Prognosis of Elevated Serum Ferritin in Allogeneic-HCT

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Abstract: *Introduction:* Serum ferritin was demonstrated to be a useful tool to predict the risk in patients who undergo hematopoietic stem cell transplantation (HCT). Still it is not clear if its predictive value solely represents iron overload (IO) and published results are sometimes contradictory. So the objective of present study was to determine relationship between elevated pre-HCT serum ferritin levels, morbidity and mortality after allogeneic hematopoietic stem cell transplantation (allo-HCT) on one side, and its correlations with various risk indexes which were developed recently to predict outcomes after allo-HCT on the other side.

Patients and Methods: In this retrospective study we have reviewed medical records of one hundred six consecutive patients (52 males and 54 females), with a median age of 32 years (range, 5 to 60), who underwent allo-HCT with unmanipulated grafts between Jan 2013 and Dec2014. We retrieved pre-allo-HCT serum ferritin levels and also calculated risk indexes before HCT. The incidence of complications and outcomes after allo-HCT was assessed. The median follow-up period was 12 (range, 4-27) months after allo-HCT.

Results: We have determined a cuttoff ferritin level of 500 ng/mL for early complications and 737 for outcomes. We found increased incidence of number of febrile neutropenic episodes (P =0.02), number of bacterial infection episodes (P =0.009), pneumonias (P =0.039), slower period of neutrophil engraftment (P=0.032), demand for multiple red blood cell (RBC) transfusions (P =0.002) within 100 days post transplantation. A significant association was found between pre-transplant ferritin concentrations and different risk indexes; European Group for Blood and Marrow Transplantation (EBMT) risk score (P=0.001), Hematopoietic cell transplantation comorbidity index (HCT-CI) (P=0.003), Pre-transplant Assessment of Mortality (PAM) score (P=0.007) and disease risk (DR) (P =0.037).

Conclusion: On the one hand we did confirmed that even moderate serum ferritin elevation is associated with increased incidence of infections, slower period to engraftment and increasing demand of RBC units transfusions, but strong correlation with pre-transplant indexes that take into account disease risk raises the question if IO is the only factor that adversely affect the outcome of HCT in patients with increased ferritin. This should be studied in prospective trials.

Keywords: Hematopoietic stem cell transplantation, Allogeneic, iron overload, Serum ferritin, Early infectious complications, Risk indexes.

INTRODUCTION

Iron overload is common in patients undergoing allogeneic hematopoietic stem cell transplantation (HCT). The main causes of IO in HCT are prolonged dys-erythropoiesis, increased intestinal iron absorption due to anemia and chemo-therapy associated mucositis which leads to increased iron absorption, transfusion burden and release of iron from injured tissues [1].

Many studies have shown an association between elevated serum ferritin before HCT (used as a surrogate measure of iron overload) and adverse outcomes [7-10, 13], mostly from an increase in infection risk [2-7] and non-relapse mortality (NRM) [7, 8], although some of these studies also suggested an association between IO and acute graft versus host disease (GvHD) [9, 10] or hepatic veno-occlusive disease (VOD) [12]. However, these studies were retrospective and mostly based on surrogate measures of iron burden, especially serum ferritin.

Different cutoffs ferritin was used in several studies, *e.g.*, (Altes *et al*. 2002 (F≥3000)[23]; Armand *et al*. 2007 (F≥2515) [24]; Kataoka *et al*. 2009 (F≥599) [8]; Mahindra *et al*. 2009b (F≥1910) [9]; Lee *et al*.2009 (F≥1000) [26]; Sucak *et al*. 2010 (F≥500) [7], so the optimal cutoff ferritin which accurately reflects increased risk of complications after HCT is not well estimated.

In the other hand when pre-transplant IO was measured using liver magnetic resonance imaging (R2-MRI), found contradictory results. There was no association between pre-transplant IO defined by R2-

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MRI measured liver iron concentration (LIC) and overall survival (OS), NRM, relapse rate, GvHD, bacterial, viral or fungal infections [14, 15]. This results doesn't contradict the previous data that used serum ferritin for assessment of IO, because these studies had limited number of patients included and small studies in HCT usually have limited power to demonstrate the impact of single factor, like IO, on the outcomes. So ferritin level is still accepted as a standard risk assessment tool in HCT recipients.

Many accepted risk indexes likes; Disease risk index(DR), Hematopoietic cell transplantation co-morbidity index (HCT-CI), European Group for Blood and Marrow Transplantation (EBMT) risk score and Pre-transplant Assessment of Mortality Score (PAM) were developed to predict outcomes and complications of allo-HCT[17-20]. Some of them include parameters that could be attributed to IO, but there is limited data on correlation between these indexes and ferritin levels.

Summarizing, a substantial number of studies have been published on the topic of serum ferritin in HCT, but still there are several controversial points. So we focused our study on the impact of pre allo-HCT serum ferritin on different events post allo-HCT; Early infectious complications, mucositis, engraftment, veno-oclusive disease, GvHD, and on determining the optimal cutoff for increased risk of complications and mortality. Also we evaluated how a serum ferritin level was correlated with the other tools of HCT risk assessment, like pre-transplant indexes.

MATERIALS AND METHODS

We have retrospectively evaluated a group of one hundred and six consecutive patients who underwent unmanipulated allo-HCT in R.M. Gorbacheva Memorial Institute of Children Oncology, Hematology and Transplantation at the St. Petersburg Pavlov State Medical University, St. Petersburg, between 01/01/2013 and 31/12/2014. The inclusion criteria were as follows: (1) First allo-HCT from HLA-compatible related, unrelated and haplo-identical donors; (2) Primary malignant or non-malignant disease; (3) Age: 5 to 60 years; (4) Karnofsky performance status ≥70 %.

