Allogeneic hematopoietic stem cell transplantation in myelofibrosis: the 20-year experience of the *Gruppo Italiano Trapianto di Midollo Osseo* (GITMO)

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ABSTRACT

Background

Allogeneic stem cell transplantation is a potentially curative treatment for myelofibrosis, although its use is limited by a high rate of transplant-related mortality. In this study, we evaluated the outcome of patients with myelofibrosis who underwent allogeneic stem cell transplantation, and the impact of prognostic factors.

Design and Methods

One hundred patients were transplanted in 26 Italian centers between 1986 and 2006. We analyzed the influence of the patients' characteristics and the clnical features of their disease before stem cell transplantation and of transplant procedures on transplant-related mortality, overall survival, and relapse-free survival by means of univariate and multivariate analyses.

Results

The median age of the patients at the time of stem cell transplantation was 49 years (range, 21-68) and 90% of them had an intermediate or high Dupriez score. Forty-eight percent received a myeloablative conditioning regimen and 78% received stem cells from matched sibling donors. The cumulative incidence of engraftment at day 90 after transplant was 87% (95% CI, 0.87-0.97). The cumulative 1-year and 3-year incidences of transplant-related mortality were 35% and 43%, respectively. The estimated 3-year overall and relapse-free survival rates after stem cell transplantation were 42% and 35%, respectively. In multivariate analysis, negative predictors of transplant-related mortality were year of stem cell transplantation before 1995, unrelated donor, and a long interval between diagnosis and transplantation. There was a trend towards longer overall and relapse-free survival in patients receiving peripheral blood stem cells rather than bone marrow as the source of their graft (p=0.070 and p=0.077, respectively). The intensity of the conditioning regimen (myeloablative versus reduced intensity regimens) did not significantly influence the outcome.

Conclusions

We conclude that the outcome of myelofibrosis patients who underwent allogeneic stem cell transplantation significantly improved after 1996 due to the reduction in transplant-related mortality. We observed that a reduction in transplant-related mortality was associated with the choice of a matched sibling donor, whereas longer overall survival was associated with the use of peripheral blood as the source of stem cells.

Key words: primary myelofibrosis, allogeneic stem cell transplantation, prognostic factors, reduced-intensity regimens.

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Introduction

Myelofibrosis is a clonal hematopoietic stem cell disorder that is clinically characterized by progressive anemia, marked splenomegaly, extramedullary hematopoiesis, constitutional symptoms and a significant risk of evolution into acute leukemia.[1,2] Myelofibrosis can appear as a primary or idiopathic disorder or, less frequently, as a secondary complication of essential thrombocythemia or polycythemia vera, with a clinical presentation and course similar to those of the idiopathic form.[3] The disease affects mainly elderly people, with the median age at diagnosis being about 65 years.[4] It is a heterogeneous disorder in terms of presentation and evolution, with a median overall survival (OS) varying between 2 and 15 years, depending on the presence or absence of clinically defined prognostic factors, such as those defined by Dupriez *et al.*[5] and Cervantes *et al.*[6] Drug therapy is aimed at alleviating the symptoms, and has not been shown to improve survival. Allogeneic hematopoietic stem cell transplantation (HSCT) is the only treatment with the potential to cure myelofibrosis. Several retrospective analyses of the outcome of patients treated with myeloablative allogeneic HSCT reported a transplant-related mortality (TRM) of about 30% and a graft failure risk of 10%, which makes the procedure feasible only in young individuals with poor prognostic factors.[7-12] Reduced-intensity conditioning (RIC) regimens might theoretically be applied to a large number of older patients, including those with comorbidities, while maintaining the potential for eradicating the disease based on the graft-versus-myelofibrosis effect.[13-17] Studies in small series of patients who underwent RIC HSCT demonstrated the feasibility of the procedure, with a lower TRM rate, although the follow-up of these studies were short. Several issues are currently under debate, such as the choice of the conditioning regimen, the use of unrelated donors, and the timing of the procedure.

In order to identify prognostic factors influencing the outcome after HSCT, we retrospectively analyzed the influence of patients' characteristics and the clinical features of their disease before HSCT and of transplant procedures on TRM and OS in 100 patients with myelofibrosis who underwent allogeneic HSCT in 26 Italian transplant centers that are part of the *Gruppo Italiano Trapianto Midollo Osseo* (GITMO).

Design and Methods

Data collection

The data related to the patients with myelofibrosis receiving HSCT were drawn from the GITMO registry. GITMO is a voluntary working group of more than 50 Italian transplant centers, the participants of which are required, once a year, to report all consecutive HSCT and follow-ups.

