ARTICLE

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Survival and late effects of hematopoietic cell transplantation in patients with thalassemia major

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In this retrospective study, we evaluated long-term survival and late effects in 137 patients affected by thalassemia major (TM) who received an allogeneic hematopoietic cell transplantation (HCT). Median age at HCT was 10.1 years. After a median follow-up of 30 years, 114 (83.2%) patients are living and 108 (78.8%) are cured. The cumulative incidence of nonrelapse mortality and thalassemia recurrence was 9.5% at 1 year and 10.2% at 39 years respectively. The 39-years cumulative incidence of overall survival and disease-free survival were 81.4% and 74.5%. One hundred twenty-three patients who survived more than 2 years after HCT were evaluated for late effects concerning hematological disorders, iron burden, growth, obesity, diabetes mellitus, thyroid and gonadal function, eye, heart, liver, lung, kidney, gastrointestinal, neurologic and psychiatric system, osteoarticular system, secondary solid cancer (SSC), performance status, and Covid-19 infection. Fertility was preserved in 21 males whose partners delivered 34 neonates and 25 females who delivered 26 neonates. Fifteen cases of SSC were diagnosed for a 39-year cumulative incidence of 16.4%. HCT represents a definitive cure for the majority of TM patients at the price, however, of a non-negligible early and late mortality which in the long run affects survival and disease-free survival.

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INTRODUCTION

ß-Thalassemia major (TM) is an inherited hemoglobinopathy associated with defective synthesis of ß-globin subunits, leading to ineffective erythropoiesis and massive hemolysis. In the last 4 decades, the prognosis for affected individuals has improved due to advances both in red cell transfusion management and in the prevention and treatment of complications due to iron overload. Most of well-treated patients survive over the fifth decade of life [1]. A new medical therapy based on the use of luspatercept (formerly ACE-536) has been recently described [2, 3]. Moreover, hematopoietic stem-cell gene therapy is now expanding with encouraging results [4–7]. In this scenario, since its first application in 1981, allogeneic hematopoietic cell transplantation (HCT) has greatly expanded to many countries around the world with continuously improving results in terms of survival and decreased transplant-related mortality [8, 9]. Current experience world-wide with HCT is that about 90% of patients now survive transplant with disease-free survival (DFS) being around 70–80% [10]. Similar results made it possible to consider HCT as standard clinical practice in TM patients. Age at transplantation and donor type predict overall survival (OS), disease-free survival (DFS) and graft rejection [11]. An international expert panel reported on consensus-based recommendations about indications for HCT and transplant management [12]. Very few studies have described very late follow-up and long-term effects after HCT [13–16]. We have previously reported on pregnancy outcome and incidence of secondary solid cancer (SSC) in TM patients after a long follow-up time (24 years) following HCT [17, 18]. Therefore, considering that the median follow-up time of survivors reached 30 years, we have extended our study with the aim to explore both OS and DFS and the occurrence of post-transplant late effects affecting the main organs and systems.

METHODS

Study design

This retrospective non-interventional study was approved by the local institutional review board. Informed consent for HCT was obtained from all patients and donors or their legal guardians in accordance with the Declaration of Helsinki. OS and DFS in all patients who received HCT were the study's primary endpoint. Secondary endpoint included the evaluation of long-term late effects in patients surviving at least 2 years after transplantation. All patients were observed longitudinally until death or last follow-up visit.

Study cohort

Our study included 137 consecutive caucasian patients affected by TM who were transplanted between May 1983 and February 2018.

Transplant procedure and follow-up plan

Details about the transplant protocol have been described elsewhere [19]. The histological grading and staging of chronic hepatitis were made following the classification of Ishak [20]. Acute GvHD (aGvHD) was

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diagnosed according to Glucksberg's criteria [21], and chronic GVHD (cGvHD) according to the modified Seattle criteria (for categorization of cGVHD as clinical limited or clinical extensive) [22]. Chimerism was done on genomic DNA extracted either from bone marrow cells or peripheral whole blood by short tandem repeats (STR) technique according to standard methods [23]. Hepatitis C virus (HCV) RNA level was measured with a real-time polymerase chain reaction–based assay. The liver stiffness measurement (LSM) for the assessment of post-transplant liver fibrosis was made only in patients with detectable HCV RNA by shear wave transient elastography by using Fibroscan Philips Epiq7 G. The METAVIR liver fibrosis (MLF) stage was determined according to transient elastography and/or liver biopsy [24]. Coronavirus disease-2019 (Covid-19) infection was evaluated by evidence of SARS-CoV-2 on reverse transcriptase-polymerase chain reaction testing performed on nasopharyngeal swab specimens.