The cohort included 52 men and 54 women with a median age at transplantation of 31.8 (range, 5 to 60) years. Underlying diseases were acute myeloid leukemia (n=78), myelodysplastic syndrome (n= 12), primary myelofibrosis (n=3), aplastic anemia (n=11) and B-thalassemia major (n=2). Stem cell transplants were from HLA-identical siblings (n=26), haplo-identical (n=9), or unrelated volunteer donors (n=71). The conditioning regimens were myeloablative (MAC) in 26 patients, and reduced intensity (RIC) in the rest of this group. The study was approved by the Institutional Review Board at the St.Petersburg State Pavlov Medical University. Each patient has given an informed consent for the use of personal data. The median follow-up period was 12 (range, 4-27) months after allo-HCT. Baseline characteristics of the patients are given in (Table **1**).

Mucositis was graded on a scale of 0 to 4, according to Common Toxicity Criteria, version 4, from the National Cancer Institute. The International Consensus on opportunistic fungal infections was established by the EORTC/MSG [16]. Bacterial infection was defined as recovery of a recognized pathogen from, at least, 2 blood or urine cultures yielding the same organism. For each patient, the numbers of bacterial, viral and fungal infections were calculated, according to CIBMTR Instructions for 100 Days post-HCT (Data Form 2100). Document Number**:** A00531 version 1.1 (7/20/2012). Neutrophil recovery was defined as an ANC of ⩾500/μL for the first 3 consecutive days. (DR) index, (HCT-CI), (EBMT) risk and (PAM) scores were calculated according to Armand *et al.* [17], Sorror ML *et al.* [18], A Gratwohl [19] and Parimon T *et al.* [20], respectively. PAM score and DR index were calculated only in malignant diseases. Acute GvHD was graded according to Gratwohl criteria [21]. Graft-*vs*-host disease - relapse free survival (GRFS) was considered according to Shernan G. Holtan, *et al.* [22]. A diagnosis of sinusoidal obstruction syndrome (Hepatic veno-occlusive disease) was made on the basis of Seattle criteria, as described by McDonald *et al.* [11]. Continuous variables in the 2 groups were compared by means of the Mann-Whitney test. Categorical variables were compared using the Chi-square test. GRFS, OS, NRM and event-free survival (EFS) were calculated using the Kaplan-Meier method. Possible risk factors were tested using the log-rank test. Relapse was counted as competing risk for non-relapse mortality. Cutoff levels of ferritin amounts were determined using ROC - analysis. The association between serum ferritin levels and different risk indexes (DR, EBMT, HC-CI and PAM score) was calculated according to Kruskal-Wallis Test. Correlation between ferritin and C-reactive protein (CRP) was evaluated using Spearman method. The calculations were made with SPSS software (version 17).

Table 1: Patients Baseline Characteristics

		%	Range
Total number of patients	106		
Male	52	49	
Female	54	51	
Median age (years)	31.8		(5-60)
Diagnosis			
Acute myeloid leukemia	78	73.6	
Myelodysplastic syndrome	12	11.32	
Primary myelofibrosis	3	2.8	
Aplastic anemia	11	10.3	
B-Thalassemia major	2	1.8	
Status of malignant disease (n=93)			
Complete remission	60	64.5	
Incomplete remission	33	35.5	
Conditioning regimen			
MAC	26	24.5	
RIC	65	75.5	
Source of stem cells			
BM	48	45.2	
PBSC	55	51.8	
PBSC+BM	3	2.80	
Stem cell dose CD34+ / $*10^6$	4.9		(1.1-14.1)
Lymphocyte dose CD3+ / $*10^7$	12.3		(1.2-39.5)
Donor			
M / F	69/37	65/35	
Median donor age	32.4		(3.9-56)
Median ferritin level pre-allo-HCT ng/mL.	772.5		(12.1-4247)
Median hemoglobin concentration g/L	104		(50-155)
Type of allo-HCT			
MRD	26	24.5	
MUD	59	55.6	
haplo	9	8.4	
MMUD 8-9/10	12	11	
Median EBMT risk score	3		(0-6)
HCT-CI ≥1	52	49.5	
Disease risk index (high + very high)	38	35.8	
Median Pre-transplant Assessment of Mortality Score(PAM)	17.5		(10.7-27.6)
Median karnofsky performans status %	90		(70-100)

Note; MAC= Myeloablative conditioning regimen; RIC = Reduced intensity conditioning regimen; BM = Bone marrow; PBSC =Peripheral blood stem cell; MRD = Matched related donor; MUD =Matched unrelated donor; MMUD = Mismatch unrelated donor

RESULTS

According to the area under the curve (AUC) and with using receiver operating characteristic curve (ROC) analysis in many indexes like febrile neutropenia, bacterial infections, fungal infections, pneumonia, NRM and OS, the cutoff of ferritin was between 412.2 to 612 ng/mL for infectious complication and 737 ng/mL for NRM & OS (Table **2**).

As the cutoffs the majority of infectious complications was near the 500 ng/mL value, which is in the literature considered moderate IO, we used it to analyze infectious complications, mucositis, febrile neutropenia, graft engraftment, hemorrhagic cystitis and number of RBC units transfused after allo-HCT. For NRM and OS analysis we used the higher cutoff 737 ng/mL, which was determined statistically.

Sixty-three (59.4%) patients (pts) had increased serum ferritin concentrations (≥500 ng/mL), and were classified as the high-ferritin group (HF), with a median of 1134.8 ng/mL (range, 511-4247). The rest of patients (n=43, 40.6%) were classified as LF group, and had serum ferritin concentrations (<500 ng/mL) with a median ferritin at 281.8 ng/mL (range, 12.1-466.3).