The information required for entry into the study was as follows: demographic data of the recipient, date of transplant, cell source, donor, type of conditioning regimen (myeloablative or reduced intensity), engraftment, follow-up to December 2006, date of relapse, date and cause of death, and development of acute and chronic graft-versus-host disease (GVHD). A total of 100 HSCT recipients from 26 transplant centers met these eligibility criteria.

Further data collected were: date of diagnosis of myelofibrosis, previous treatment, clinical, hematologic and cytogenetic characteristics of the disease before transplantation, Dupriez score[5] at transplant, combinations of drugs, drug doses and irradiation dose delivered during the conditioning regimen, and GVHD prophylaxis. These data were not considered essential for participation in the study and were provided by 20 out the 26 centers.

Patients

Twenty-one centers transplanted five or fewer patients each, whereas the centers in Palermo, Pescara, Udine, Rome and Genoa performed 7, 7, 8, 9 and 26 transplants, respectively.

Informed consent was signed before transplantation and the procedures were performed according to each center's protocol. The protocol for each institution was approved by the institutional review board. The results of 15 patients included in this study have been previously reported[7,13] and are here updated with a longer follow-up. Patient and disease characteristics are presented in Table 1. At the time of HSCT, the patients' median age was 49 years (range, 21-68), 13% of them were 60 years or older, 65% were male, the median time between diagnosis and HSCT was 14 months (range, 3-300), and one-third of the procedures were performed 3 or more years after diagnosis. Eighty-two percent of the patients had primary myelofibrosis and 57% had previously received chemotherapy, mainly hydroxyurea or busulfan. Ninety percent of the patients had an intermediate or high Dupriez score, 54% had previously received red cell transfusions, 24% had circulating blasts in the peripheral blood, 56% had splenomegaly, and 38% had previously undergone splenectomy. Abnormal karyotypes were detected in 24% of the patients. Nine patients with a low Dupriez score were considered eligible for transplantation: these patients were agend between 34 and 55 years, all had splenomegaly, five had a hemoglobin concentration <11 g/dL, and three had bone marrow cytogenetic abnormalities.

Transplant-related factors are given in Table 2. All the transplant procedures were performed between November 1986 and June 2006, and 65 of them between 2001 and 2006. Stem cells came from matched sibling donors for 78% of the patients, mismatched sibling donors for 4% of patients, and unrelated donors for the remaining 18% of the patients. The matched unrelated donors were matched for HLA-A, -B, and DRB1 loci. Serological HLA class I typing was accepted until 2000, after which a molecular confirmation was required; molecular class II typing was always performed. Stem cells from unrelated or mismatched donors were used in one of 11 cases (9%)

Table 1. Clinical and hematologic characteristics of the patients at allogeneic stem cell transplantation.

Total n. of patients	100
Sex:male	65/100 (65%)
Age (years)	
Median (range)	49 (21-68)
Diagnosis	
Idiopathic myelofibrosis	73/89 (82%)
Secondary myelofibrosis	16/89(18%)
Time diagnosis-transplant (months)	
Median (range)	14 (3-300)
≤12	46/100
13-35	21/100
≥36	33/100
Previous treatment	
Chemotherapy	45/78 (57%)
Prednisone	20/78 (26%)
Thalidomide	3/78 (4%)
Androgens	8/78 (10%)
Interferon	2/78 (3%)
Dupriez score at transplantation	
Low	9/87 (10%)
Intermediate	51/87 (58%)
High	27/87 (32%)
Previous transfusions	42/78 (54%)
Karyotype	
Normal	50/65 (76%)
Abnormal	15/65 (24%)
Previous splenectomy	35/92 (38%)
Splenomegaly at transplantation	49/88 (56%)
Spleen ≤6 cm below costal margin	32/49 (65%)
Spleen >6 cm below costal margin	7/49 (35%)
Circulating blasts at transplantation	21/85 (24%)
Hemoglobin level at transplantation (g/dL)	
Median (range)	9.0 (5.6-15.0)
White cell count at transplantation ($\times 10^6$/L)	
Median (range)	7.0 (0.1-118)

Table 2. Transplant-related characteristics.

