AIM OF THE WORK

The aim of this work is to investigate the association between pre-transplant serum ferritin level and hepatitis C viral (HCV) infection on the outcome of fully matched sibling donor (MSD) peripheral blood stem cell transplantation (PBSCT) in patients with β-thalassemia major (BTM).

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PATIENTS AND METHODS

Patients

Forty-four patients with β-thalassemia major who received allogeneic PBSC transplantation using BU /CY/ATG conditioning regimen after the consent of the Ethical Committee of Nasser institute Hospital during the period from March 2013 to April 2014 were included in this study with a mean follow up period of 9.8 months. The age ranged between 2-18 years with mean age of 5.6 ± 3.587.The male/female ratio was 1:2.38.

Inclusion criteria:

β-thalassemia patients of any risk class according to Pesaro risk stratification who have matched sibling donor. [278]

Exclusion criteria:

The established exclusion criteria of matched sibling peripheral stem cells transplantation were applied, including any patients with abnormal organ function, active infection and concomitant life-threating illness .[279]

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Methods

Pre-transplant evaluation for recipients and donors

All patients and donors will be subjected during pre-transplantation assessment to:

I. Full history taking

II. Complete physical examination

III. Dental examination for patients only

IV. Laboratory investigation including :

- **Hematological investigation :** [280]
  - Complete blood picture & reticulocytic count
  - Bone marrow examination
  - Hemoglobin electrophoresis
  - ABO blood grouping & Rh testing
  - HLA matching [281]
- **Biochemical analysis :** [282]
  - Liver function tests (SGOT, SGPT, bilirubin total & direct and ALP).
  - Kidney function tests (urea, Creatinine and Uric acid).
  - Electrolytes (Na, K, Ca and Mg)
  - Serum ferritin by ELISA
- **Viral profile for :**
  - HIV Ab, HBs Ag, HCV Ab, CMV Ab, Herpes simplex Ab (by ELISA).[283]
  - Testing for toxoplasma IgG, Ig M
  - RT-PCR for HCV RNA in ELISA positive patients. [284]
- **Imaging studies :**
  - Plain X-Ray for patients only
  - Abdomen & pelvic U/S for patients only
  - Echocardiography for all patients and donors (above the age of 40 years)

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CVL insertion

CVLs made of polyethylene or polyvinyl chloride, which are less resistant to adhesion of micro-organisms than are CVCs made of Teflon, silicone or polyurethane are used. CVLs used are a single lumen lines fixed in the operating theatre and under general anesthesia, 4 doses of 3rd generation cephalosporin and vancomycin prophylaxis are given 6 hourly with the first dose given 4-6 hours before the time of line fixation.

A subclavian site is preferred as lower extremity sites are associated with a higher risk of infection (and deep venous thrombosis). Subclavian sites also reduce the risk of infection compared to jugular sites.

Conditioning regimen:

All Patients received oral busulfan (16-20 mg/kg) divided in 4 days from d-11to d-8 ,and epanutin prophylaxis is given from the start of conditioning regimen till 7 days after the last dose of busulfan .Intravenous cyclophosphamide (120 mg/kg) divided in to 4 days starting from d-5 to d-2 with prophylactic urometoxane at a dose equal 120 % of the cyclophosphamide dose divided in to 6 doses with the first dose 4 hours before the first dose of CY the every 6 hours till 16 hours after the dose of CY . Intravenous ATG (110 mg/kg) divide into 10 doses starting from d-5 till d-1, infusion of stem cells take place at day zero, then ATG post infusion of stem cells from d+1 till d+5. Antiemetics in the form of ondansetron was given to all patient through out the conditioning regimen starting from d-11 till the end of conditioning.

GVHD prophylaxis

All patients received cyclosporine A (CSA) 3 mg/kg/day i.v. by continuous infusion from day -1, then trough level is estimated 3 times per week keeping the level around 200 mcg/L. In addition to methyl prednisone 2 mg/kg i.v. on day -7 till day +4 then gradual withdrawal by 50 % over 3 weeks . Following engraftment, when the patient became able to eat, CSA is converted to oral administration and tough level is performed weekly keeping the level around 200 mcg/L to day 180-300 and tapered thereafter.

Donor PBSCT mobilization

All donors received GCSF 10 mg/kg/day divide in to two doses every 12 hours till the apheresis day where the donor receive the total dose as a single early morning dose GCSF is given for 5 days before peripheral stem cells collection.

Apheresis

Donors with good veins were subjected to apheresis using their peripheral veins, after receiving the full dose of GCSF 2-4 hours before apheresis, while those with bad veins were subjected to double lumen CVL before apheresis. CD34+ cells yield is counted using flow cytometry, with a minimum cutoff of cells equal to 3×10^{6} per Kg recipient weight.

Stem cell infusion

The collected stem cells are given at day zero directly in the CVL after giving the patient pre-medication including antihistaminic, hydrocortisone and antipyretic, then the patient is monitored during the whole infusion procedure by measuring blood pressure, pulse, temperature and central venous pressure every 15 minutes till the end of infusion.