Active infections within last month prior to HCT were observed only in HF group. There were sixteen cases; (8) pts with probable invasive pulmonary aspergillosis, (5) pts with bacterial infections, (2) pts with fever of unknown origin and (1) case hepatitis B, with a median ferritin level of 1080.5 ng/mL (606.1-1875). Hence, fungal infections prevailed among total infections by the date of allo-HCT, and 25.4% of patients in HF group with increased ferritin levels, *P*= 0.001; [OR], 0.73; 95% [CI], (0.62–0.86).

Complications in the Early Period after Allogeneic-HCT

Mucositis

Post-transplant mucositis was observed in eighty patients; 33 pts (52.3%) in HF and 21 (48%) in LF group had mucositis grade I-II, *P* =0.43; [OR], 1.15 ;

Table 2: Results of AUC & ROC Analysis to Determine the Cutoff Ferritin in Different Indexes

Indexes	AUC	Sensitivity	Specificity	Cutoff Ferritin ng/mL
Bacterial infection >2 episodes	0.599	0.717	0.567	608.55
Febrile neutropenia >1 episode	0.600	0.879	0.438	412.20
Septicemia	0.586	0.719	0.527	612.00
Pneumonia	0.573	0.870	0.398	412.20
Fungal infections	0.640	0.933	0.385	412.20
VOD	0.664	0.500	0.941	1603.50
NRM+OR	0.608	0.692	0.591	737.25

95% [CI], (0.53–2.5). 12 pts (19 %) in HF group *versus* 14 (31.57%) in LF group had mucositis grade III-IV, *P* =0.08*;* [OR], 0.48 ; 95% [CI], (0.19–1.19). No statistical differences were observed between the two groups.

Febrile Neutropenia

Febrile neutropenia was observed in eighty cases; 51(81%) in HF and 29 (67.4%) in LF (*P* > 0.05). Despite the lack of a statistically significant difference between the groups, we revealed a linear correlation between increasing ferritin level and numbers of febrile neutropenia episodes. The median for HF group was 1.4 febrile episodes/ patient (rang, 0 to 6) *vs* 0.9 (0 to 3) in LF patients, *P* = 0.02; [OR], 2.87; 95% [CI], (1.14–7.21). In the same context, the number of patients who required granulocyte colony-stimulating factor (G-CSF) injections to boost hematopoiesis and to shorten neutropenic period was; 30 (47.3%) *vs* 9(21%) in the HF and LF groups, respectively, *P* =0.007; [OR], 3.4 ; 95% [CI], (1.14–8.3).

Bacterial Infections

Although the percentage of patients exposed to bacterial infections was similar in both HF and LF groups (resp., 85.7, 84.2%), there was a distinct correlation between the increased pre-HCT ferritin and median number of infectious episodes/ patient, *i.e*., 2.6(0 to 7) in HF group, and 2.0 (0 to 6) in LF sample, *P* = 0.009; [OR], 3; 95% [CI], (1.33–6.9). Incidence of blood-borne infections (septicemia) was more frequent in the HF group (n=23; 36.5%) *vs* (n=9; 21%) in LF patients, a statistical difference was absent, *P* = 0.06; [OR], 2.17; 95% [CI], (0.88-5.32).

Pneumonia

Increasing incidence of pneumonia was observed, regardless of its causes, in the first group (ferritin ≥500 ng/mL) compared to the second group; 18 cases (28.6%) in HF *vs* 5 cases (11.6%) in LF, *P* =0.039, [OR], 3; 95%[CI], (1–8.9) . Mixed pathogens as causes of pneumonia were observed in a large proportion of patients (bacterial, fungal and viral pathogens); 55.5% and 60% in HF and LF groups, respectively. Isolated bacterial pneumonia was recorded only in HF group and formed 33.3% of causes of pneumonia.

Fungal Infections

In our cohort study, fungal infections were observed in 12 pts (19%) of HF group and 3 pts (7%) in LF group, with no statistically significant difference *P* =0.06, [OR], 3,1; 95%[CI], (0.8–11.8).

Cytomegalovirus(CMV) Infections

During the early period after allo-HCT, CMV reactivation was observed in 40 pts (37.7% of total), including 25(39.7%) in HF, and 15(34.8%) in LF group, without statistically significant difference *P* =0.38, [OR], 1.22 ; 95%[CI], (0.54–2.74). There was no differences in the incidence of other viral infections like polyomavirus (BK, JC) between the two groups; 27% *vs* 21% in HF and LF, respectively, *P* =0.31, [OR], 1.39 ; 95%[CI], (0.55–3.50).

Engraftment

Median time of neutrophil (Neu ≥ 500 $*10^9$) and platelet recovery (Plt ≥ 20 $*10^9$) was, respectively, 22.3 days (range, 13-43) and 18 days (10-38) in HF group, *versus* 20.3 days (11-36) and 16.6 days (11-36) in LF group. Neutrophil recovery was slower in the high ferritin group than in the low ferritin group, *P* =0.032, [OR], 0.2 ; 95%[CI], (0.04–0.97). That was independent of the stem cell dose; 5 (1.1-14.1)$*10^6$ *versus* 4.7 (1.5-10.1)$*10^6$ in HF and LF. Intensity of conditioning regimens did not affect the engraftment rates in our cohort: MAC/ RIC; 25.3/ 74.6 % and 23.2/76.8 % in HF

and LF groups, respectively. No statistically significant difference was observed in platelet recovery , *P*> 0.05.

Hepatic Veno-Occlusive Disease (VOD)

Only 4 cases were observed in total cohort; 3 in HF and 1 in LF group. The pre-HCT ferritin levels in these cases were as follows; 4247, 1631, 827.3, 112 ng/mL, and one case with primary myelofibrosis had severe VOD with multiorgan failure, and died on D7+ after transplantation.

