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RESEARCH ARTICLE

The Impact of Ferritin Levels on Outcomes of Allogeneic Hematopoietic Stem Cell Transplantation: Real-Life Data

Ferritin Düzeylerinin Allojeneik Hematopoietik Kök Hücre Nakli Sonuçları Üzerine Etkisi: Gerçek Yaşam Verileri

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ABSTRACT

Objective: This retrospective study aimed to evaluate the effects of ferritin level on outcomes of allogeneic hematopoietic stem cell transplantation (allo-HSCT) including the neutrophil/platelet engraftment, febrile neutropenia, transplant related mortality (TRM), graft versus host disease (GvHD), sinusoidal obstruction syndrome (SOS)/veno-occlusive disease (VOD) and overall survival (OS).

Materials and Methods: Sixty-nine patients with ferritin values measured at the beginning of allo-HSCT between 2018 - 2021 were enrolled in this study. The ferritin cut-off value was determined as 1000ng/mL and the patients were divided into 2 groups (<1000 ng/mL vs ≥1000 ng/mL).

Results: The median age was 32 years (23-49) and 38 (55.1%) of them female in all cohorts. Most of them (47.8%) were diagnosed with acute myeloid leukemia, followed by aplastic anemia (18.8%) and acute lymphoblastic leukemia (17.4%), respectively. The median ferritin level was 1080 ng/mL (505-1650) in all cohorts. Ferritin levels had no statistically significant effect on the engraftment, the febrile neutropenia, TRM and SOS/VOD (p>.05). The median OS in patients with ferritin level ≥1000 ng/mL and ferritin level <1000 ng/mL 4 months (95% CI: 1.4-6.6) and 8 months (95% CI: 0-24.2), respectively. There was no statistically significant correlation between ferritin value and OS (p=0.206). There was no statistically significant difference between the ferritin groups on both acute and chronic GvHD (p=0.713 and p=0.999, respectively).

Conclusion: Our study did not demonstrate any negative effects of serum ferritin levels on allo-HSCT outcomes; however, large-scale prospective studies are needed to clarify the effect of iron overload on the outcomes of allo-HSCT.

Keywords: Ferritin, hematopoietic stem cell transplantation, prognostic factors

ÖZET

Amaç: Bu retrospektif çalışma ferritin düzeyinin; nötrofil/trombosit engraftmanı, febril nötropeni, transplantasyonla ilişkili mortalite (TRM), greft-versus host hastalığı (GvHH), sinüzoidal obstrüksiyon sendromu (SOS)/venöz oklüzyon hastalığı (VOH) ve genel sağkalım (OS) gibi allojeneik hematopoietik kök hücre transplantasyon (allo-HKHT) sonuçları üzerindeki etkilerini değerlendirmeyi amaçlamıştır.

Gereç ve Yöntemler: 2018-2021 yılları arasında allo-HSCT başlangıcında ferritin değerleri ölçülen 69 hasta bu çalışmaya dâhil edilmiştir. Ferritin eşik değeri 1000 ng/mL olarak belirlenmiş ve hastalar 2 gruba ayrılmıştır (<1000 ng/mL ve ≥1000 ng/mL).

Bulgular: Çalışmada ortanca yaş 32 (23-49) yıl olup, bunların 38'i (%55,1) kadındı. Çoğu (%47,8) akut miyeloid lösemi tanılı olup, bunu sırasıyla aplastik anemi (%18,8) ve akut lenfoblastik lösemi (%17,4) izledi. Tüm kohortta ortanca ferritin düzeyi 1080 ng/mL (505-1650) olup ferritin düzeyinin engraftman, febril nötropeni, TRM ve SOS/VOH üzerinde istatistiksel olarak anlamlı bir etkisi yoktu (p>.05). Ferritin düzeyi ≥1000 ng/mL ve ferritin düzeyi <1000 ng/mL olan hastalarda ortanca OS sırasıyla 4 ay (%95 CI: 1,4-6,6) ve 8 ay (%95 CI: 0-24,2) olarak bulunmuştur. Ferritin değeri ile OS arasında istatistiksel olarak anlamlı bir ilişki bulunmamıştır (p=0,206). Akut ve kronik GvHH açısından ferritin grupları arasında istatistiksel olarak anlamlı bir fark bulunmamıştır (sırasıyla p=0,713 ve p=0,999).