Peritransplant care

All blood products are irradiated and filtered. Red blood cell and platelet transfusions are given to maintain a hemoglobin concentration of >80 g/l and platelet count >20 ´ 10^9/l ,respectively. Infection prophylaxis during the peri-transplant period consists of acyclovir 1500 mg/m^2 / d. every 8 h. Trimethoprim-sulfamethoxazole 5 mg/ kg/d – bid (till d-2), and when engraftment occurs all patients will received twice-weekly trimethoprim-sulfamethoxazole, and antifungal prophylaxis fluconazole 6-12 mg /kg. All patients received broad-spectrum antibiotics for neutropenic fever and fever workup including blood culture from peripheral veins and CVL , CRP is done . 3rd generation cephalosporin plus amikacin are administered after the first fever spike then if the fever is not controlled within 5 days upgrading to carbapenem and amphotericin B or voriconazole takes place, and C/T sino-pulmonary and upper cuts abdomen is performed at that time. Patients who experienced acute GVHD are managed with the addition of 2 mg/kg methylpridnesolone in to divided doses till at least one week after total control of all signs and symptoms of GVHD ,then tapered slowly over a minimum of 3-4 weeks ,and for patients observed in the outpatient clinic who had any signs or symptoms of chronic GVHD are treated with methylprednisolone at a dose of 1 mg/kg till at least 2-3 months from the complete disappearance of all signs and symptoms of chronic GVHD .SOS was suspected in our patients after developing weight gain , jaundice and tender hepatomegaly ,then U/S abdomen and hepatic duplex are requested for the possibility of ascites ,hepatomegaly reversed blood flow or any other abnormality .Then when VOD is suspected or diagnosed patients are managed by fluid restriction ,close monitoring of fluid balance , diuretics and monitoring their body weight .

Assessment of engraftment and response

Neutrophil recovery is defined as first 3 consecutive days with absolute neutrophil count $\geq 0.5 \times 10^9$/L and platelets, $\geq 20 \times 10^9$/L, unsupported for 7 days. Acute grade II-IV GVHD and chronic GVHD are graded using standard criteria. Disease free survival (DFS) is defined as survival without graft failure.

Data for monthly follow up will include

- Thorough clinical examination.
- Complete Blood Picture.
- Liver and kidney function tests.
- Cyclosporine A level

Statistical Analysis

- Input data were processed using computer-based software (SPSS version 20 interface).
- Data are expressed as mean ± standard deviation (± SD).
- The ordinary data were compared with the use of student's t-tests: paired t-test was applied for comparing the mean of variables inside the group and unpaired t-test was applied for comparing the mean of variables between the studied subjects.
- Non ordinary data were compared with the use of Mann-Whitney U-test between the studied subjects.
- Chi-square test was used to compare the data that expressed as the percentage changes.
- For all statistical analysis a significant P value was considered when P value was less than 0.05.
- r will always be between -1.0 and +1.0. if the correlation is negative, we have a negative relationship; if it's positive, the relationship is positive.

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RESULTS

Patients' characteristics

Table (13) summarizes patients' characteristics of the whole group of patients as regard age, gender, pre-transplant serum ferritin levels, disease risk class at the time of BMT and viral markers.

The age ranged between 2-18 years with mean age of 5.6 ± 3.587.The male/female ratio was 1:2.38. Thirty-six Patients were transplanted at risk class 2 and eight patients at risk class 3. Six patients had HCV Ab positive and 11 were HCV PCR positive, regarding serum ferritin 18 patients had serum ferritin > 2000 ng/dl while patients with ferritin below 2000 ng/dl were 26 cases.

Table 13: Patients' characteristics of the 44 β-thalassemia patients included in the study:

Variable	**Number (%)**
Age (years)	Mean ± *SD* = 5.6 ± 3.587(range 2-18)
Gender	
Male	31 (70.5%)
Female	13 (29.5%)
Risk class at BMT (Pesaro)	
Risk-class 2	36(81.8%)
Risk-class 3	8 (18.2%)
Virology	
HCV Ab positive	6 (13.6%)
HCV PCR positive	11(25%)
Pre-transplant Serum ferritin level	26(59.1%)
≤ 2000 ng/dl	18(40.9%)
> 2000 ng/dl	

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Transplant data

Relation between CD34 & Neutrophilic and platelets engraftment (Table 14)

- The total CD34+ cells/kg patients' weight ranged from 3.2 x 10^6 to 36.5x10^6 with a mean of 14.09 ± 8.725 x 10^6 cells/kg patients' weight.
- The mean numbers of days till TLC reached ≥ 1000/µl (Neutrophilic engraftment) was 21days in those patients who received CD 34+ ≤ 7 x 10^6 cells/kg patient's weight, while the mean numbers of days till TLC reached ≥ 1000/µl was 19.0 in those patients who received CD 34+ > 7 x 10^6 cells/kg patients' weight and difference was statistically non-significant between the two groups of patients

  (p= 0.404)
- The mean numbers of days till platelets reached 25,000/µl (platelets engraftment) was 42.3 in those patients who received CD 34+ ≤ 7 x 10^6 cells/kg patients' weight while the mean numbers of days till platelets reached 25,000/µl was 31.4 in those patients who received CD 34 > 7x10^6 cells/kg patients' weight and difference was statistically non-significant between the two groups of patients

  (p= 0.103)
- In general, there was non-significant relation between CD 34+cell dose and engraftment. (Table23).