Data collection and post-transplant clinical observation

All surviving patients were asked to carry out a clinical screening which included the most appropriate haematological, clinical chemistry and instrumental tests either at the Pescara transplant center or at their trusted medical center in their city of residence within 6 months prior to the final censoring date (March 20, 2022). The physician who took care of the patient was asked to complete an anamnestic questionnaire in order to evaluate the appearance of late effects of the main organs and systems: hematological disorders, iron burden, growth, obesity, diabetes mellitus, thyroid and gonadal function, eye, heart, liver, lung, kidney, gastrointestinal, neurologic and psychiatric system, osteoarticular system, secondary solid cancer (SSC), performance status, and Covid-19 infection and vaccination. The functional status of the patient at last follow-up visit was made on the basis of the Eastern Cooperative Oncology Group Performance Status (ECOG PS). A partially modified SF-36 questionnaire was administered to all living patients at the time of the last follow-up visit to evaluate the perception of the health-related quality of life (HRQoL). Finally, in patients aged less than 10 height and weight were evaluated at time of transplant and at 20 years after HCT. Data were plotted as z-scores to eliminate variability of age and gender.

Outcome definitions

Non relapse mortality (NRM) was defined as death from any cause except original disease. Thalassemia recurrence (TR) was defined as return to the pretransplant pattern of ß-globin chain synthesis and red cell transfusion requirement. OS was defined as time to death from any cause. DFS was defined as survival without TR or death.

Statistical analysis

A descriptive analysis of all variables was performed including mean, median, standard deviation, range, minimum and maximum value for continuous variables, absolute and relative frequencies for categorical variables. Using parametric and nonparametric statistical procedures, the possible interdependence between 2 or more variables was evaluated and a P value of 0.05 was considered significant.

Taking into consideration the competing risks, the probabilities of NRM, TR, secondary solid cancer (SSC), aGvHD and cGvHD were studied by fitting cumulative incidence function. The probabilities of OS and DFS were calculated with the method of Kaplan–Meyer and the curves of various subgroups were compared using the log-rank test. The estimated probabilities were summarized along with 95% Confidence Interval (95% CI). Statistical analyses were performed with the use of R Statistical Software (version 3.3.3; R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Table 1 shows characteristics of patients, donors and transplantation. The patient's median age at HCT was 10.1 years (range, 1–29).

Table 1. Patient, Donor and Transplant Characteristics.

	N.	%
Patients	137	
Gender, male/female	67/70	49/51
Median age, years (range)	10.1 (1–29)	
<10 years	65	47
10–17 years	46	34
≥18 years	26	19
RBC transfusions before HCT, median (range)	125 (2–900)	
Median ferritin, ng/mL (range)	1385 (258–8962)	
Left ventricular ejection fraction, % median (range)	70 (47–75)	
Splenectomy before transplant	30	22
Previous HBV infection	38	28
Previous HCV infection	77	56
Liver biopsy before HCT	88	64
Mild liver fibrosis	44	50
Moderate liver fibrosis	26	29.5
Severe liver fibrosis	16	18.2
Definitive cirrhosis	2	2.3
Donors	137	
Gender, male/female	77/60	56/44
Median age, years (range)	10 (1–48)	
N. with beta-thalassemia trait	89	65
Relationship and HLA compatibility		
HLA genotipically identical sibling	127	93
HLA phenotipically identical parent	6	4
Unrelated donor (10/10)	4	3
Transplant procedure		
Conditioning regimen		
BU 13–14 mg/Kg p.os. + CY 200 mg/Kg	121	88
BU 12.8 mg/Kg i.v. + TH 8 mg/Kg + FLU 160 mg/m^2	12	9
TREO 42 g/m^2 + TH 8 mg/Kg + FLU 160 mg/m^2	4	3
GvHD prophylaxis		
CSA	37	27
CSA + short course MTX	100	83
Stem cell source		
BM	135	98
PBSC	1	1
CB (identical sibling)	1	1

RBC red blood cell, *HCT* hematopoietic cell transplantation, *HBV* hepatitis B virus, *HCV* hepatitis C virus, *BU* busulfan, *CY* cyclophosphamide, *TH* thiotepa, *FLU* fludarabine, *TREO* treosulfan, *GvHD* graft-versus-host disease, *CSA* cyclosporine A, *MTX* methotrexate, *BM* bone marrow, *PBSC* peripheral blood stem cells, *CB* cord blood.

Survival

Post-transplant outcome is shown in Table 2. Thirteen patients (9.5%) died for transplant-related causes between day 12 and 212 (median 42) post-transplant. One patient died for accidental cause at 2 years after transplant. One patient died for late transplant-related cause (cGvHD-related multiorgan failure) 12.9 years after transplantation. The cumulative incidence of NRM was 9.5% (95% CI 4.6–14.4%) and 10.3% (95% CI 5.2–15.4%) at 1 and 15 years after transplant, respectively (Fig. 1a). One hundred twenty-three (89.8%) patients (60 males) survived more than 2 years following HCT and entered the study for evaluation of late effects. Their median follow-up was 30 years (range, 4 to 39 years). During

Table 2. Post-transplant outcome.

