iron such as the release of toxic iron radicals by intensive treatments, ineffective hematopoiesis, a consequence of the treatment leading to growth and differentiation factor (GDF-15) over-expression which inhibits the production of hepcidin in the liver, or a mutated HFE genotype [35, 36]. Our results confirm that excess body iron might be present after HCT even in patients not heavily transfused.

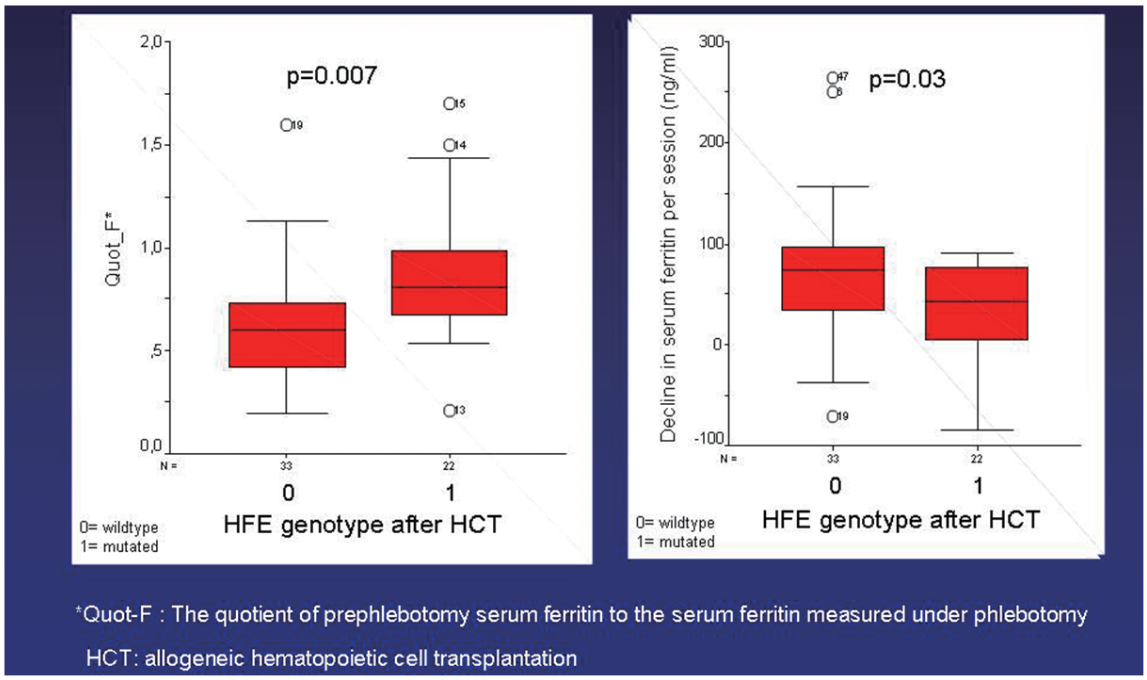

Figure 3. Posttransplantational mutant HFE variants correlate with a slower iron mobilization by phlebotomy. HFE genotype after HCT correlated with both Quot-F (p=0.007) and the reduction of serum ferritin per phlebotomy session (p=0.03). Patients with wild type HFE genotype after HCT had a median Quot-F of 0.6 (range 0.2-1.6), and a median of 74.4 (range 71.3-264) ng/ml ferritin reduction per session compared to a median Quot-F of 0.8 (range 0.2-1.7) (p=0.002), and a median ferritin reduction per phlebotomy of only 42.4 (range 84.8-91) ng/ml (p=0.02) in patients with mutated HFE genotype after HCT irrespective of the type of mutation.