Acute and Chronic Graft-Versus-Host Disease (GvHD)

Acute GvHD grade I-II and grade III-IV was diagnosed in 23 pts (37%) and 6 (9.5%) in HF *vs* 19 pts (44.2%) and 2 (4.6%) in LF, respectively. This difference was not statistically significant, *P* =0.46. The cumulative incidence of extensive chronic GvHD at 1 year after transplantation was 16% in HF and 28% in LF without reaching statistically significant, *P* =0.1, [OR], 0.48 ; 95%[CI], (0.17–1.3).

Blood Transfusion

Number of RBC units transfused was counted for each patient within 100 days post-HCT, and the median number of RBC units/patient was 8.8 units (range, 0 to 36) and 3.8 units (0 to 27) in HF and LF groups respectively, *P* =0.002 ,[OR], 5.23 ; 95%[CI] , (1.65–16.55).

The results of analysis regarding early and infectious complications are summarized in Figure **1**

One Year Outcomes of Allo-HCT

With the estimated cutoff level ≥ 737 ng/mL the cumulative incidence of NRM within one year was higher in HF (25%) than in LF (10.3 %), *P* =0.024. The same pattern was observed for OS; 85% in LF *vs* 63% in HF, *P* = 0.002. On the contrary, there was no statistical differences in EFS and GRFS between HF and LF groups; 54% *vs* 66% (*P* =0.14) for EFS and 40% *vs* 45% *(P* =0.4*)* for GRFS. However, on multivariate analysis we found that donor other than matched related (MRD) was the only factor that was associated with higher NRM and lower OS, *P* =0.044, [HR], 2.66; 95%[CI], (1.02–6.96) for NRM and , *P* =0.021 , [HR], 2.76 ; 95%[CI], (1.72–6.50) for OS. Ferritin was not a significant factor in multivariate for both NRM *(P* =0.28) and OS *(P* =0.55) (Table **3**).

The results of outcomes (NRM, OS, EFS and GRFS) analysis are summarized in Figure **2** (**a,b,c,d**)

Correlation of the pre-HCT serum ferritin and C-reactive protein (CRP)

To exclude that the elevated pre-transplant ferritin was not due to IO, but the systemic inflammatory response (SIRS), we analyzed the association between serum ferritin and pre-transplant CRP levels. There was no statistically significant correlation between CRP> 5 and ferritin levels (*P* =0.055). So the observed differences in early complications based on ferritin level were not associated with presence of SIRS pre-HCT, but more likely due to IO.

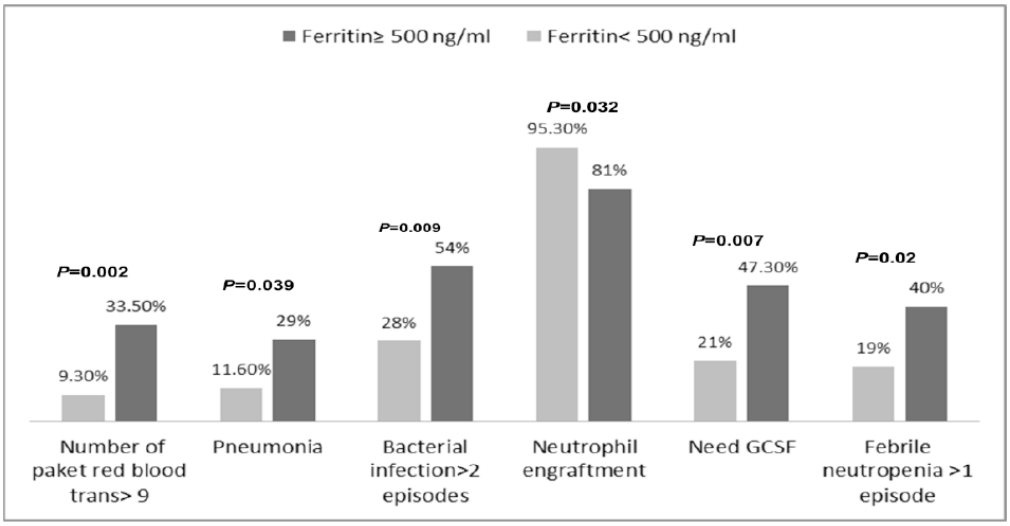

Figure 1: The percentage of early and infectious complications post allo-HCT in two groups of ferritin. We calculated recurrent febrile neutropenia >1 episode, recurrent bacterial infectious >2 episodes, need of stimulating factor (GCSF), incidence of pneumonia, multiple blood transfusion >9 units and the percentage of neutrophil engraftment in two ferritin groups.

Table 3: Univariate and Multivariate Analysis of Factors Effecting 1 Year Cumulative Non Relapse Mortality and Probability of overall Survival

Factor	Univariate, P-Value	Multivariate, P-Value	HR	HR 95% CI
Non-relapse mortality				
Ferritin ≥737	0.024	0.28		
Active disease at the time of HCT	0.011	0.27		
Matched related *vs* other donor	0.002	0.044	2.66	(1.02-6.96)
CRP > 5 prior HCT	0.015	0.69		
Probability of overall survival				
Ferritin ≥737	0.002	0.55		
Active disease at the time of HCT	<0.0001	0.13		
Matched related *vs* other donor	0.004	0.019	2.76	(1.17-6.50)
CRP > 5 prior HCT	0.007	0.74		