	N.	%	Outcome or causes of death
Total patients	137	100	
Full engraftment	133	97.1	
Primary graft failure	4	2.9	Second HCT in 4
Secondary graft failure	2	1.5	Second HCT in 2
Thalassemia recurrence	12	8.8	Second HCT in 4
Early death due to transplant-related causes (<1 year)	13	9.5	Heart failure 3, infection 3, aGvHD 3, encephalopathy 2, VOD 2
Late death due to transplant-related cause (>1 year)	1	0.7	cGvHD-related MOF 1 (at 12.9 years after transplant)
Death due to other causes	1	0.7	Accidental (2 years)
Patients who survived >2 years and were evaluated for late effects	123	89.8	Males 60, females 63
Late deaths (>2 years)	8	5.8	Secondary cancer 4, septic shock 1, heart failure 1, JCV-PML 1, second HCT 1
Second HCT	10	7.3	Dead 2, living and cured 6, living with TR 2
Total dead	23	16.8	
Total living	114	83.2	
• Cured	108	78.8	
• Living with TR and red cell transfusions	6	4.4	
Chimerism at last follow-up visit in 108 cured patients			
• Full chimerism with 99–100% donor cells	103	95.4	
• Mixed chimerism with <95% donor cells	5	4.6	

HCT hemopoietic cell transplantation, *aGvHD* acute graft-versus-host disease, *VOD* veno-occlusive disease of the liver, *cGvHD* chronic graft-versus-host disease, *MOF* multiorgan failure, *JCV-PML* JC virus-progressive multifocal leukoencephalopathy, *TR* thalassemia recurrence.

follow-up 8 patients died for nontransplant-related causes between 4.5 and 31 years after transplantation (median 12.6 years). At the final censoring date, 114 (83.2%) patients (56 males) were living. Of them, 108 were cured and 6 received regular erythrocyte transfusion support and iron chelation therapy following TR. The median age of living patients at time of final censoring date was 43.8 years (range, 14.4 to 56 years). Of 108 patients who were cured, full chimerism with 99–100% donor cells was documented in 103 (95.4%) patients and mixed chimerism (MC) was present in 5 (4.6%) ranging from 20% to 94% donor cells (median 80%) with untransfused hemoglobin levels ranging from 8.8 to 15.6 g/dL (median 10.9 g/dL).

TR occurred in 12 (8.7%) patients (6 males). In 9 (75%) of 12 patients TR was diagnosed in the first year following transplantation. In 3 cases TR occurred at 8.6, 27.4 and 31.2 years after HCT respectively. The cumulative incidence of TR was 10.2% (95% CI 4.3–16.1%) at 39 years after transplantation (Fig. 1b). Of 12 patients with TR, 6 patients are currently alive and are receiving regular erythrocyte transfusion support and iron chelation therapy. Three patients underwent successful second transplant from the same donor and are living and cured. Three patients died for hearth failure ($n = 1$), secondary cancer ($n = 1$), and aGvHD following second transplant ($n = 1$). Overall, the 39-years cumulative incidence of OS and DFS was 81.4% (95% CI 74.5–88.9%) and 74.5% (95% CI 67.0–83.0%) (Fig. 1c). No statistically significant difference in OS and DFS was found comparing sex and age of recipients and time of transplant (Table 3).

Graft-versus-host disease

The cumulative incidence of both aGvHD and cGvHD was 36.5% (95% CI 28.5–44.5%) and 13.1% (95% CI 7.4–18.8%) at 100 days and at 3 years after transplant respectively. At last follow-up visit, no patient showed active cGVHD and none was receiving immunosuppressive therapy. However, 3 patients showed severe ocular damage as a consequence of cGvHD-related sicca syndrome which in 2 cases produced unilateral amaurosis.

Late effects

Table 4 provides the last hematological counts, the hematologic disorders and the iron burden as well as the prevalence of impaired organ function at last follow-up visit.