Sonuç: Çalışmamız, serum ferritin düzeylerinin allo-HKHT sonuçları üzerinde herhangi bir olumsuz etkisini göstermemiştir; ancak, demir yükünün allo-HKHT sonuçları üzerindeki etkisini açıklığa kavuşturmak için büyük ölçekli prospektif çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Ferritin, hematopoietik kök hücre nakli, prognostik faktörler

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INTRODUCTION

Allogeneic hematopoietic cell transplantation (allo-HCT) is still only curative treatment methods for many benign and malign hematological diseases which increased in recent years with the increase in donor options and regulation of conditioning regimens (1-3). However, transplant related mortality (TRM) including infections, graft versus host disease (GvHD), and therapy-related toxicity are still major complications for allo-HCT (4,5). Iron overload is an adverse prognostic factor for patients undergoing allo-HCT which is related to increased poor outcomes, including high infection rates, high TRM, and lower survival. Additionally iron overload was associated with an important risk factor for hepatic sinusoidal obstruction syndrome as well as for both acute GvHD and chronic GvHD in allo-HCT (6-8). Serum ferritin, magnetic resonance imaging (MRI) of liver (T2 or R2 MRI), superconducting susceptometry (SQUID) and liver biopsy were some methods of evaluating iron overload (5, 9, 10).

Ferritin is an iron storage protein and measurement in the plasma which is commonly used in clinical practice to assess iron overload due to its inexpensive, noninvasive method and easy accessibility (11, 12). In the literature, a serum ferritin level exceeding 1000 µg/L (or >1000 ng/mL) is commonly used as the threshold for detecting iron overload, which correlates with a higher likelihood of complications. Elevated pre-transplant ferritin levels have been reported to increase the risk of non-relapse mortality following HCT and adversely impacts on overall survival and increases the GVHD after allogeneic HCT in the literature (13-15). We aimed to evaluate the effects of ferritin level on outcomes including the neutrophil/platelet engraftment status, febrile neutropenia rate, TRM, GvHD, sinusoidal obstruction syndrome (SOS)/veno-occlusive disease (VOD) and overall survival (OS) of allo-HCT in this study.

MATERIALS AND METHODS

Sixty-nine patients with ferritin values measured at the beginning of transplantation who underwent allo-HCT between January 2018-June 2021 were included in this study. The clinical and laboratory parameters were collected from the hospital registry system. This study was formally approved by Inonu University Health Sciences Non-Interventional Clinical Research Ethics Committee (permission date: 29.06.2021, approval number: 2021/2250). It was performed following the ethical principles set forth in the Declaration of Helsinki.

Study design

Laboratory parameters were including alanine aminotransferase (ALT), creatinine, ferritin, C-reactive protein (CRP), and lactate dehydrogenase (LDH) were evaluated. Using a ferritin cutoff of 1000 ng/mL, patients were divided into 2 groups: ferritin levels <1000 ng/mL vs. ≥ 1000 ng/mL. The outcomes, including the neutrophil/platelet engraftment status, febrile neutropenia rate, TRM in the first 30 days, and OS were evaluated according to the ferritin level. The following provides the definitions of the outcomes analyzed in this study. Neutrophil engraftment; the first of three consecutive days with an absolute neutrophil count ≥ 0.5×10^9/L after stem cell infusion. Platelet engraftment; the first of three consecutive days with a platelet count ≥ 20×10^9/L in the absence of transfusion. Febrile neutropenia; the occurrence of a body temperature ≥ 38 °C in the setting of a neutrophil count < 0.5×10^9/L. Transplant related mortality; the first 30 days after allo-HSCT without any evidence of disease relapses or progression after stem cell infusion. Overall survival; the time interval from the date of the first day of allo-HCT to death from any cause or last follow-up.