Table 14: Relation between CD34 & Neutrophilic and platelets engraftment

CD+34 Dose		TLC ≥ 1000/µl	PLT ≥ 25,000/µl
≤ 7 x 10^6 /kg patients' wt	n=	14	14
	Mean ±SD (days)	21.4 ±8.0	42.3 ±19.9
> 7 x 10^6 kg patients' wt	n=	30	30
	Mean±SD (days)	19.0 ± 7.4	31.4 ± 20.2
P value		0.404	0.103

Graft Vs host disease (GVHD)

1. Relation between GVHD and CD34: (Table 15 and Figure 8)

- **Acute graft versus host disease (AGVHD)**

AGVHD occurred in 7 out of the 44 (15.9%) BTM patients included in this study. One/14 (7.14%) of patients who received a CD34+ stem cell dose ≤ 7 x 10^6 cells/kg patients' weight developed AGVHD, compared to 6/30 (20%) of patients who received a CD34+ stem cell dose > 7 x 10^6 cells/kg patients' weight. The difference in the incidences of AGVHD between both groups was statistically non-significant (p=0.288).

- **Chronic graft versus host disease (CGVHD)**

CGVHD occurred in 4 out of the 44 (9.1%) BTM patients included in this study. 1/14 (7.14%) of patients who received a CD34+ stem cell dose ≤ 7 x 10^6 cells/kg patient's weight group developed CGVHD compared to 3/30 (10 %) of patients who received a CD34+ stem cell dose > 7 x 10^6 cells/kg patient's weight. The difference in the incidences of CGVHD between both groups was statistically non-significant (P= 0.765). (Figure 8)

Table 15: Correlation between GVHD (acute and chronic) and number of CD34+ cells:

CD34Dose		AGvHD	CGVHD
≤ 7 x 10^6 cells patients' weight n=14	Mean±SD	1.0714±.26726	1.0714±.26726
	n= Cases affected	1	1
	%	7.14%	7.14%
> 7 x 10^6 cells/kg patients' weight n=30	Mean±SD	1.20±.40684	1.10.± .30513
	n= cases affected	6	3
	%	20%	10%
r =		0.283	0.379
P value		0.288	0.765

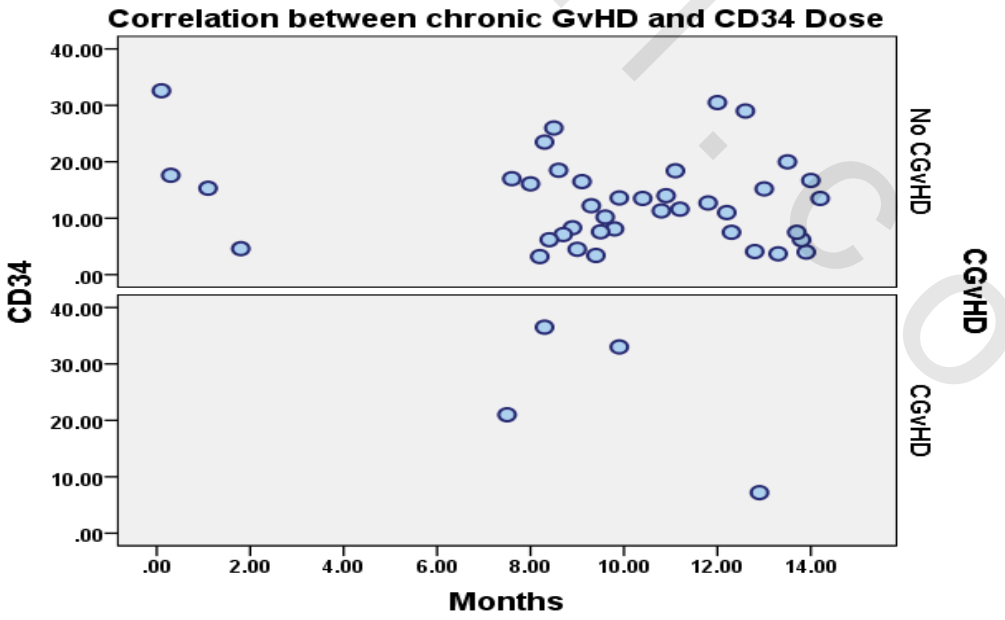

Fig. 8: Correlation between Chronic GVHD and CD34+ cells dose

2. Relation between GVHD and risk class at BTM

Regarding disease status, 13.9% and 5.6% of patients transplanted in risk class 2 suffered from acute and chronic GVHD respectively, while the incidences of both AGVHD and CGVHD in risk class 3 patients were 25%. The differences in incidences of AGVHD and CGVHD between the two risk groups were statistically non-significant (p= 0.449 and 0.087 respectively) (table 16).