Median hemoglobin level was 12.6 g/dL. Among 123 surviving patients, we observed 3 (2.4%) cases of idiopathic thrombocytopenic purpura (ITP). The first case of ITP was diagnosed in a 10-yr old female at 6 years after HCT and was definitively resolved with steroid therapy. The second case of ITP occurred in a 37-yr old male at 28 years after HCT. The disease has been resistant to both steroid therapy and splenectomy and showed complete response to romiplostim therapy. The third case of ITP was occasionally diagnosed at last follow-up visit in a 40-yr old male at 33 years after HCT. The patient is currently under observation with a platelet count of 39×10^9/L. In patients aged less than 10, median weight and height z score at transplant were lower if compared with the normal population, −0.33 and −0.62 respectively. When evaluated at 20 years after transplant, median z-score for weight was normal (+0.04), whereas median z-score for height was not modified (−0.58). Most living patients (90.4%) showed an ECOG PS of zero. We were unable to analyze HRQoL because only 20% of patients completed the questionnaire. Twenty-nine (25.4%) patients had evidence of Covid-19 infection. Of them, 23 (79.3%) had received at least 2 doses of the vaccine and 6 were not vaccinated. In all cases, Covid-19 infection presented with few or no symptoms in all infected patients and none required hospitalization or progressed to critical disease and death.

At time of HCT, 77 patients (56%) showed anti-HCV antibodies. Among them, HCV RNA was detected post-transplant in 33 patients with a median viral load of 1.5×10^6 copies/ml. They were treated either with interferon-alpha (IFN) between 1993 and 2009 or with directly acting agents (DAAs) between 2015 and 2021. Results of treatment of HCV-related chronic hepatitis are shown in Table 5. All 33 patients obtained persistent clearance of HCV RNA from blood.