The sequels of persistent iron overload in survivors of HCT are not yet thoroughly researched and require meticulous measurements of different organ functions in long-term follow-up studies. Nevertheless and consistent with previous reports, venesection in our cohort positively influenced liver biochemical measurements with significant improvement in elevated ALT, AST, AP, and bilirubin. Chronic liver disease after HCT is not uncommon with iron overload being one of the most frequent causes. Through phlebotomies improvement in liver function could be achieved [12, 16, 37-38]. Iron overload might mimic, trigger or exacerbate hepatic GVHD possibly through the induction of an inflammatory cascade caused by damage to cellular lipids, proteins and nucleic acids by reactive oxygen species [39]. The severity of liver dysfunction and the impact of venesection on hepatic GVHD in our cohort could not be answered because of the small number of patients and the need for an additional liver biopsy which was not justified in patients with improving values. Intriguing was the observation that the improvement of liver dysfunction under phlebotomy occurred independent and irrespective of the decline in serum ferritin. It is known that once transferrin saturation exceeds 75%, non –transferrin bound iron (NTBI) may be detected and lead to the formation of labile plasma iron (LPI) and reactive oxygen species resulting in cellular damage. In a subgroup analysis evaluating the effect of a once-daily oral iron chelator deferasirox on LPI levels, there was a significant and sustained reduction in preadministration LPI that reached the normal range at week four [40]. Although it is not known how rapidly phlebotomy can normalize NTBI, the observed improvement in liver function suggests that phlebotomy, similar to what is seen with iron chelators, might result in a rapid reduction of the deleterious LPI.

Remarkable was the significant rise in Hb values under phlebotomy without erythropoietin support. This is comparable to what has been observed in patients with MDS where the oxidative stress in red blood cells, platelets and polymorphonuclear leukocytes was ameliorated by a short incubation with the iron-chelators, deferrioxamine and deferiprone [41]. This suggests that phlebotomy might mitigate suppres-

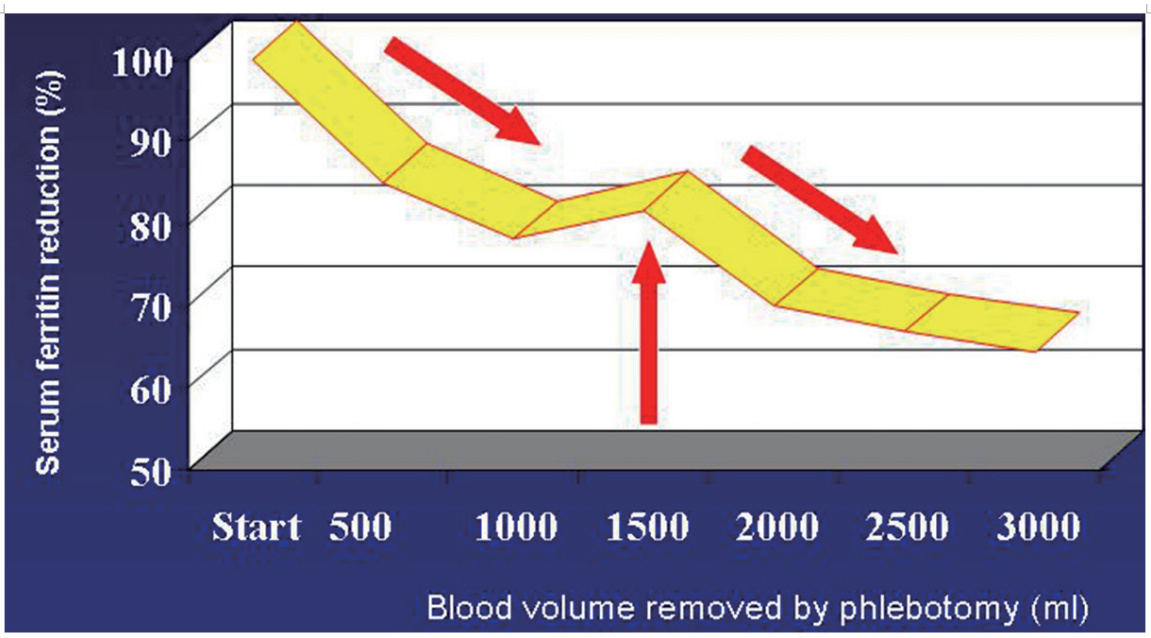

Figure 4. The removal of excess body iron by phlebotomy reflected by a reduction in serum ferritin is not steady in all patients. In 58% of patients, a plateau after an initial steady decline in SF was followed by a second decline under further phlebotomy.

sion of erythropoiesis attributable to iron and/or oxidative stress. Another explanation for the improvement of Hb might be related to hepcidin changes under venesection. Both hepcidin and serum ferritin respond similarly to changes in iron stores, hepcidin transcription is usually upregulated by inflammatory stimuli and an elevated hepcidin expression is a proposed cause of anemia of chronic inflammation [29]. By reducing hepcidin levels with such therapy, iron release might be promoted and bioavailability for hemopoiesis increased. Actually, an inverse correlation of hepcidin levels with Hb values was observed in the cohort of patients with AML, high transfusional burden, and elevated pre- and post-HCT hepcidin concentrations [31].