Table 16: Relation between GVHD (acute and chronic) and pesaro risk class:

Risk Class (Pesaro)		AGvHD	CGVHD
Class 2 n=36	Mean±SD	1.14±.35074	1.05 ± .23231
	%	13.9%	5.5%
Class 3 n=8	Mean ± SD	1.25±.46291	1.25±.46291
	%	25%	25%
P value		0.449	0.087

Veno-occlusive disease

Six out of 44 (13.6%) BTM patients included in this study developed hepatic veno-occlusive disease based on Seattle Criteria. VOD occurred in 2/33 (6.1%) patients in HCV negative group versus 4/11 (36.4%) patients in HCV positive group. The difference in the incidences of VOD between both groups was statistically significant (p=0.010*) (table 17).

Table 17: Relation between VOD and HCV

VOD				
HCV status	Mean±SD	n=	N0.of VOD	% of Total No.
HCV Negative	1.0606±.24231	33	2	6.1%
HCV positive	1.3636±.50452	11	4	36.4%
Relation between VOD & HCV status		*P value* =0.010*		

* P value is considered significant when it is less than 0.05

Relation between VOD and risk class at BMT

VOD was reported in 6 cases (13.6%) in the whole group of patients. Patients transplanted in risk class 2 suffered from VOD in 3/36 (8.3%) of patients compared to 3/8 (37.5%) patients transplanted in risk class 3. The difference in the incidences of VOD between both groups was statistically significant (p=0.030*) (table 18).

Table 18: Relation between VOD and risk class at BMT

VOD		
Risk Class	**No. of VOD**	**% of Total No.**
Class 2 n= 36	3	8.3%
Class 3 n=8	3	37.5%
		P value
VOD * Risk Class Between Groups (Combined)		0.030*

* P value is considered significant when it is less than 0.05

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Relations between OS and DFS with Serum Ferritin, HCV-status and Pesaro risk class

Overall survival (OS) and disease free survival (DFS) was 86.4 % and 77% respectively, in all patients included in this study, while subgroup analysis of patients with serum ferritin below 2000 were 96% and 88% for OS and DFS respectively, and for those with levels above 2000 were 72% and 61% for OS and DFS respectively (p value 0.02) . Cases with HCV PCR positivity had OS and DFS of 73 % and 64% respectively, while those with negative HCV PCR had OS and DFS of 90 % and 82% respectively (p value 0.13). Regarding OS and DFS in different risk class groups, it was reported 91% and 83% for OS and DFS, respectively in risk class 2 patient, while for cases with risk class 3 the OS and DFS was 62.5% and 56% respectively (p value 0.04*) .

Table 19: Relations between OS and DFS and Serum Ferritin, HCV-status and Pesaro risk class:

	n =	**Overall Survival (OS)**	**P-value**	**Disease Free Survival (DFS)**	**P-value**
All patients	44	86.4%		77%	
	Serum Ferritin:				
≤ 2000 ng/dl	26	96%	0. 02*	88%	0.02*
> 2000 ng/dl	18	72%		61%	
	HCV-status (HCV-PCR):				
HCV-PCR negative	33	90%	0. 09	82%	0.13
HCV-PCR positive	11	73%		64%	
	Risk groups:				
Class II	36	91%	0. 02*	83%	0.04*
Class III	8	62.5%		56%	