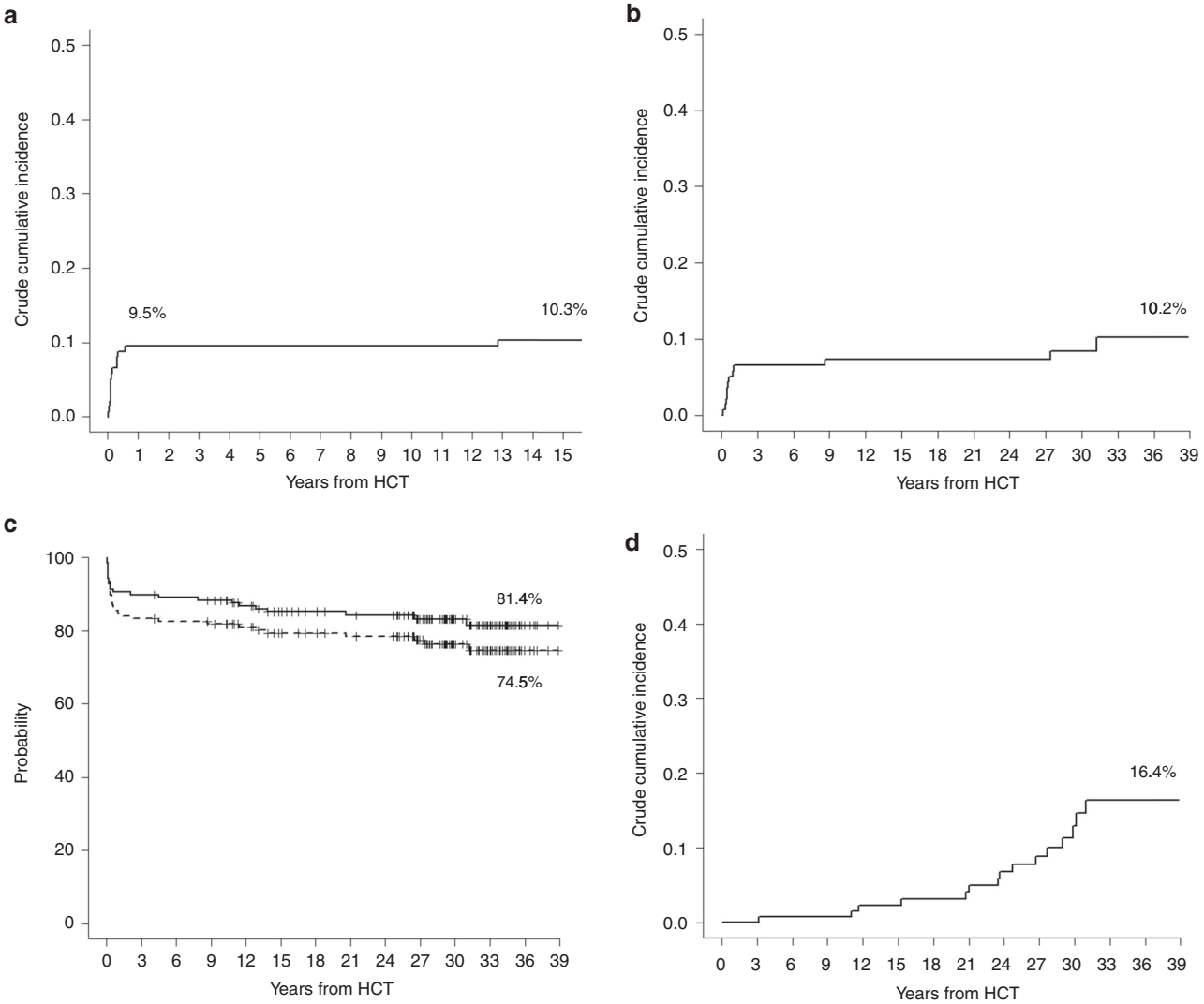

Fig. 1 Outcomes of allogeneic cell transplantation in all patients affected by thalassemia major. a Cumulative incidence of nonrelapse mortality. **b** Cumulative incidence of thalassemia recurrence. **c** Probability of overall survival (continuous line) and disease-free survival (dotted line). **d** Cumulative incidence of secondary solid cancer.

Table 3. Overall survival and disease-free survival according to gender and age of recipients and time of transplant.

	N.	Median time of OS, years	OS% (95% CI)	*P* value	Median time of DFS, years	DFS% (95% CI)	*P* value
Gender							
Male	67	>38	82.9 (74.1–92.7)	0.889	>38	75.3 (64.7–87.6)	0.815
Female	70	>39	79.5 (68.8–91.8)		>39	74.2 (64.2−85.8)	
Age							
<10 years	65	>39	84.7 (75.6–94.8)	0.561	>39	78.4 (68.3–90.0)	0.634
10−17 years	46	>35	80.9 (70.3–93.1)		>35	72.2 (60.2–86.5)	
≥18 years	26	>34	74.3 (56.8–97.2)		>34	69.5 (51.4–94.0)	
Time of HCT							
1983–1992	71	>39	82.7 (74.2–92.2)	0.495	>39	77.0 (67.7–87.6)	0.813
1993–2002	41	>29	76.5 (63.8–91.7)		>29	73.9 (60.9–89.8)	
2003–2018	25	>19	90.7 (79.0–100)		>19	75.6 (60.3–94.7)	

HCT hemopoietic cell transplantation, *OS* overall survival, *DFS* disease-free survival, *CI* confidence interval.

Table 4. Prevalence of impaired organ function at last follow-up visit.

Organ function	N. tested	Finding (%)	Observations
Blood counts in cured patients	108		
Hemoglobin, median, g/dL, (range)		12.6	(8.8–16.8)
Leukocyte count, median, x10^9/L, (range)		7.05	(3.1–15.3)
Neutrophil count, median, x10^9/L, (range)		3.58	(1.2–9.9)
Platelet count, median, x10^9/L, (range)		242	(43–479)
Hematological disorders			
Idiopathic thrombocytopenic purpura	123	3 (2.4)	
Iron burden			
N. treated with phlebotomy program	108	42 (39)	Median duration 12 months
N. treated with oral iron chelators	108	23 (21)	Deferasirox in all cases
Ferritin, median, ng/mL, (range)	108	215	(6–3523)
Growth in patients <10 years at transplant			
mean z score for weight at transplant, (range)	60	−0.33	(−2.49 to +2.17)
mean z score for weight at 20 years, (range)	55	+0.04	(2.09 to +1.91)
mean z score for height at transplant, (range)	60	−0.62	(−4.80 to +2.60)
mean z score for height at 20 years, (range)	55	−0.58	(−2.40 to +2.40)
Obesity			
Class II (BMI 30.0-39.9 Kg/m^2)	123	8 (6.5)	
Class III (BMI ≥ 40 Kg/m^2)	123	1 (0.8)	
Thyroid			
Hypothyroidism	123	13 (10.5)	All cases under substitutive treatment
Hyperthyroidism	123	1 (0.8)	Thyroidectomy 4
Hashimoto thyroiditis	123	3 (2.4)	
Multinodular goiter	123	6 (4.9)	
Diabetes mellitus	123	4 (3.2)	
Type I		3 (2.4)	
Type II		1 (0.8)	
Gonads in 60 males			
Median age at puberty in prepuberal patients, yr (range)	29	13	
Azoospermia		22 (52)	
N. of men who fathered a child	42	20 (33)	
N. of pregnancies		32	(10–17)
N. of living sons		34	Natural conception 24, FIVET 8
Gonads in 63 females			
Median age at puberty in prepuberal patients, yr (range)	33	13	(10–19)
Primary amenorrhea	62	20 (32.2)	Natural conception 35, FIVET 6
Secondary amenorrhea	62	12 (19.3)	
N. of women who became pregnant		25 (39.7)	
N. of pregnancies		41	
N. of living sons		36	
Eye			
Cataract	123	3 (2.4)	Associated with unilateral amaurosis in 2 cases
Severe ocular sicca	123	3 (2.4)	
Retinal vein thrombosis	123	1 (0.8)	
Cardiovascular			
Hypertension	123	18 (14.6)	
Ejection fraction, %, (range)	123	67	(55–75)
Valvular disease	123	6 (4.9)	Mitral valve prolapse 2, aortic valve insufficiency 2, tricuspid valve insufficiency 2