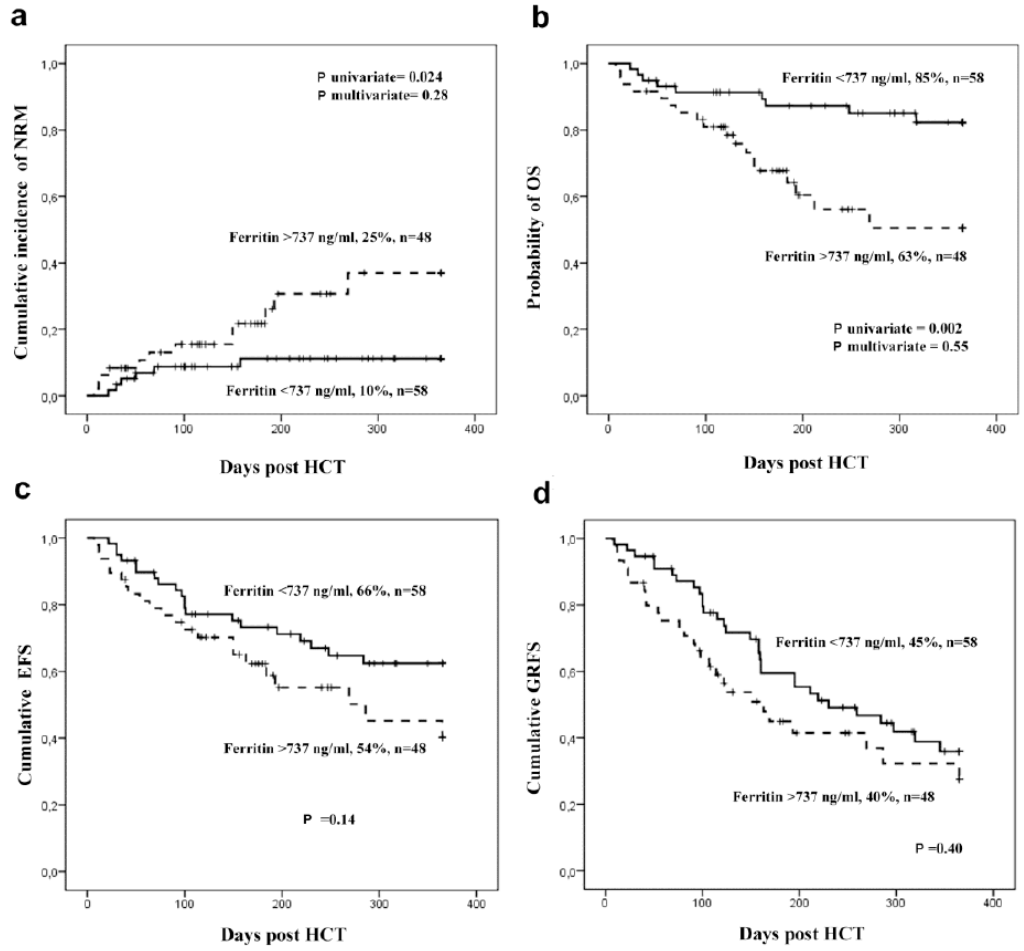

Figure 2: Differences in 1 year outcomes between two ferritin groups with pre allo-HCT ferritin level cutoff >737 ng/mL (**a**) Cumulative incidence of 1 year non-relapse mortality (**b**) Probability of 1 year overall survival (**c**) Cumulative incidence of 1 year event free survival (**d**) Cumulative incidence of 1 year graft *vs* host disease -free relapse survival.

Associations between pre allo-HCT ferritin levels and different risk indexes

Surprisingly, we found statistically significant association between pre-transplant ferritin and all currently existing risk assessment indexes; EBMT score (*P* =0.0001), comorbidity index (HCT-CI) (*P* =0.003), Pre-transplant Assessment of Mortality Score (PAM) (*P* =0.007) and disease risk (DR)(*P* =0.037).

Index	EBMT score	N pts	Mean±(SD)	HCT-CI	N pts	Mean±(SD)	DR index	N pts	Mean±(SD)
	0	2	184.1 (123.74)	0	54	628.75 (548.18)	1	3	102.53 (97.07)
	1	11	714 (621.53)	1	40	1031.11 (750.42)	2	52	744.52 (764.64)
	2	30	535.24 (556.34)	2	8	507.92 (352.49)	3	30	699.22 (454.01)
	3	29	701.17 (340.97)	3	2	288.05 (237.65)	4	8	1055.95 (604.4)
	4	11	768.04 (393.14)	4	1	1315.3			
	5	20	1326.5 (936.09)	5	0				
	6	3	776.6 (673.49)	6	1	740			
Total		106			106			93	
p value	0.0001			0.003			0.047		

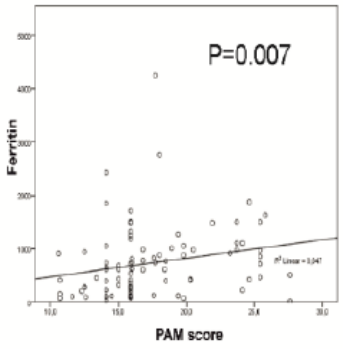

Figure 3: The association between pre allo-HCT serum ferritin and risk assessment indexes; EBMT score, comorbidity index (HCT-CI), Pre-transplant Assessment of Mortality Score (PAM) and disease risk (DR). [N pts: Number of patients. Mean ±SD: Mean ferritin level ± Standard deviation] were calculated for every degrees of risk indexes. Disease risk index and PAM score were calculated only for malignant diseases (n=93). In the right liner correlation between pre allo-HCT ferritin and PAM score was observed (r2=0.047).

Despite, absence of a clear trend of increasing ferritin in risk groups from different scoring systems, there was a clear evidence of lower ferritin level in patients with lowest risk. Also there was a positive correlation between the ferritin level and PAM (r2=0.047, Figure **3**).