The Glucksberg scale was used in the assessment of acute GvHD (16). Similarly, the National Institutes of Health (NIH) Consensus Criteria were used in the assessment of chronic GvHD (17). Baltimore criterion was used for diagnosis of SOS/VOD (18).

Statistical analyses

The analysis of all data was performed with the SPSS 22.0 program. Categorical variables were summarized using frequencies (n) and percentages (%), while continuous variables were presented as medians and quartiles (quartile 1 [Q1], quartile 3 [Q3]), values. The Mann–Whitney U test was applied to compare non-normally distributed continuous variables, and categorical variables were assessed using either Pearson's chi-square test or Fisher's exact test, depending on the data. Univariate survival outcomes were analyzed using Kaplan–Meier methods with weighted Log-rank tests. The possible significant parameters with p≤0.20 in univariate tests were included in the multivariate analysis. Multivariable analysis was performed using Cox's proportional hazard model. A p<0.05 was regarded as statistically significant for all analyses.

RESULTS

The study population had a median age of 32 years (23-49) and 38 (55.1%) of them female. The most common diagnosis was acute myeloid leukemia (AML) (47.8%), followed by aplastic anemia (AA) (18.8%) and acute lymphoblastic leukemia (ALL) (17.4%), respectively. In addition to these diseases, 3 (4.3%) patients were diagnosed with myelodysplastic syndrome (MDS), 3 (4.3%) patients with chronic myeloid leukemia (CML), 3 (4.3%) patients with primary myelofibrosis (MF), 1 (1.4%) patient with post-essential thrombocythemia MF and 1 (1.4%) patient with paroxysmal nocturnal hemoglobinuria. The median ferritin level was 1080 ng/mL (505-1650) in all groups. The patients' demographic and clinical characteristics at the time of transplantation were summarized in Table 1 after stratification according to ferritin levels. Comparative analysis between the groups showed no significant differences with respect to demographic characteristics (age and sex) or biochemical parameters, including CRP, ALT, LDH, and creatinine (p > 0.05). AML diagnosis was higher in the group with ferritin ≥1000 ng/mL (61.5%) than in the group with ferritin <1000 ng/mL (30%). Again, patients with non-AML, ALL and AA diagnoses were more in the group with ferritin <1000 ng/mL (26.7%) than in the group with ≥1000 ng/mL (7.7%). A statistically significant difference in diagnostic status was found between the ferritin groups (p = 0.043).

Table 1. The demographic and clinical characteristics of the patients at the time of transplantation according to the ferritin level

	Ferritin <1000 ng/mL n:30 (%)	Ferritin ≥1000 ng/mL n:39 (%)	p value
Median age (Q1-Q3)	34 (23-48)	32 (23-49)	0.856
Sex			
Male	15 (50)	16 (41)	0.618
Female	15 (50)	23 (59)	
Diagnosis			
AML	9 (30)	24 (61.5)	0.043*
ALL	6 (20)	6 (15.4)	
AA	7 (23.3)	6 (15.4)	
Others	8 (26.7)	3 (7.7)	
The median time to allo-HSCT from diagnosis, months (Q1-Q3)	4 (2-7)	5 (3-10)	0.279
LDH level			
Normal	14 (46.7)	27 (69.2)	0.084
High	16 (53.3)	12 (30.8)	
CRP level			
Normal	9 (30)	11 (28.2)	0.999
High	21 (70)	28 (71.8)	
ALT level			
Normal	23 (76.7)	26 (66.7)	0.429
High	7 (23.3)	13 (33.3)	
Creatinine level			
Normal	29 (97.1)	38 (97.4)	0.999
High	1 (2.6)	1 (2.9)	
Median ferritin, ng/mL (Q1-Q3)	382.2 (255.1-606.1)	1650.0 (1247.3-1975.7)	NA

Allo-HSCT; allogeneic hematopoietic stem cell transplantation, AML; acute myeloid leukemia, ALL; acute lymphoblastic leukemia, AA; aplastic anemia, ALT; alanine aminotransferase, LDH; lactate dehydrogenase, CRP; C-reactive protein.NA; not-applicable
* Pearson's chi-squared test, p<0.05