The incidence of HFE gene mutations detected in our cohort was comparable to that published for European populations [17-19].

The observation that patients with mutated HFE genotype after HCT are "slow-reducers" of body iron under phlebotomy is interesting. It is known that HFE is one of the proteins elegantly regulating hepatic hepcidin expression, the central regulator of iron homeostasis, but the exact mechanisms by which HFE influences iron uptake and efflux from cells are not very well understood [42]. HFE protein is expressed not only in gastrointestinal epithelial cells but also in macrophages, granulocytes, monocytes and even in B-lymphoid cell lines [43, 44]. Thus, in recipients of hematopoietic precursors with mutant HFE variants a sort of "mixed chimerism" of HFE in different body tissues is possibly created. This might lead to a change in the set point for iron regulation or a blunted response to iron sensing. The routine screening for HFE genotype prior to and after HCT is investigational, but it might be indicated in patients with non-transfusional iron overload

The decline in serum ferritin consistent with removal of excess body iron by continuous phlebotomy is not steady in all patients. The observed plateau despite continued venesection after the initial decline in serum ferritin is usually followed by a second steady decrease in serum ferritin. Awareness of this phenomenon is clinically relevant as this plateau is usually transient, does not indicate futility of phlebotomy, does not require a change in treatment regimen, and might reflect iron mobilisation from various body tissues as liver, heart...etc.

One limitation of phlebotomy is the fact that it could not be initiated in the pre-, peri-, and very early posttransplantational setting. This might

hamper its value as an instrument to improve results after HCT. However, bearing in mind that excess body iron could persist for many years after HCT with the potential to long-term deleterious sequels, phlebotomy remains a convenient, and cost-effective option for therapy of iron overload in survivors of HCT with a conceivable potential to improve long-term outcome. Further research elucidating the precise consequences of persistent excess body iron, the safety and the value of the various iron depletive therapies is required.

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References

[1] Pullarkat V. Iron overload in patients undergoing hematopoietic stem cell transplantation. Adv Hematol 2010; pii: 345756.

[2] Kanda J, Kawabata H, Chao NJ. Iron overload and allogeneic hematopoietic stem-cell transplantation. Expert Rev Hematol 2011; 4: 71-80.

[3] Lee JW, Kang HJ, Kim EK, Kim H, Shin HY, Ahn HS. Effect of iron overload and iron-chelating therapy on allogeneic hematopoietic SCT in children. Bone Marrow Tranplant 2009; 44: 793-797.

[4] Majhail NS, Lazarus HM, Burns LJ. Iron overload in hematopoietic cell transplantation. Bone Marrow Transplant 2008; 41: 997–1003.

[5] McKay PJ, Murphy JA, Cameron S, Burnett AK, Campbell M, Tansey P, Franklin IM. Iron overload and liver dysfunction after allogeneic or autologous bone marrow transplantation. Bone Marrow Tranplant 1996; 17: 63–66.

[6] Chotsampancharoen T, Gan K, Kasow KA, Barfield RC, Hale GA, Leung W. Iron overload in survivors of childhood leukemia after allogeneic hematopoietic stem cell transplantation. Pediatric Transplantation 2009; 13: 348–352.

[7] Lichtman SM, Attivissimo L, Goldman IS, Schuster MW, Buchbinder A. Secondary hemochromatosis as a long-term complication of the treatment of hematologic malignancies. American Journal of Hematology 1999; 61: 262–264.

[8] Lucarelli G, Angelucci E, Giardini C, Baronciani D, Galimberti M, Polchi P, Bartolucci M, Muretto P, Albertini F. Fate of iron stores in thalassaemia after bone-marrow transplantation. Lancet 1993; 342: 1388-1391.

[9] Majhail NS, Lazarus HM, Burns LJ. A prospective study of iron overload management in allogeneic hematopoietic cell transplantation survivors. Biol Blood Marrow Transplant 2010; 16: 832-837.

[10] Busca A, Falda M, Manzini P, D'Antico S, Valfrè A, Locatelli F, Calabrese R, Chiappella A, D'Ardia S, Longo F, Piga A. Iron overload in patients receiving allogeneic hematopoietic stem cell transplantation: quantification of iron burden by a superconducting quantum interference device (SQUID) and therapeutic effectiveness of phlebotomy. Biol Blood Marrow Transplant 2010; 16:115-122.