DISCUSSION

IO associates with increased susceptibility to different infections. Iron deprivation was found to be the key factor in the antimicrobial host defense. Several previous studies showed the impact of elevated serum ferritin on the increasing the incidence of bacterial, blood stream, fungal infections [25, 27, 28] and pneumonia [7] post allo-HCT. On the contrast, latest recent study, M Tanaka1 *et al.* [29], showed that the cumulative incidence of infection within 100 days after HCT, was not different between the two groups with (cutoff >1000 ng/mL). In our study we found that elevated ferritin more than 500 ng /mL pre allo-HCT associated with increasing number of febrile neutropenia episodes (*P* =0.02), increasing number of bacterial episodes, increased pneumonia incidence, predominantly the bacterial one. The observed results reflect a well-known negative effect of IO on anti-infectious immunity [31-34]. In contrast to the published studies we didn't find increased incidence of fungal infections, that may be related to aggressive center policy in pre-transplant fungal infection screening and secondary prophylaxis [35]. Also in accordance with published studies we found delayed engraftment and increased percentage of patients who required G-CSF stimulation in HF group [29].

Also in this study we observed that patients who had high pre allo-HCT ferritin levels needed more blood transfusions than those who were in LF group. This may be explained in part by the presence of antibodies in heavily transfused patients [39] and by increasing incidence of febrile neutropenia and bacterial infections. In these inflammatory conditions the hepcidin is secreted as a defensive mechanism, which causes inhibition of iron realizing from its stores and thus prevents use iron in normal hematopoiesis and leads to slower recovery of erythroid lineage after HCT[40]. History of heavy blood transfusion ≥20 RBC units in last year before transplantation was recorded in 14 pts, the median ferritin levels in these pts was 1464.5 ng/mL (range, 650-4247). Only 3 patients were on chelation therapy with Exjade at the date of allo-HCT and they discontinued it just prior conditioning regimen.

Interestingly, the statistical cutoffs for infectious complications and delay in the engraftment were lower (>500 ng/mL), than in published papers [3,4,8-10], which was usually 1000-3000 ng/mL. This may raise the question that some of the patients with moderate elevation of ferritin may have not the actual IO, but the systemic inflammatory response (SIRS) pre-transplant. Several studies postulated that moderately increased ferritin is associated with SIRS in the HCT and non-HCT setting [30,41], but in our patient series we found no correlation between CRP and ferritin. Furthermore, CRP level was not statistically different in patients with ferritin above or below 500 ng/mL. So this may indicate that even moderate IO may be associated with increased incidence of complications in HCT recipients and may advocate use of chelation therapy before HCT

in patients with lower level of ferritin than 1000-2000 ng/mL. This observation should be confirmed with large, adequately powered studies with MRI assessment of IO.

Many studies have shown an association between elevated serum ferritin before HCT (used as a surrogate measure of IO) and adverse overall survival [7-10,13,29] and NRM [7,8]. These studies used various cutoff levels (500-3000 ng/mL). On the other hand when pre-transplant IO was measured with MRI, there such differences were not observed in the two studies; Trottier *et al*, found no association between pre-transplant IO defined by R2-MRI measured LIC and OS, NRM, relapse rate or GvHD [14], similar results have been reported by Armand *et al*. [15]. In our study in multivariate analysis we found no association between pre-transplant ferritin, NRM and OS, although in univariate, the significant differences were observed. This may be due to relatively small group size and heterogeneity of indications for HCT and conditioning regimens. Partly, this is confirmed by the strong observed correlation between ferritin levels and all of the HCT risk assessment indexes, including EBMT, HCT-CI, DR, PAM. As all of them were developed using very large cohorts of patients, with larger study group, the observed differences in NRM and OS in HF and LF groups might have reached the statistical significance.

Furthermore, based on analysis of ferritin and transplant indexes, several interesting conclusions could be drawn. On the one hand, we found association between ferritin and NRM predictive indexes, like HCT-CI and PAM. These scoring systems include such parameters as liver, heart, kidney and lung dysfunction and infectious complications that at least partially may be associated with IO [36-38]. Indeed in our recurrent infections and liver dysfunctions were more common in HF group. So IO is one of the factors that increase the risk of HCT predicted by HCT-CI and PAM. On the other hand, pre-transplant ferritin was associated with DR scores, which indicates that some proportion of the risk is not due to IO, but due to underlying disease itself, higher number of previous chemotherapy courses and poorer status at the time of HCT.

In conclusion, as was previously published, we observed increased incidence of infectious complica-tions and adverse impact on engraftment, but also found that lower level of ferritin is associated with increased risk of these events. Also association of ferritin levels with major HCT mortality risk indexes, makes it simple and effective tool to predict the outcome of treatment.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

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

[2] Chow JK, Werner BG, Ruthazer R, Snydman DR. Increased serum iron levels and infectious complications after liver transplantation. Clin Infect Dis 2010; 51: e16-e23

[3] Ozyilmaz E, Aydogdu M, Sucak G, Aki SZ, Ozkurt ZN, Yegin ZA, Kokturk N. Risk factors for fungal pulmonary infections in hematopoietic stem cell transplantation recipients: The role of iron overload. Bone Marrow Transplant 2010; 45: 1528-1533
http://dx.doi.org/10.1038/bmt.2009.383

[4] Tachibana T, Tanaka M, Takasaki H, Numata A, Ito S, Watanabe R, Hyo R, Ohshima R, *et al*. Pretransplant serum ferritin is associated with bloodstream infections within 100 days of allogeneic stem cell transplantation for myeloid malig-nancies. Int J Hematol 2011; 93: 368-374
http://dx.doi.org/10.1007/s12185-011-0784-0

[5] Kanda J, Mizumoto C, Ichinohe T, Kawabata H, Saito T, Yamashita K, *et al*. Pretransplant serum ferritin and C-reactive protein as predictive factors for early bacterial infection after allogeneic hematopoietic cell transplantation. Bone Marrow Transplant 2011; 46: 208-216
http://dx.doi.org/10.1038/bmt.2010.108