Table 4. continued

Organ function	N. tested	Finding (%)	Observations
Severe arrhythmia	123	2 (1.6)	Atrial fibrillation 1, fibrillo-flutter 1
Heart failure	123	1 (0.8)	Primary cause of late death
Ischemic ictus	123	1 (0.8)	Associated to unilateral hemiparesis
Lung	123	1 (0.8)	Cystic bronchiectasis disease with severe COPD
Kidney	123	1 (0.8)	Acute renal failure 1
Gastrointestinal	123	0	
Neurologic and psychiatric disorders			
Seizures	123	4 (3.2)	Grand-mal in all cases
Persistent anxiety and depression	123	6 (4.9)	
Psychosis and chronic delirium	123	1 (0.8)	
Schizophrenia	123	1 (0.8)	
Bone health			
Osteoporosis	123	12 (9.8)	Males 3, females 9
Hip disease	123	4 (3.2)	Coxarthrosis 2, hip dysplasia 2
Osteonecrosis of the femoral head	123	4 (3.2)	Hip prosthesis 4
Secondary solid cancer	123	15 (12.2)	Oral cavity 5, thyroid 3, colon 2, uterus 1, melanoma 1, Merkel cell carcinoma 1, parotid 1, breast 1
ECOG PS in living patients	114		
Score 0		103 (90.4)	
Score 1		7 (5.6)	Six with TR and 1 with depression, osteoporosis, and hypothyroidism
Score 2		3 (2.6)	1 with COPD, 1 with severe ocular sicca and unilateral amaurosis, 1 with depression associated to schizophrenia
Score 3		1 (0.9)	1 with hemiparesis, severe arrhythmia and diabetes mellitus
Covid-19 disease			
Clinical evidence of infection	114	29 (25.4)	
N. who received vaccination	114	98 (86)	
N. who refused vaccination	114	16 (14)	

BMI body mass index, *FIVET* fertilization in vitro and embryo transfer, *COPD* chronic obstructive pulmonary disease, *ECOG PS* Eastern Cooperative Oncology Group Performance Status, *TR* thalassemia recurrence, *Covid-19* coronavirus 2019.

Table 5. Post-transplant treatment of HCV-related chronic hepatitis.

Treatment	N. treated	SVR (%)	Median time from completion of therapy, years (range)	MLF at last follow-up visit
IFN ± RIBA	21	13 (62)	26 (11–30)	F0 = 11
				F1 = 2
DAAs*	20[§]	20 (100)	4 (1–7)	F0 = 9
				F1 = 7
				F2–F3 = 2
				F4 = 2

HCV hepatitis C virus, *IFN* interferon-alpha, *RIBA* ribavirine, *DAAs* directly acting agents, *SVR* sustained virological response, *MLF* Metavir liver fibrosis.
*DAAs included various combination of sofosbuvir, dasabuvir, daclatasvir, elbasvir, glecaprevir, grazoprevir, ledispavir, ombitasvir, paritaprevir, pibrentasvir, ritonavir, simeprivir, velpatasvir.
[§]DAAs was the first line therapy for 12 patients and the second line therapy in 8 patients who failed IFN therapy.

Fifteen patients (12.2%) (6 males) developed SSC at a median of 23.6 years (range, 3.1 to 30.9 years) since HCT. Details of the 15 patients who were diagnosed with SSC are shown in Table 6. The patient's median age at HCT and at time of SSC diagnosis was 13.08 years (range, 2.0 to 22.05) and 37.04 years (range 13.09 to 49.07) respectively. The median interval between HCT and diagnosis of SSC was 23.06 years (range, 3.01 to 30.08 years). The 39-years cumulative incidence of SSC was 16.4% (95% CI 8.4–24.4%) (Fig. 1d). Four patients out of 15 (26.6%) (2 with tumor of oral cavity, 1 with Merkel cell carcinoma, and 1 with tumor of parotid) died because of tumor progression between 6 months and 5.6 years after SSC diagnosis. One patient, who was diagnosed with uterus carcinoma, died because of JC virus-progression multifocal leukoencephalopathy 18 years after SSC diagnosis. Ten (66.6%) patients are currently living between 2.03 and 13.07 years (median, 5.08 years) after SSC diagnosis. None of them is now receiving anti-tumor therapy. As determined by both univariate and multivariate analysis about factors described in our previous study, we didn't find any factor that was significantly associated with an increased cumulative incidence of SSC [18]. We compared

Table 6. Details of 15 patients who were diagnosed with SSC.

