ARTICLE

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

β-Thalassemia major (TM) is an inherited hemoglobinopathy associated with defective synthesis of β-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 transplantation 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 evaluated. Our study included 137 consecutive caucasian patients affected by TM who were transplanted between May 1983 and February 2018.

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
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 EpiQ 7 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 β-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–Meier 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).

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 multigorgan 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 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 genotypically identical sibling | 127 | 93 |
| HLA phenotypically identical parent | 6 | 4 |
| Unrelated donor (10/10) | 4 | 3 |
| Transplant procedure | |
| Conditioning regimen | |
| BU 13–14 mg/Kg p.o. + CY 200 mg/Kg | 121 | 88 |
| BU 12.8 mg/Kg l.v. + TH 8 mg/Kg + FLU 160 mg/m² | 12 | 9 |
| TREG 42 g/m² + TH 8 mg/Kg + FLU 160 mg/m² | 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 thiopeta, FLU fludarabine, TREG treosulfan, GvHD graft-versus-host disease, CSA cyclosporine A, MTX methotrexate, BM bone marrow, PBSC peripheral blood stem cells, CB cord blood.
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 died for heart 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 hematologic 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 because only 20% of patients completed the questionnaire.

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. |
|---------------------------------------------------------------|
| Gender | N. | Median time of OS, years | OS% (95% CI) | P value | Median time of DFS, years | DFS% (95% CI) | P value |
|--------|----|--------------------------|--------------|---------|----------------------------|---------------|---------|
| 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) | 0.815 |
| Age    |    |                          |              |         |RFC318|                        |RFC319       |         |
| <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) | 0.634 |
| ≥18 years | 26 | >34                      | 74.3 (56.8–97.2) | >34 | 69.5 (51.4–94.0) | 0.634 |
| Time of HCT |    |                          |              |         |RFC320|                        |RFC321       |         |
| 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) | 0.813 |
| 2003–2018 | 25 | >19                      | 90.7 (79.0–100) | >19 | 75.6 (60.3–94.7) | 0.813 |

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, x10⁹/L, (range)             |           | 7.05        | (3.1–15.3)                                        |
| Neutrophil count, median, x10⁹/L, (range)            |           | 3.58        | (1.2–9.9)                                         |
| Platelet count, median, x10⁹/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/m²)                        | 123       | 8 (6.5)     |                                                   |
| Class III (BMI ≥ 40 Kg/m²)                            | 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                                               | 108       | 3 (2.4)     |                                                   |
| Type II                                              | 123       | 1 (0.8)     |                                                   |
| Gonads in 60 males                                   |           |             |                                                   |
| Median age at puberty in prepuberal patients, yr (range) | 29 | 13 |                                                   |
| Azoospermia                                          | 123       | 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        | 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 |
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

| Organ function                      | N. tested | Finding (%) | Observations                                      |
|-------------------------------------|-----------|-------------|---------------------------------------------------|
| Severe arrhythmia                   | 123       | 2 (1.6)     | Atrial fibrillation, 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                               |
| 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**

| Score 0 | 103 (90.4) | Six with TR and 1 with depression, osteoporosis, and hypothyroidism |
| Score 1 | 7 (5.6)    |                                                   |
| 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**

| N. who received vaccination | 98 (86) |
| N. who refused vaccination | 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.

| 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, ledipasvir, omibitasvir, paritaprevir, pibrentasvir, ritonavir, simprevir, velpatasvir.

§DAAs was the first line therapy for 12 patients and the second line therapy in 8 patients who failed IFN therapy.
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 |
|-----|-----|-------------------|-----|-----|-------|-----------------------|-----------------------|------------------------|------------|----|----|-------------------------|
| 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 7.2 years |
| 128 | F   | 10.02             | Neg | Neg | No    | 1203                  | 398                   | 26.2                   | Breast     | Yes| Yes* | living after 3 years |
| 130 | M   | 10.02             | Pos | Pos | No    | 1037                  | 650                   | 30.2                   | Thyroid    | No | Yes | living after 9.9 years |
| 140 | M   | 13.08             | Neg | Pos | Mild  | 1532                  | 976                   | 27.8                   | Colorectal | Yes| No | living after 2.3 years |
| 257 | M   | 22.02             | Neg | Pos | Severe| 3324                  | 2850                  | 11.8                   | Tongue     | No | Yes | cancer progression, dead after 2 years |
| 275 | M   | 14.02             | Neg | Pos | No    | 728                   | 395                   | 21.0                   | Tongue     | Yes| No | cancer progression, dead after 5.6 years |
| 290 | M   | 19.04             | Neg | Neg | No    | 1557                  | 410                   | 24.8                   | Colorectal | No | No | living after 9 years |
| 292 | F   | 19.11             | Neg | Neg | No    | 1557                  | 410                   | 24.8                   | Melanoma   | No | No | living after 2.4 years |

**SSC** secondary solid cancer, **unique patient number**, **UPN** female, **HCT** hemopoietic cell transplantation, **HBV** hepatitis B virus antibody, **HCV** hepatitis C virus antibody, **cGvHD** chronic graft-versus-host disease, **CH** chemotherapy, **RT** radiotherapy, **PPY** JCPyV-induced acute liver failure, **PMI** progressive multifocal leukoencephalopathy, **PML** progressive multifocal leukoencephalopathy.

**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 considered with caution. 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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