* P value is considered significant when it is less than 0.05

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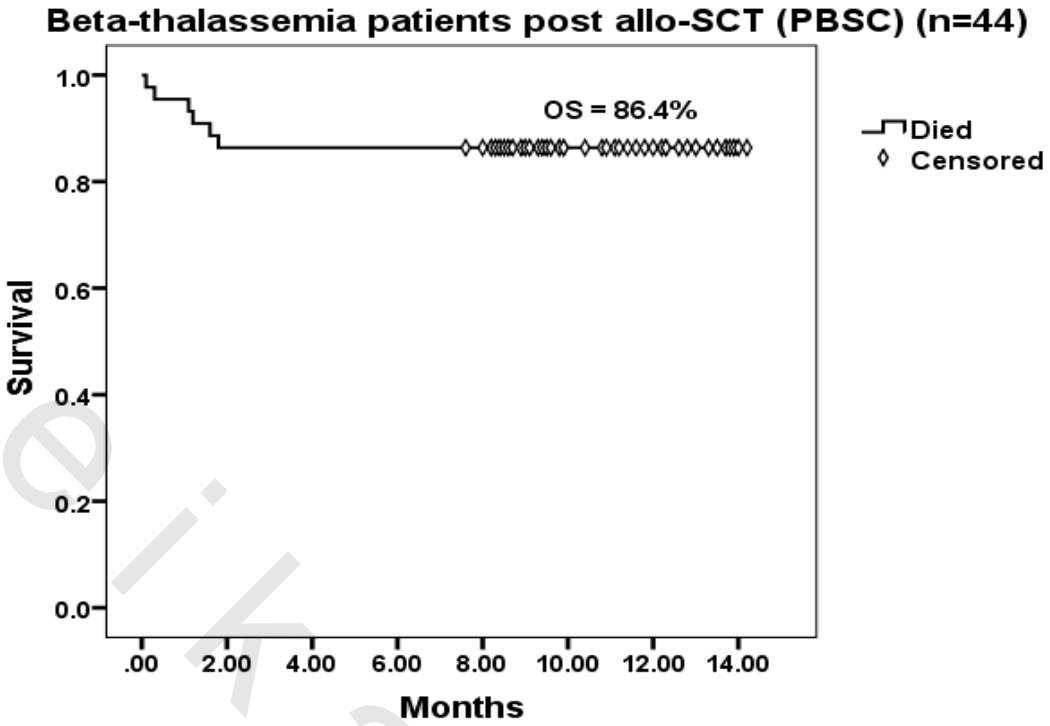

Fig. 9: Overall survival of the 44 β-thalassemia cases post allo- SCT (PBSC)