UPN	Sex	Age at HCT years	HBV	HCV	cGvHD	Ferritin at HCT ng/mL	Ferritin at SSC ng/mL	Interval HCT / SSC years	Site of SSC	CH	RT	Status at last follow-up
23	F	2.0	Neg	Neg	No	800	2550	29.0	Merkel-cell	Yes	No	cancer progression, dead after 2 years
32	F	2.01	Neg	Neg	No	879	298	11.0	Parotid	Yes	No	cancer progression, dead after 6 months
103	M	7.07	Neg	Pos	No	2233	525	29.6	Cheek	No	No	living after 5.1 years
109	F	14.01	Neg	Pos	No	1327	304	20.7	Thyroid	No	No	living after 13.8 years
128	F	4.03	Neg	Neg	No	1203	398	26.2	Breast	Yes	Yes	living after 7.2 years
130	M	10.02	Pos	Pos	No	1037	650	30.2	Thyroid	No	Yes*	living after 3.8 years
136	F	12.05	Pos	Pos	No	2445	515	27.8	Thyroid	No	Yes*	living after 5.9 years
140	M	13.08	Neg	Pos	No	1532	976	23.6	Colorectal	No	No	living after 9.9 years
145	F	18.08	Pos	Pos	No	1385	104	30.8	Tongue	No	No	living after 2.3 years
257	M	22.02	Neg	Pos	Mild	1224	840	15.3	Cheek	No	Yes	living after 12.9 years
275	M	14.02	Neg	Pos	Severe	3324	2850	11.8	Tongue	Yes	No	cancer progression, dead after 2 years
278	F	18.08	Neg	Pos	No	2360	348	23.6	Colorectal	Yes	Yes	living after 3.9 years
290	M	13.04	Neg	Neg	Severe	728	395	21.0	Tongue	No	Yes	cancer progression, dead after 5.6 years
292	F	19.11	Neg	Neg	No	1557	410	24.8	Melanoma	No	No	living after 2.4 years
384	F	22.05	Pos	Neg	No	3937	2031	3.1	Uterine cervix	No	No	dead after 18 years, JCPyV PML

SSC secondary solid cancer, *UPN* unique patient number, *M* male, *F* female, *HCT* hemopoietic cell transplantation, *HBV* hepatitis B virus antibody, *HCV* hepatitis C virus antibody, *cGvHD* chronic graft-versus-host disease, *Pos* positive, *Neg* negative, *CH* chemotherapy, *RT* radiotherapy, *Yes** radiometabolic therapy, *JCPyV* JC Polyomavirus, *PML* progressive multifocal leukoencephalopathy.

the occurrence of SSC in transplanted patients with that of 133 relative donors. In the donor cohort, only one developed breast cancer 28 years after marrow donation and died for cancer progression. The donor 35-year cumulative incidence of SSC was 2.05% (95% CI 0–4.42%). Compared to the cumulative incidence of SSC in transplanted patients, the difference is statistically significant ($P = 0.002$).

DISCUSSION

This single center study of 137 allotransplanted TM patients represents the report with the longest median follow-up time (30 years) of survivors even described, to our knowledge. The importance to monitor long-term healthcare in patients transplanted for hemoglobinopathy has been outlined in guidelines published in 2018 [25]. We sought to characterize some important aspects of allogeneic transplantation focusing either on clinical outcome and survival and on the occurrence of late effects that involved the main organ functions and systems in order to provide a detailed profile of health after transplant. From a general point of view, most patients (90.4%) enjoy a normal clinical condition with a performance status of zero score according to the ECOG scale. OS and DFS were similar to those reported by the studies with the longest follow-up [14, 16, 26]. Age and gender of the recipient have no significant impact on OS and DFS. The incidence of long-term MC after transplantation (4.6%) seems lower than that (10%) reported by others [27, 28]. We can confirm that our 5 patients with MC, although they have not achieved the complete eradication of the thalassemic hemopoietic clone, show a functioning graft status characterized by adequate hemoglobin level, no red cell transfusion requirement and no iron burden increment.

Looking at the early and late causes of death, we can see that 4 (2.9%) patients, aged between 9 and 18 years at transplant, died of rapidly progressive heart failure ($n = 3$) in the first 50 days post-transplant or of congestive heart failure associated to severe fibrillo-flutter ($n = 1$) that occurred at 10.5 years after transplant. Heart complication was responsible of 23% of early causes of death and 19% of all causes of death. Studies have shown that the overall incidence of life-threatening cardiotoxicity during HCT is moderate (range 0.9–8.9% depending on the study) in patients mostly in adulthood [29]. Heart failure and arrhythmia were significant factors predicting mortality in TM patients conventionally treated with red cell transfusion and iron chelation therapy [30, 31]. Iron overload in myocardial tissue prior to transplantation is likely the main cause in predisposing the thalassemic patient to severe cardiotoxicity after HCT. In addition to the patient who died late from heart failure, late deaths were recorded in other 7 patients, mostly due to SSC ($n = 4$).