[6] Dadwal SS, Tegtmeier B, Liu X, Frankel P, Ito J, Forman SJ, Pullarkat V. Impact of pre-transplant serum ferritin level on risk of invasive mold infection after allogeneic hematopoietic stem cell transplantation Eur J Haematol. 2014 Aug 1. doi: 10.1111/ ejh. 12421

[7] Sucak GT, Yegin ZA, Ozkurt ZN, Aki SZ, Yağci M. Iron overload: predictor of adverse outcome in hematopoietic stem cell transplantation. Transplant Proc 2010; 42(5): 1841-1848
http://dx.doi.org/10.1016/j.transproceed.2009.11.049

[8] Kataoka K, Nannya Y, Hangaishi A, Imai Y, Chiba S, Takahashi T, Kurokawa M. Influence of pretransplantation serum ferritin on nonrelapse mortality after myeloablative and nonmyeloablative allogeneic hematopoietic stem cell transplantation. Biol Blood Marrow Transplant 2009; 15: 195-204
http://dx.doi.org/10.1016/j.bbmt.2008.11.012

[9] Mahindra A, Bolwell B, Sobecks R, Rybicki L, Pohlman B, Dean R, *et al*. Elevated pretransplant ferritin is associated with a lower incidence of chronic graft-versus-host disease and inferior survival after myeloablative allo-geneic haematopoietic stem cell transplantation. Br J Haematol 2009; 146: 310-316
http://dx.doi.org/10.1111/j.1365-2141.2009.07774.x

[10] Alessandrino EP, Della Porta MG, Bacigalupo A, Malcovati L, Angelucci EM, Van Lint T, *et al*. Prognostic impact of pretransplan-tation transfusion history and secondary iron overload in patients with myelodysplastic syndrome undergoing allogeneic stem cell transplantation: a GITMO study. Haematologica.2010; 95: 476-484
http://dx.doi.org/10.3324/haematol.2009.011429

[11] McDonald GB, Hinds MS, Fisher LD, Schoch HG, Wolford JL, Banaji M, *et al*. Veno-occlusive disease of the liver and multi-organ failure after bone marrow transplantation: a

cohort study of 355 patients. Ann Int Med 118: 255.1993

[12] Simone CM, Angelo M, Alexandre MA, Marta C, Luis FB, Marcio N. Serum ferritin as risk factor for sinusoidal obstruction syndrome of the liver in patients undergoing hematopoietic stem cell transplantation. Blood, 6 august 2009,V 114, N 6

[13] Storey JA, Connor RF, Lewis ZT, Hurd D, Pomper G, Keung YK, *et al*. The transplant iron score as a predictor of stem cell transplant survival. J Hematol Oncol 2009; 2: 44-52 http://dx.doi.org/10.1186/1756-8722-2-44

[14] Trottier BJ, Defor TE, Burns LJ, Defor TE, Cooley S, Majhail NS. Association of iron overload with survival and complications in allogeneic hematopoietic cell transplant recipients: prospective cohort study using R2-MRI measured liver iron content. Blood 2013; 122: 1678-1684 http://dx.doi.org/10.1182/blood-2013-04-499772

[15] Armand P, Sainvil MM, Kim HT, Rhodes J, Cutler C, Ho VT, *et al*. Does iron overload really matter in stem cell transplantation? Am J Hematol.2012; 87: 569-572 http://dx.doi.org/10.1002/ajh.23188

[16] Ascioglu S, Rex JH, de Pauw B, Bennett JE, Bille J, Crokaert F, *et al*. Invasive Fungal Infections Cooperative Group of the European Organization for Research and Treatment of Cancer; Mycoses Study Group of the National Institute of Allergy and Infectious Diseases: Defining opportunistic invasive fungal infections in immuno-compromised patients with cancer and hematopoietic stem cell transplants: an international consensus. Clin Infect Dis 34: 7, 2002 http://dx.doi.org/10.1086/323335

[17] Armand P, Gibson CJ, Cutler C, Ho TV, Koreth J, Alyea EP, *et al*. Disease risk index for patients undergoing allogeneic stem cell transplantation.Blood, 26 JULY 2012.V 120, N 4

[18] Sorror ML, Maris MB, Storb R, Baron F, Sandmaier BM, Maloney DG, Storer B. Hematopoietic cell transplantation (HCT)-specific comorbidity index: a new tool for risk assessment before allogeneic HCT. Blood.2005; 106(8): 2912-2919 http://dx.doi.org/10.1182/blood-2005-05-2004

[19] A Gratwohl. The EBMT risk score. Bone Marrow Transplantation (2012) 47, 749-756; doi: 10 . 1038/bmt.2011.110; published online 6 June 2011

[20] Parimon T, Au DH Martin PJ, Chien JW. A risk score for mortality after allogeneic hematopoietic cell transplantation. Ann Intern Med. 2006 Mar 21; 144 (6): 407-14 http://dx.doi.org/10.7326/0003-4819-144-6-200603210-00007

[21] Gratwohl A, Hermans J, ApperleyJ, Arcese W, Bacigalupo A, Bandini G, *et al*. Acute graft-versus-host disease: grade and outcome in patients with chronic myelogenous leukemia. Working Party Chronic Leukemia of the European Group for Blood and Marrow Transplantation. Blood.1995, 86, 813-818

[22] Holtan SG, DeFor TE, Lazaryan A, Bejanyan N, Arora M, Brunstein CG, *et al*. Composite end point of graft-versus-host disease-free, relapse-fre survival after allogeneic hematopoietic cell transplantation. Blood, 19 FEBRUARY 2015,V125, N 8