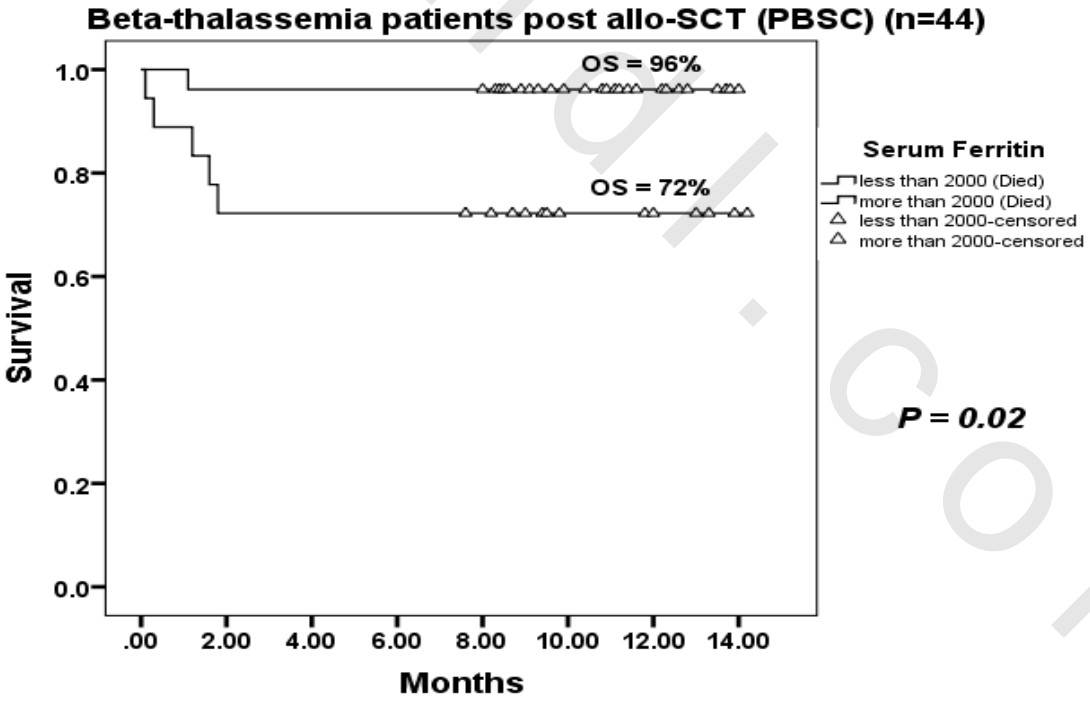

Fig. 10: Relation between serum ferritin level and OS

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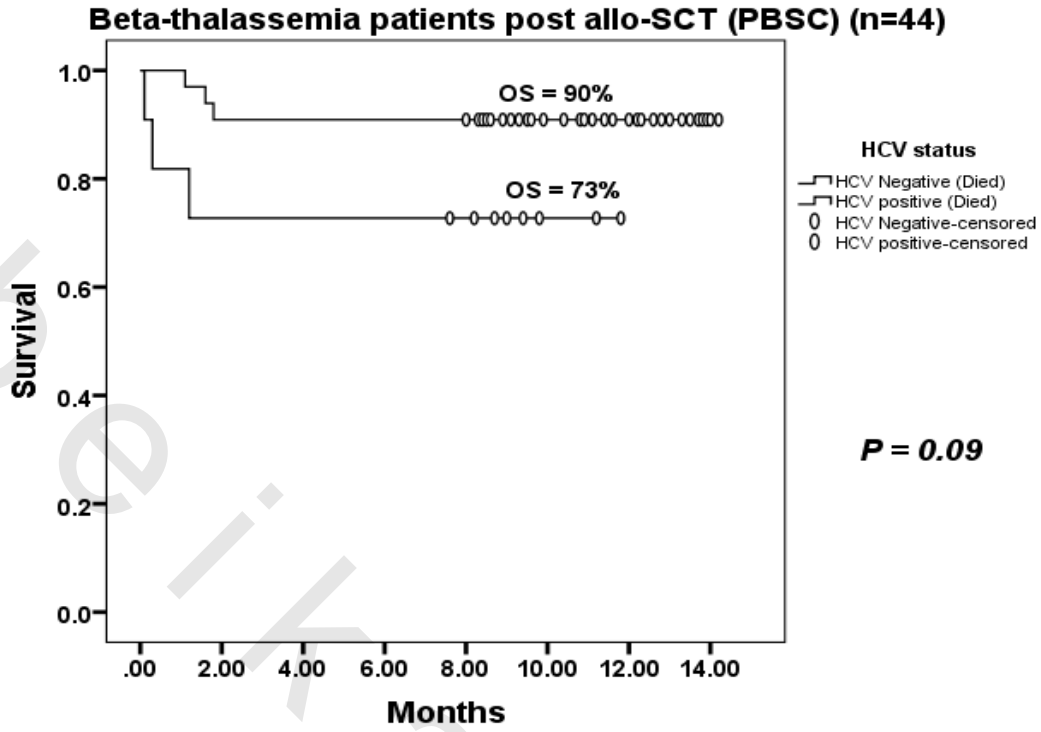