In the context of late effects, impaired gonadal function was the most frequent complication encountered in both males and females. Azoospermia was diagnosed in 22 of 42 (52%) males and both primary and secondary amenorrhea affected in total 32 of 62 females (51.5%). These findings are not different from those found in TM patients treated with conventional therapy [31, 32]. However, although fertility was impaired in a large number of patients, 41 pregnancies were observed in 25 transplanted females and resulted in 36 healthy infants. Moreover, 32 pregnancies were registered in partners of 21 transplanted males and resulted in 34 healthy infants. These findings confirm and extend what we reported in 2016 [17].

One of the most relevant and also unexpected late effects was the occurrence of ITP in 3 patients (2.4%). This finding is much higher than the incidence found in the normal population which was estimated to be 2 to 5 per 100,000 persons [33, 34]. The pathogenesis of ITP remains unclear although both antibody-mediated and/or T cell-mediated platelet destruction are key processes. In addition, impairment of T cells, cytokine imbalances,

and the contribution of the bone marrow niche have been recognized to be important [35]. All 3 patients had full donor engraftment with normal platelet counts above 200×10^9/L. None of them showed cGvHD or other autoimmune diseases. The link between this pathology and HCT is not clear and it is not possible at the moment to give a sure explanation for this apparently high incidence of ITP in our patients.

A very worrying and in some ways alarming finding is the high incidence of SSC. Fifteen patients have been diagnosed with SSC for a 39-yr cumulative incidence of 16.4%. What is really impressive is the young age (37.3 years) at which patients developed SSC. The occurrence of hematological malignancies and solid cancer have been well described in TM patients treated with conventional therapy [36]. We believe that this high incidence may be explained by the sum of thalassemia-related factors per se to which are to be added several transplant-related factors, in particular use of busulfan, HCV and HBV infection, chronic GvHD, prolonged immune deficiency, persistence of residual iron overload over time.

Other complications noted in our study such as diabetes mellitus, obesity, thyroid dysfunction, vision abnormalities, hypertension, altered lung function, renal function impairment, psychiatric disorders, and bone health impairment were similar to those described in HCT survivors of malignant and nonmalignant disorders.

In the present study we reported the approach to chronic HCV infection and treatment either with IFN or with DAAs. Treatment of chronic HCV infection with DAAs has been recently described in HCT patients [37]. The main findings in our series are:(1) HCV RNA is undetectable in all 33 patients who received therapy; (2) most patients ($n = 29$) show a normal or low grade of liver fibrosis (F0-F1); (3) liver fibrosis was moderate (F2-F3) in 2 patients or advanced (F4) in other 2 patients, although the liver disease in these patients is clinically stable with normal serum biomarkers.

A strength of our study is the very long follow-up of the surviving patients who in most cases received the transplant after an identical busulfan-based conditioning regimen. Moreover, all patients were closely followed over time and those who were living were evaluated uniformly in the previous 6 months before the date of study closure. However, some limitations of the study are evident. First of all, the cohort of patients examined is small and this leads us to believe that our results should be confirmed with a multicenter study on a much larger number of patients. Furthermore, our study did not allow us to detect the quality of life perceived by the subjects concerned following the poor compliance to complete the questionnaire that was administered. In summary, HCT represents a definitive cure for the majority of TM patients at the price, however, of a non-negligible early and late mortality which in the long run affects OS and DFS. It is also true that most patients (112 out of 137, 82%) were transplanted in the 1980s and 1990s with an inevitable impact on the final results. The most relevant positive finding is that a certain number of patients have maintained fertility intact and this has led to the birth of many healthy children. The most significant adverse late effect is the high incidence of SSC. Considering that the cumulative incidence curve of SSC doesn't show a plateau over time since HCT, systematic prospective monitoring and close clinical observation is mandatory for all survivors after transplant. Most importantly, the development of cancer screening guidelines is strongly recommended, so that physicians can provide state-of-the-art counsel and care for the benefit of all patients.

DATA AVAILABILITY

The datasets generated during and/or analyzed during the current study are available upon reasonable request from the corresponding authors.

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AUTHOR CONTRIBUTIONS

SS, PDB contributed patients, designed the study, analyzed the data, and wrote the manuscript; SA performed statistical study and contributed to the interpretation of the results; AN, DV, RS, PC contributed to data acquisition, analyzed the data, and wrote the paper; FP performed HLA typing and evaluated the chimerism; EDL, GI performed the liver evaluation by transient elastography. All authors read and critically reviewed the manuscript and approved the final version.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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