Year of the transplant	
Before 1995	11/100
1996-2000	24/100
After 2001	65/100
Donor	
HLA matched sibling	78/100
Unrelated or HLA "mismatched" sibling	22/100
Source of stem cells	
Bone marrow	48/100
Peripheral blood	50/100
Bone marrow and peripheral blood	2/100
Conditioning regimen	
Standard myeloablative conditioning	48/100
Busulfan+Cy	24/100
TBI +Cy	6/100
Thiotepa 15 mg/kg + Cy 120 mg/kg ± melphalan	14/100
Unspecified	4/100
Reduced-intensity conditioning	52/100
Thiotepa 10 mg/kg + Cy 100 mg/kg	24/100
Fludarabine + busulfan (or treosulfan)	14/100
Fludarabine + TBI	4/100
Fludarabine + melphalan	6/100
Unspecified	4/100
GVHD prophylaxis	
Cyclosporine	10/100
Cyclosporine + methotrexate	70/100
Cyclosporine + methotrexate+ ATG	18/100
Cyclosporine + mycophenolate mofetil	2/100

Cy: cyclophosphamide; TBI: total body irradiation; ATG: antithymocyte globulin.

before 1995 and increased to five out of 24 cases (21%) in the period 1996-2000, and to 16 out of 65 (25%) after 2001. Forty-eight percent of patients received bone marrow, 50% peripheral blood, and 2% stem cells from both sources because of inadequate mobilization of peripheral blood stem cells in a donor followed by a bone marrow harvest. Peripheral blood was chosen for 3 out of 11 patients (27%) before 1995, for 12 out of 24 patients (50%) between 1996 and 2000, and for 35 out of 65 patients (54%) afterwards. Conditioning regimens were heterogeneous. They were given at myeloablative doses to 48 patients: 24 received oral busulfan plus cyclophosphamide, 6 received total body irradiation plus cyclophoasphamide, 8 received thiotepa 15 mg/kg plus a standard dose of cyclophoasphamide, with the addition of melphalan in another 6 cases. In 4 cases, the myeloablative regimen was unspecified. Busulfan doses were not regularly adjusted according to plasma levels (targeted busulfan). Fifty-two patients received RIC regimens as follows: thiotepa 10 mg/kg plus cyclophoasphamide 100 mg/kg (24 patients), fludarabine with the addition of busulfan (14 patients) or melphalan (6 patients) or 2 Gy total body irradiation (4 patients), and unspecified drug combinations in the remaining 4 patients. RIC regimens were not administered before 1995, 7 out of 24 patients (29%) and 45 out of 65 patients (69%) received such a regimen between 1996-2000 and after 2001, respectively. GVHD prophylaxis consisted of cyclosporine plus methotrexate in 88 patients, with the addition of antithymocyte globulin in all 18 cases of HSCT from unrelated donors. The median follow-up of the whole population after HSCT was 13 months (range, 1-234).

Criteria for engraftment, response, and graft-vs-host disease

The day of engraftment was defined as the first of 3 consecutive days on which the blood granulocyte count rose to 0.5×10^9/L. Primary graft failure was diagnosed if the granulocyte engraftment end-point was not reached by day 50. Complete hematologic remission was defined as the disappearance of all clinical signs (including constitutional symptoms, splenomegaly and hepatomegaly) and of peripheral blood and cytogenetic abnormalities due to the disease.

Relapse was defined as the reappearance of host cells, the presence of morphological criteria of myelofibrosis after initial clearance of the marrow, or the detection of previously existing abnormalities. Acute and chronic GVHD were graded according to standard criteria.[18,19]

Statistical analysis

Data were collected in an XLS database and imported into Stata/SE 9.0 for Windows for the statistical analysis. The close-out date for analysis was December, 2006.

The end-points were engraftment, acute and chronic GVHD, relapse, TRM, OS, and relapse-free survival (RFS). TRM was defined as death due to all causes not related to myelofibrosis. The cumulative incidence method was used to estimate the rate of engraftment, acute and chronic GVHD, TRM, and relapse. OS was defined as the time (in months) from the date of transplant to either death or last observation. RFS was defined as the time from the date of transplant to relapse, death or last observation. OS and RFS were described using the Kaplan-Meier approach. Survival was analyzed using Cox proportional hazard models, after the proportional hazard assumption had been verified. In univariate analysis, variables considered as possible prognostic factors were: primary diagnosis (primary or secondary myelofibrosis), Dupriez score at transplant (low, intermediate, or high), transplant time (before 1995, 1996-2000, after 2001), interval between diagnosis and transplantation (months), transfusions before transplant, hemoglobin levels, white cell counts, circulating blasts, karyotype, previous splenectomy, splenomegaly at transplantation, age at transplantation, intensity of conditioning regimen (standard myeloablative or reduced intensity), donor (matched sibling or mismatched sibling and unrelated), source of stem cells (bone marrow or peripheral blood), acute GVHD (grade 0-I or grade II-IV), and chronic GVHD (absent or present).

Acute and chronic GVHD were treated as time-dependent variables. Multivariate stepwise analyses included all variables significant at $p \leq 0.10$ in univariate analysis. Retention in the stepwise model required that the variable be significant at $p \leq 0.05$ in a multivariate analysis.