The median interval from diagnosis to allo-HSCT was 4 months (1-158 months). Peripheral blood served as the only stem cell source in all groups. The median counts of infused stem cells were 7.20x106/kg (6.10-8.13x106/kg) in all cohorts. The HLA-full-matched related donor was used most frequently as the donor type, which was used in 52 (75.4%) patients. A reduced conditioning regimen was used most frequently as the conditioning regimen, which was used in 40 (58%) patients. Fludarabine plus cyclophosphamide plus anti-thymocyte globulin (ATG) regimen was primarily used as a reduced-intensity regimen, which was used in 15 (37.5%) patients. Treosulfan plus fludarabine plus ATG and fludarabine plus amsacrine plus cytarabine (FLAMSA) therapy were the other most used RIC regimens which were used in 9 (22.5%) and 9 (22.5%) patients, respectively. Busulfan plus cyclophosphamide was used as the MAC regimen, which was used in 26 (89.7%) patients. Cyclosporine A plus methotrexate combination was used in all patients due to GvHD prophylaxis. The transplantation characteristics and outcomes were shown according to the ferritin levels in Table 2. Comparison of the ferritin levels revealed no significant differences in $CD34^+$ stem cell dose, donor type, or conditioning regimen ($p > 0.05$). Ferritin levels had no statistically significant effect on the transplant outcomes, including engraftment status/times, the febrile neutropenia rates, hospitalization times, the rates TRM and SOS/VOD ($p>0.05$).

Response assessment could be performed in 47 (68.1%) patients at 3 months after transplantation. Twenty-two (31.9%) patients died before response assessment could be performed. Complete response was detected in 38 (80,9%) patients in all cohorts. There was no statistically significant effect of ferritin

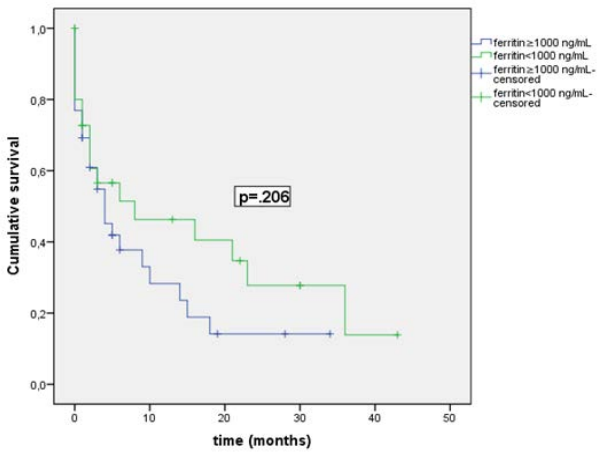

Figure 1. Kaplan–Meier curves for overall survival according to the ferritin level

Table 2. The transplantation characteristics and outcomes according to the ferritin levels

	Ferritin <1000 ng/mL n: 30 (%)	Ferritin ≥1000 ng/mL n: 39 (%)	p value
Median infused CD34+ count (Q1-Q3), x106/kg	7.45 (7.00-8.80)	6.95 (5.90-7.90)	0.166
Donor type			
Matched sibling	25 (83.3)	27 (69.2)	
Matched unrelated	3 (10)	6 (15.4)	0.380
Haploidentical	2 (6.7)	6 (15.4)	
Conditioning regimen			
Myeloablative	12 (40)	17 (43.6)	0.957
Reduced intensity conditioning	18 (60)	22 (56.4)	
Neutrophil engraftment status			
Yes	23 (76.7)	30 (76.9)	0.999
No	7 (23.3)	9 (23.1)	
Median time of neutrophil engraftment (Q1-Q3), day	15 (13-20)	16 (13-20)	0.766
Platelet engraftment status			
Yes	23 (76.7)	30 (76.9)	0.999
No	7 (23.3)	9 (23.1)	
Median time of platelet engraftment (Q1-Q3), day	15 (14-20)	16 (14-22)	0.678
Febrile neutropenia rate			
Yes	20 (66.7)	31 (79.5)	0.355
No	10 (33.3)	8 (20.5)	
Median hospitalization time (Q1-Q3), day	18 (16-23)	20 (16-25)	0.456
Transplant related mortality rates, (%)	8 (26.6)	9 (23)	0.783
Response at 3 months	n:21	n:26	
CR	19 (90.5)	19 (73.1)	
PR	0	1 (3.8)	0.287
Refractory	2 (9.5)	6 (23.1)	
Acute GvHD status			
Yes	4 (13.3)	3 (7.7)	0.713
No	26 (86.7)	36 (92.3)	
Chronic GvHD status			
Yes	6 (20)	8 (20.5)	0.999
No	24 (80)	31 (79.5)	
SOS/VOD			
Yes	4 (13.3)	4 (10.3)	0.987
No	26 (86.7)	35 (89.7)	