Fig. 11: Relation between HCV status and OS

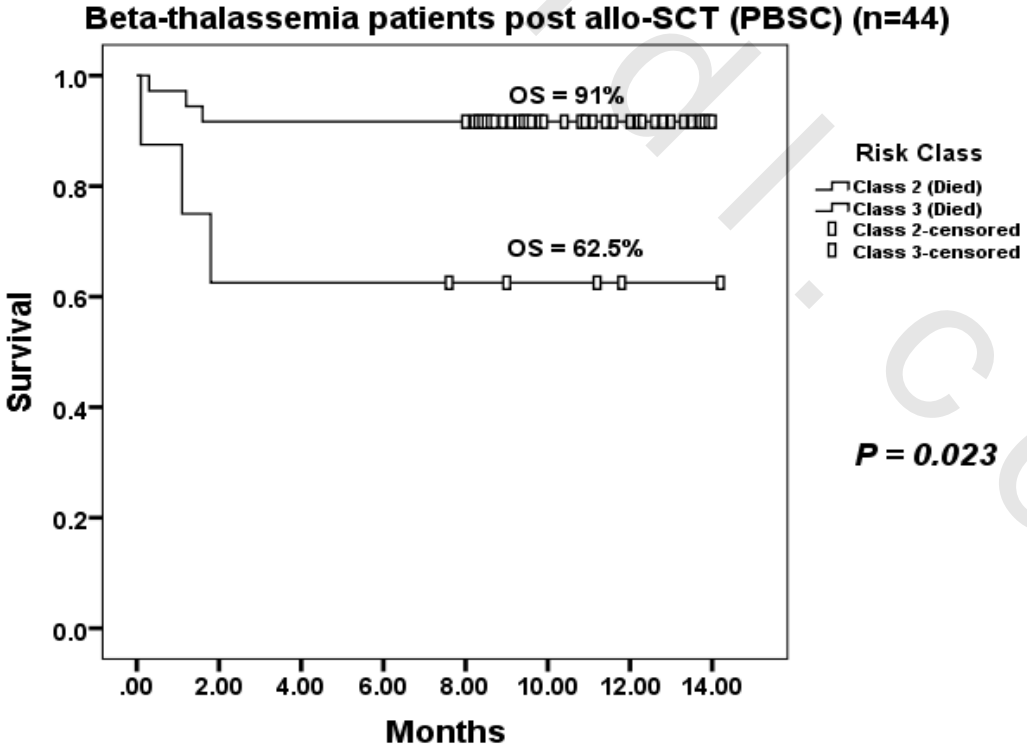

Fig. 12: Relation between risk class and OS

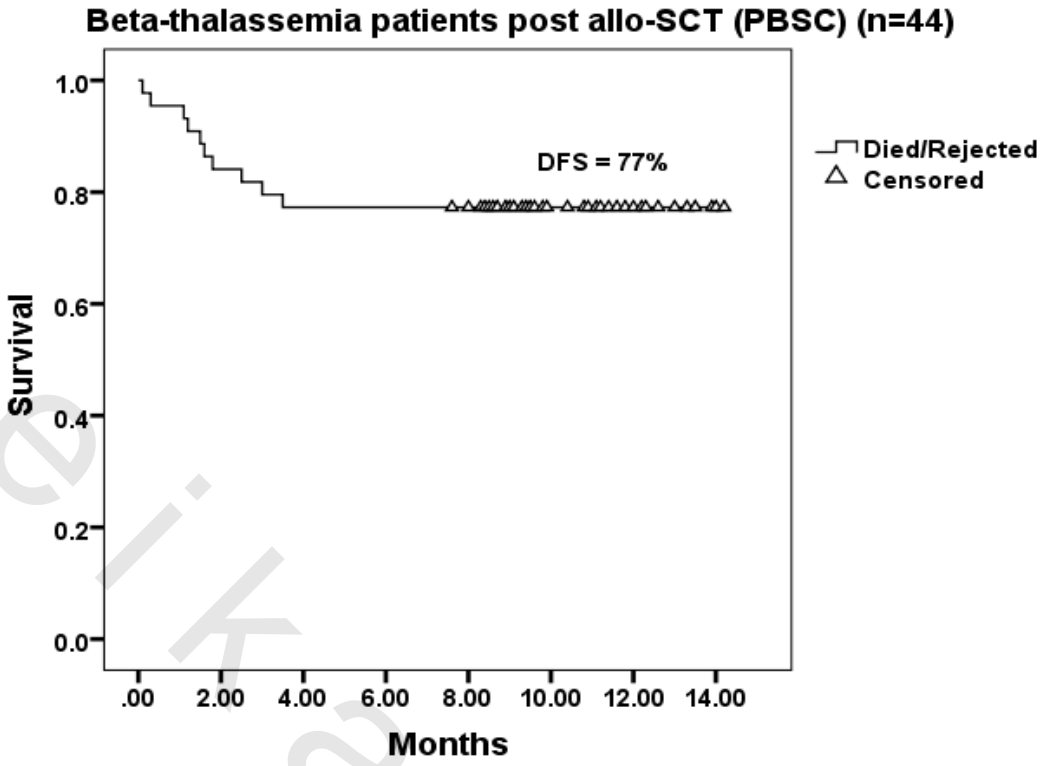

Fig. 13: DFS of the 44 β-thalassemia cases post allo- SCT (PBSC)