[23] Altes A, Remacha AF, Sureda A, Martino R, Briones J, Canals C, *et al*. Iron overload might increase transplant-related mortality in haematopoietic stem cell transplantation. Bone Marrow Transplant 2002; 29: 987-989 http://dx.doi.org/10.1038/sj.bmt.1703570

[24] Armand P, Kim HT, Cutler CS, Ho VT, Koreth J, Alyea EP, *et al*. Prognostic impact of elevated pretransplantation serum ferritin in patients undergoing myeloablative stem cell transplantation. Blood 2007; 109: 4586-4588 http://dx.doi.org/10.1182/blood-2006-10-054924

[25] Altes A, Remacha AF, Sarda P, Sancho FJ, Sureda A, Martino R, *et al*. Frequent severe liver iron overload after stem cell transplantation and its possible association with invasive aspergillosis. Bone Marrow Transplant 2004; 34(6): 505-509 http://dx.doi.org/10.1038/sj.bmt.1704628

[26] Lee JW, Kang HJ, Kim EK, Kim H, Shin Y, Ahn HS. Effect of iron overload and iron-chelating therapy on allogeneic hematopoietic SCT in children. Bone Marrow Transplant 2009; 44: 793-797 http://dx.doi.org/10.1038/bmt.2009.88

[27] Pullarkat V, Blanchard S, Tegtmeier B, Dagis A, Patane K, Ito J, Forman S.J. Iron overload adversely affects outcome of allogeneic hematopoietic cell transplantation. Bone Marrow Transplant.2008; 42: 799-805 http://dx.doi.org/10.1038/bmt.2008.262

[28] Kontoyiannis DP, Chamilos G, Lewis RE, Giralt S, Cortes J, Raad II, *et al*. Increased bone marrow iron stores is an independent risk factor for invasive aspergillosis in patients with high-risk hematologic malignancies and recipients of allogeneic hematopoietic stem cell transplantation. Cancer. 2007; 110: 1303-1306 http://dx.doi.org/10.1002/cncr.22909

[29] Tanaka M, Kanamori H, Matsumoto K,Tachibana T, Numata A, Ohashi K, *et al*. Clinical significance of pretransplant serum ferritin on the outcome of allo-geneic hematopoietic SCT: a prospective cohort study by the Kanto Study Group for Cell Therapy. Bone Marrow Transplantation; 2 March. 2015, 1 - 7; doi: 10.1038/bmt

[30] Douglas B. Kell and Etheresia Pretorius. Serum ferritin is an important inflammatory disease marker, as it is mainly a leakage product from damaged cells. *Metallomics*, 2014,6, 748-773DOI: 10.1039/C3MT00347G http://dx.doi.org/10.1039/c3mt00347g

[31] Boelaert JR, Vandecasteele SJ, Appelberg R, Gordeuk VR. The Effect of the Host's Iron Status on Tuberculosis. The Journal of Infectious Diseases 2007; 195: 1745-53 http://dx.doi.org/10.1086/518040

[32] Bullen JJ, Rogers HJ, Spalding PB, Ward CG. Natural resistance, iron and infection: a challenge forclinical medicine. Journal of medical microbiology. 2006 Mar; 55(Pt 3): 251-8 http://dx.doi.org/10.1099/jmm.0.46386-0

[33] Pieracci FM, Barie PS. Iron and the risk of infection. Surg Infect (Larchmt). 2005; 6 Suppl 1S41-6. http://dx.doi.org/10.1089/sur.2005.6.s1-41

[34] Mencacci A, Cenci E, Johan RB, Bucci P, Mosci P, Cristiana Fe` d'Ostiani, *et al.* Iron Overload Alters Innate and T Helper Cell Responses to Candida albicans in Mice. The Journal of Infectious Diseases 1997; 175: 1467-76 http://dx.doi.org/10.1086/516481

[35] Popova MO, Volkova A, Soulaiman SE, Vavilov V, Bondarenko S, Slesarchuk O, *et al*. Allo-geneic hematopoietic stem cell transplantation in patients with invasive aspergillosis. BMT: Mar. 50[41st meeting of European Society of blood and marrow transplan-tation] BMT.2015/ 27. S-33, 0056

[36] Lucarelli G, Galimberti M, Polchi P, Angelucci E, Baronciani D, Giardini C, *et al*. Bone marrow transplantation in adults with thalassemia: Treatment and long-term follow-up. Ann N Y Acad Sci 2005; 1054: 196-205 http://dx.doi.org/10.1196/annals.1345.024

[37] Liu Q, Sun L, Tan Y, Wang G, Lin X, Cai L. Role of iron deficiency and overload in the pathogenesis of diabetes and diabetic complications. PMID: Curr Med Chem 2009; 16(1): 113-29. PMID: 19149565

[38] Ozkurt S, Acikalin MF, Temiz G, Akay OM, Soydan M. Renal hemosiderosis and rapidly progressive glomerulonephritis asso-ciated with primary hemochromatosis. Renal Failure, June 2014, Vol. 36, No. 5: Pages 814-816 http://dx.doi.org/10.3109/0886022X.2014.892391

[39] Fluit CR, Kunst VA, Drenthe-Schonk AM. Incidence of red cell antibodies after multiple blood transfusion. Transfusion.1990 Jul-Aug; 30(6): 532-5 http://dx.doi.org/10.1046/j.1537-2995.1990.30690333485.x

[40] Guido D'Angelo. Role of hepcidin in the pathophysiology and diagnosis of anemia. Blood Res2013; 48: 10-5 http://dx.doi.org/10.5045/br.2013.48.1.10

[41] Tbahriti HF, Bouchenak M, Mekki K. Inflammatory status in chronic renal failure: The role of homocysteinemia and pro-inflammatory cytokines. World J Nephrol. 2013 May 6; 2(2): 31-7 http://dx.doi.org/10.5527/wjn.v2.i2.31

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