[11] Muretto P, Angelucci E, Lucarelli G. Reversibility of cirrhosis in patients cured of thalassemia by bone marrow transplantation. Ann Intern Med 2002; 136: 667-72.

[12] Kamble RT, Selby GB, Mims M, Ozer H, George JN. Iron overload manifesting as apparent exacerbation of hepatic graft-versus-host disease after allogeneic hematopoietic stem cell transplantation. Biol Blood Marrow Transplant 2006; 12: 506-510.

[13] Rose C, Ernst O, Hecquet B Maboudou P, Renom P, Noel MP, Yakoub-Agha I, Bauters F, Jouet JP. Quantification by magnetic resonance imaging and liver consequences of posttransfusional iron overload alone in long-term survivors after allogeneic hematopoietic stem cell transplantation. Haematologica 2007; 92: 850–853.

[14] Li CK, Lai DH, Shing MM, Chik KW, Lee V, Yuen PM. Early iron reduction programme for thalassaemia patients after bone marrow transplantation. Bone Marrow Transplant 2000; 25: 653–656.

[15] Angelucci E, Muretto P, Lucarelli G, Ripalti M, Baronciani D, Erer B, Galimberti M, Giardini C, Gaziev D, Polchi P. Phlebotomy to reduce iron overload in patients cured of thalassemia by bone marrow transplantation. Italian Cooperative Group for Phlebotomy Treatment of Transplanted Thalassemia Patients. Blood 1997; 90: 994–998.

[16] Tomás JF, Pinilla I, García-Buey ML, García A, Figuera A, Gómez-García de Soria VGG, Moreno R, Fernández-Rañada JM. Long-term liver dysfunction after allogeneic bone marrow transplantation: clinical features and course in 61 patients. Bone Marrow Transplant 2000; 26: 649-655.

[17] Pedersen P, Milman N. Genetic screening for HFE hemochromatosis in 6020 Danish men: penetrance of C282Y, H63D, and S65C variants. Ann Hematol 2009; 88: 775-784.

[18] Fix OK, Kowdley KV. Hereditary hemochromatosis. Minerva Med 2008; 99: 605-617.

[19] Hanson EH, Imperatore G, Burke W. HFE gene and hereditary hemochromatosis: a HuGE review. Human Genome Epidemiology. Am J Epidemiol 2001; 154: 193-206.

[20] Lyon E. Mutation detection using fluorescent hybridization probes and melting curve analysis. Expert Review of Molecular Diagnostics 2001; 1: 92–101.

[21] Wood JC. Diagnosis and management of transfusion iron overload: the role of imaging. American Journal of Hematology 2007; 82: 1132–1135.

[22] Jacobi N, Grosse R, Aktas T, Herich L, Nielsen P, Fischer R, Zabelina T, Bacher U, Kroeger N. Measurement of liver iron concentration by quantum interference device biosusceptometry (SQUID) validates serum ferritin as prognostic parameter for allogeneic stem cell transplantation. Blood 2011; 118: Abstract 1018.

[23] Rose C, Ernst O, Hecquet B, Maboudou P, Renom P, Noel MP, Yakoub-Agha I, Bauters F, Jouet JP. Quantification by magnetic resonance imaging and liver consequences of post-transfusional iron overload alone in long term survivors after allogeneic hematopoietic stem cell transplantation (HSCT). Haematologica 2007; 92: 850-853.

[24] Majhail NS, DeFor T, Lazarus HM, Burns LJ. High prevalence of iron overload in adult allogeneic hematopoietic cell transplant survivors. Biol Blood Marrow Transplant 2008; 14: 790-794.

[25] Wermke M, Schmidt A, Middeke JM, Sockel K, von Bonin M, Schonefeldt C, Mair S, Plodeck V, Laniado M, Weiss G, Schetelig J, Ehninger G, Theurl I, Bornhauser M, Platzbecker U. MRI-based liver iron content predicts for non-relapse mortality in MDS and AML patients undergoing allogeneic stem cell transplantation. Clin Cancer Res 2012; [Epub ahead of print].

[26] Pigeon C, Ilyin G, Courselaud B, Leroyer P, Turlin B, Brissot P, Loréal O. A new mouse liver-specific gene, encoding a protein homologous to human antimicrobial peptide hepcidin, is overexpressed during iron overload. J Biol Chem 2001; 276: 7811-7819.