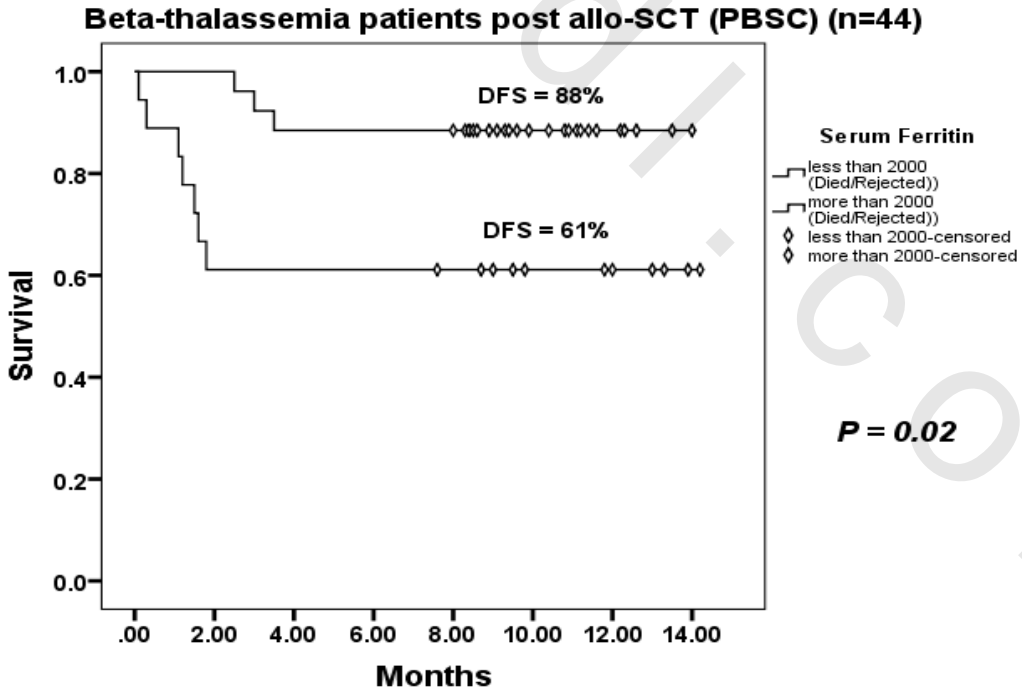

Fig. 14: Relation between DFS and pre-transplant serum ferritin level

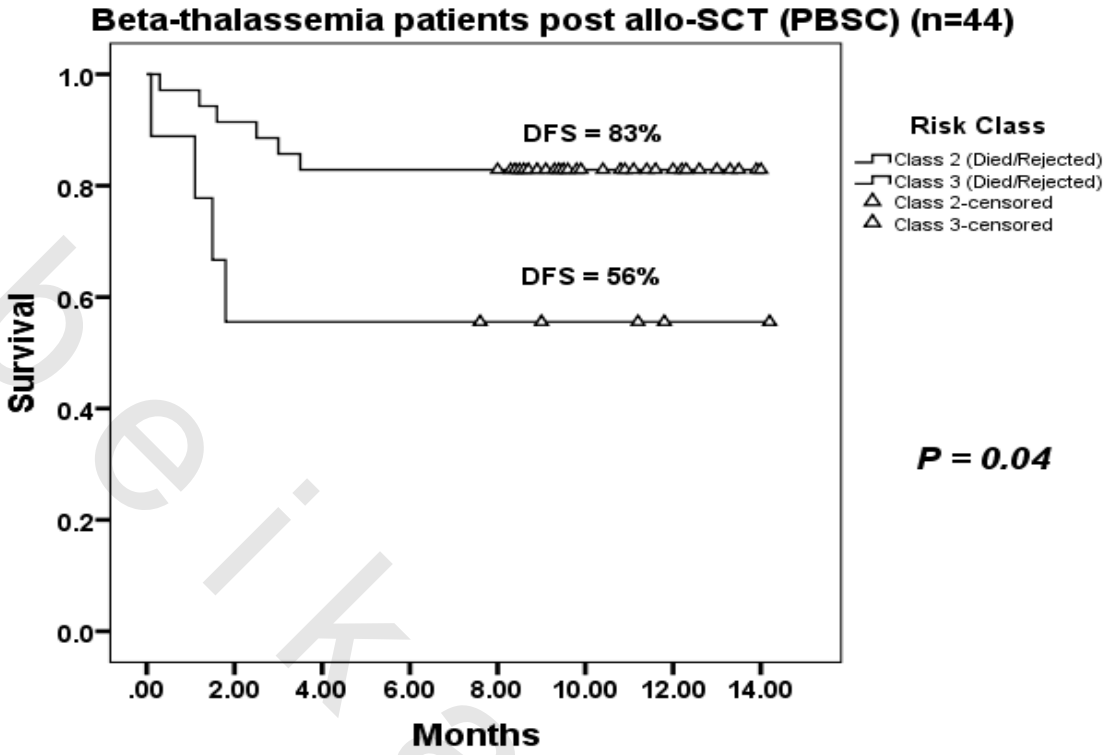

Fig. 15: Relation between risk class and DFS

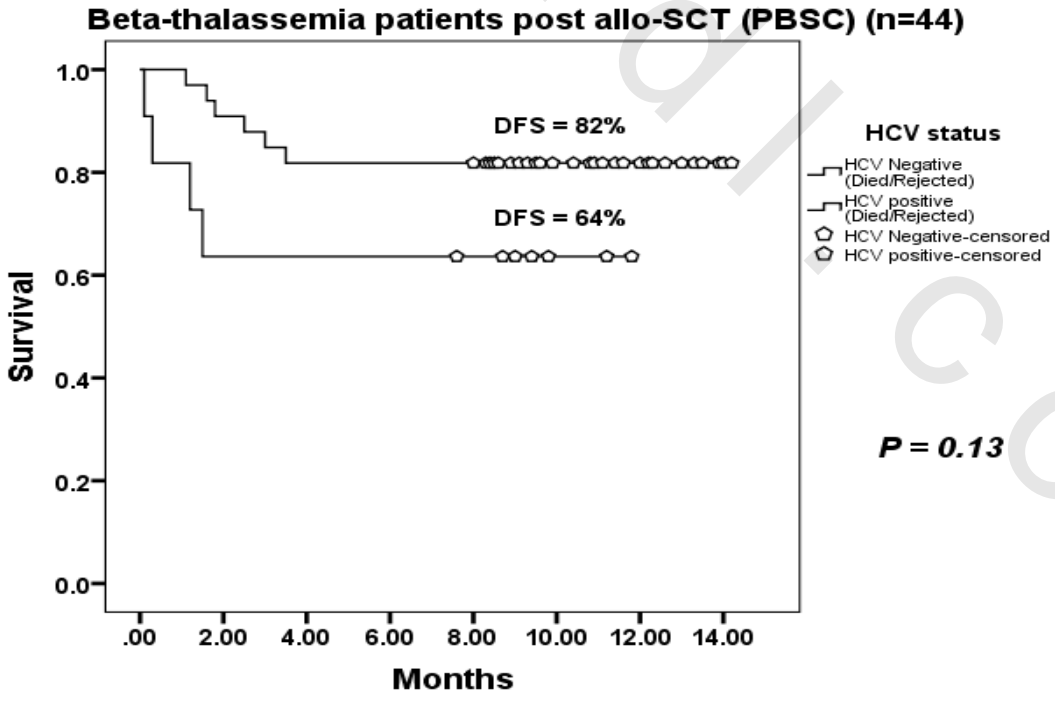

Fig. 16: Relation between HCV status and DFS