CR: complete response, GvHD: graft versus host disease, PR: partial response, SOS/VOD: Sinusoidal obstruction syndrome/veno-occlusive disease

value on response status (p=0.287) (Table 2)

The median OS was 4 months (95% CI: 1.4–6.6) in patients with ferritin levels ≥1000 ng/mL and 8 months (95% CI: 0–24.2) in those with ferritin levels <1000 ng/mL. No statistically significant association was observed between ferritin levels and OS (p = 0.206) (Figure 1). At the time of the last follow-up, 24 patients (34.7%) were alive. Acute GvHD occurred in 7 (10.1%) patients as only liver acute GvHD in 3 (4.3%) patients, only skin acute GvHD in 1 (1.4%) patient, only gastrointestinal acute GvHD in 1 patient (1.4%), and both gastrointestinal and liver acute GvHD in 2 (2.9%) patients. Grades III/IV acute GvHD occurred in 4 (5.8%) patients. Chronic GvHD occurred in 14 (20.2%) patients (9 limited and 5 extended. Comparison between the ferritin groups showed no statistically significant variation in the rates of acute or chronic GvHD (p=0.713 and p=0.999, respectively) (Table 2). In the univariate analysis, factors significantly associated with overall survival (OS) included the time from diagnosis to allo-HSCT, alanine aminotransferase (ALT) levels, creatinine levels, ferritin levels, the presence of febrile neutropenia, and infused $CD34^+$ cell counts (Table 3). In the multivariable Cox regression analysis, the time from diagnosis to allo-HSCT, creatinine levels, febrile neutropenia, and infused $CD34^+$ cell counts remained independent predictors of OS (Table 3).

DISCUSSION

In this study, the effect of ferritin levels on allo-HCT outcomes was evaluated. The ferritin levels had no effect on the early outcomes including the engraftment status, engraftment times, the febrile neutropenia rates, hospitalization times, and 1-month TRM rates (p>0.05). Gu et al. reported that no significant relationship was observed between iron levels (serum ferritin <1000 ng/mL vs. ≥1000 ng/mL) and both myeloid and platelet reconstitution (p = 0.441 and p = 0.579,

Table 3. Univariate and multivariate analysis of overall survival

Parameters for OS	Univariate analysis			Multivariate analysis		
	Hazard ratio	95% confidence interval	p	Hazard ratio	95% confidence interval	p
Age (years)	1.006	0.987-1.025	0.529			
Gender (female)	1.448	0.798-2.628	0.224			
The median time to allo-HSCT from diagnosis months	1.010	1.001-1.019	0.037	1.033	1.008-1.059	0.009
ALT level	1.006	0.999-1.013	0.119	-	-	-
Creatinine level	3.184	0.796-12.741	0.102	5.110	1.043-25.034	0.044
CRP level	0.970	0.879-1.071	0.546			
Ferritin level	1.001	0.988-0.0002	0.172	-	-	-
LDH level	0.9908	0.997-1.005	0.372			
Febrile neutropenia	2.844	1.264-6.398	0.0115	3.846	1.496-9.886	0.005
CD34+ cell counts in the transplant	1.102	0.965-1.258	0.154	1.228	1.056-1.428	0.007
Acute GvHD	0.972	0.384-2.481	0.953			
Chronic GvHD	0.631	0.308-1.290	0.207			

ALT; alanine aminotransferase, CRP; C-reactive protein, GvHD: Graft-versus-host disease, LDH; lactate dehydrogenase, OS: overall survival.