[27] Lin L, Valore EV, Nemeth E, Goodnough JB, Gabayan V, Ganz T. Iron transferrin regulates hepcidin synthesis in primary hepatocyte culture through hemojuvelin and BMP2/4. Blood 2007; 110: 2182-2189.

[28] Nemeth E, Valore EV, Territo M, Schiller G, Lichtenstein A, Ganz T. Hepcidin, a putative mediator of anemia of inflammation, is a type II acute-phase protein. Blood 2003; 101: 2461-2463.

[29] Ganz T. Hepcidin, a key regulator of iron metabolism and mediator of anemia of inflammation. Blood 2003; 102: 783-788.

[30] Nemeth E, Ganz T. Hepcidin and iron-loading anemias. Haematologica 2006; 91: 727-732.

[31] Eisfeld AK, Westerman M, Krahl R, Leiblein S, Liebert UG, Hehme M, Teupser D, Niederwieser D, Al-Ali HK. Highly Elevated Serum Hepcidin in Patients with Acute Myeloid Leukemia prior to and after Allogeneic Hematopoietic Cell Transplantation: Does This Protect from Excessive Parenchymal Iron Loading? Adv Hematol 2011; 491058.

[32] Muckenthaler MU. Fine tuning of hepcidin expression by positive and negative regulators. Cell Metab 2008; 8: 1-3.

[33] Malcovati L. Impact of transfusion dependency and secondary iron overload on the survival of patients with myelodysplastic syndromes. Leukemia Research 2007; 31: S2–S6.

[34] Gattermann N. Guidelines on iron chelation therapy in patients with myelodysplastic syndromes and transfusional iron overload. Leukemia Research 2007; 31: S10–S15.

[35] Cortelezzi A, Cattaneo C, Cristiani S, Duca L, Sarina B, Deliliers GL, Fiorelli G, Cappellini MD. Non-transferrin-bound iron in myelodysplastic syndromes: a marker of ineffective erythropoiesis? Hematol J 2000; 1: 153-158.

[36] Tanno T, Bhanu NV, Oneal PA, Goh SH, Staker P, Lee YT, Moroney JW, Reed CH, Luban NL, Wang RH, Eling TE, Childs R, Ganz T, Leitman SF, Fucharoen S, Miller JL. High levels of GDF15 in thalassemia suppress expression of the iron regulatory protein hepcidin. Nat Med 2007; 13: 1096-1101.

[37] McDonald GB. Management of hepatic disease following haematopoietic cell transplant. Alimentary Pharmacology and Therapeutics 2006; 24: 441–452.

[38] Sucak GT, Yegin ZA, Ozkurt ZN, Aki SZ, Karakan T, Akyol G. The role of liver biopsy in the workup of liver dysfunction late after SCT: is the role of iron overload underestimated? Bone Marrow Transplant 2008: 42: 461–467.

[39] Peretz G, Link G, Pappo O, Bruck R, Ackerman Z. Effect of hepatic iron concentration reduction on hepatic fibrosis and damage in rats with cholestatic liver disease. World J Gastroenterology 2006; 12: 240-245.

[40] Daar S, Pathare A, Nick H, Kriemler-Krahn U, Hmissi A, Habr D, Taher A. Reduction in labile plasma iron during treatment with deferasirox, a once-daily oral iron chelator, in heavily iron-overloaded patients with beta-thalassaemia. Eur J Haematol 2009; 82: 454-457.

[41] Ghoti H, Amer J, Winder A, Rachmilewitz E, Fibach E. Oxidative stress in red blood cells, platelets and polymorphonuclear leukocytes from patients with myelodysplastic syndrome. Eur J Haematol 2007; 79: 463-467.

[42] Schmidt PJ, Toran PT, Giannetti AM, Bjorkman PJ, Andrews NC. The transferring receptor modulates Hfe-dependent regulation of hepcidin expression. Cell Metab 2008; 7: 205-214.
[43] Parkkila S, Parkkila AK, Waheed A, Britton RS, Zhou XY, Fleming RE, Tomatsu S, Bacon BR, Sly WS. Cell surface expression of HFE protein in epithelial cells, macrophages, and monocytes. Haematologica 2000; 85: 340-345.
[44] Chitambar CR, Wereley JP. Iron transport in a lymphoid cell line with the hemochromatosis C282Y mutation. Blood 2001; 97: 2734-2740.