respectively) and the median serum ferritin levels before transplantation was 561 (223-846) µg/L in this study (19). In similar, another study reported that both neutrophil and platelet engraftment day were not affected by iron overload (p=0.710 and p=0.190, respectively). In the same study, iron overload had no poor effect on 3-months and 1-year TRM rates (iron overload group; 8.3% and 17.5%, non-iron overload group; 4.5% and 26.5%, respectively, p=0.940) (20). Our results on the effects of iron overload on engraftment and TRM are in line with the existing literature. However, Pullarkat et al. found that the high ferritin (≥1000 ng/mL) had increased day 100 mortality (median pre-transplant serum ferritin 952 ng/ml, range from 10 to 10 000 ng/ml, ferritin ≥1000 ng/mL; 20%, ferritin <1000 ng/mL; 9%, p=0.038) (6).

In a retrospective study, the patients with MDS who underwent allo-HSCT divided into two groups: the effective treatment group (ferritin <1000 µg/L) and iron overload group (ferritin ≥1000 µg/L). The effective treatment group demonstrated a significantly lower incidence of infections than the iron overload group (36.8% vs. 82.4%, p = 0.002) (19). Sivgin et al. analyzed the effects of pre-transplant serum ferritin levels (ferritin level ≥1000ng/mL or <1000 ng/mL) in patients who had undergone allo-HSCT. It was reported that patients who developed infectious complications exhibited significantly higher pre-transplant serum ferritin levels (p < 0.05) (21). Contrary to the literature, iron overload was not adversely affected on febrile neutropenia in our study.

In a prospective study, 45 patients with acute leukemia and myelodysplastic syndrome who underwent allo-HCT were evaluated. They found that pre-HCT iron overload (median ferritin 1432 ng/ml (20–6989) was not associated with increased mortality and relapse (22). In a meta-analysis including four studies, 276 patients who underwent allo-HCT was analyzed. This meta-analysis reported that serum ferritin >1000 ng/mL had significant effect on OS but not ferritin >2500 ng/mL (15). However, ferritin levels (<1000 ng/mL vs ≥1000 ng/mL) had no effect on overall survival in our study (p=0.206). There are different studies in the literature reporting the relationship between iron load and GvHD. Pullarkat et al. reported that ferritin levels >1000 ng/mL was associated with common acute GvHD (6). A separate retrospective analysis reported an association between pre-transplant ferritin concentrations >400 µg/L and a decreased incidence of chronic GvHD following allo-HCT (23). In contrast, Penack et al. found no significant relationship between serum ferritin levels and the occurrence of either acute or chronic GvHD (24). In our study, no statistically significant relationship was detected between ferritin levels and either acute or chronic GvHD (p = 0.713 and p = 0.999, respectively).

İron overload is known important risk factor for SOS/VOD in patients who underwent allo-HSCT. Several trials have reported a significant association between iron overload and SOS/VOD. In one study, elevated ferritin levels exceeding 1000 ng/mL were linked to an increased risk of SOS, with an odds ratio of 2.49 (95% CI, 1.54–4.02) (25). A retrospective study in pediatric patients identified elevated pre-transplant serum ferritin as a major risk factor for SOS/VOD. Ferritin levels >2400 ng/mL were associated with a significantly increased incidence of SOS/VOD compared with levels ≤2400 ng/mL (29.7% vs. 7.4%, p = 0.005) (26). In contrast, there was not significantly association between the serum ferritin levels and SOS/VOD in our study (p=0.987).

Study Limitations

This study has certain limitations. First, its retrospective design and the relatively small, heterogeneous cohort for allo-HCT. Second, ferritin is a positive acute-phase markers and it may not always show iron overload. However, we prefer ferritin firstly to show the iron overload because it is easily accessible and non-invasive method. For a more transparent interpretation of survival data, our cohort was not a heterogeneous group.

CONCLUSION

In conclusion, our study did not show the poor effects of the serum ferritin levels on the transplantation outcomes, but large prospective studies are needed to clarify the impact of iron overload on outcomes of allo-HCT.

DECLARATIONS

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