Results

Engraftment

Eighty-eight patients engrafted. The cumulative incidence of engraftment at day 90 after transplant was 87% (95% CI, 0.87-0.97) (Figure 1). Among patients' characteristics, clinical features of the disease, age, Dupriez score, previous transfusions and splenectomy, presence of splenomegaly, circulating blasts, and abnormal karyotype were equally distributed between patients who engrafted and those who did not. However, 9 out of the 12 patients (75%) who failed to engraft had received a RIC regimen, in comparison with 43 out of 88 (49%) who had had allogeneic hematologic reconstitution (p=0.09 n.s.). The nine RIC transplants were conditioned with thiotepa plus cyclophasphamide (5 patients) or fludarabine plus melphalan or busulfan (4 patients). Two of the three myeloablative transplants that did not engraft were performed in 1982 and in 1986. Neither donor nor source of stem cells was a significant risk factor for graft failure. Three of the 12 patients who did not engraft had a second transplant. All 12 patients who failed to engraft died within 6 months after the first HSCT because of overt disease recurrence (3 patients) or complications in the aplastic phase (9 patients).

Graft-vs-host disease

Forty patients developed acute GVHD grades II to IV for a cumulative incidence of 41% by day 100 after transplantation (95% CI, 0.9-1.3). Chronic GVHD occurred in 37 patients for a cumulative incidence of 43% at 2 years (95% CI, 0.4-0.8): it was limited in 26 patients and extensive in 11 patients. Age, Dupriez score, and presence of splenomegaly before transplantation were similar between patients who developed acute and chronic GVHD and those who did not. Transplant factors such as source of stem cell, donor type, and conditioning regimen did not influence the incidence of acute or chronic GVHD.

Patients' outcome: transplant-related mortality and causes of death

Overall, 38 patients died at a median time of 4.5 months (range, 1-48) after transplantation because of

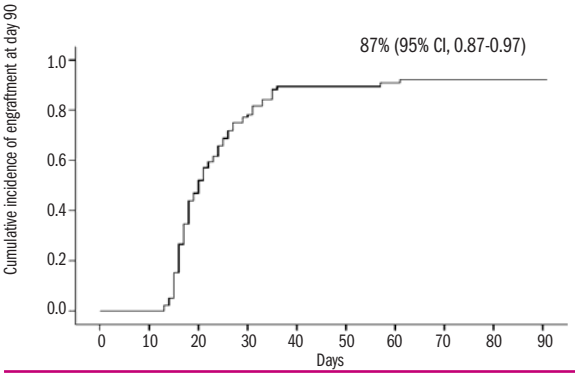

Figure 1. Cumulative incidence of engraftment at +90 days.

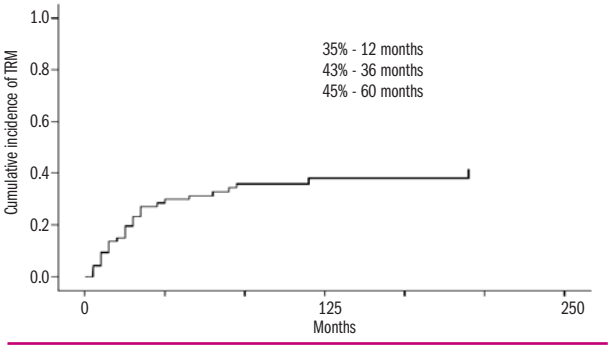

Figure 2. Cumulative incidence of transplant- related mortality.

transplant-related causes: acute GVHD (13 patients), infections (13 patients), bleeding (6 patients), veno-occlusive disease (2 patients), transplant-related microangiopathy (1 patient), second cancer (1 patient), and heart failure (2 patients). The 1-year and 3-year TRM cumulative incidence rates were 35% and 43%, respectively (Figure 2). Seventeen patients (45%) died within 100 days after transplantation and 32 (89%) within 1 year. The prognostic factors that showed a significant ($p \geq 0.10$) association with TRM in the univariate proportional hazard model were: transplant time, interval between diagnosis and transplantation, and type of donor (Table 3). These variables comprised the eligible pool of predictors for multivariate, stepwise proportional hazards models used to predict TRM.

The final TRM model included transplantation time, interval between diagnosis and transplantation, and type of donor (Table 4). Transplantation after 1996 significantly decreased the hazard of TRM in comparison with the previous transplant period. A longer time between diagnosis and HSCT resulted in a significant increase in TRM (Table 4). An unrelated or mismatched sibling donor increased the hazard ratio of death by 2.5 times compared to the risk associated with a transplant from a matched sibling (Table 4).

Table 3. Univariate analysis of transplant-related mortality data.

Factor	*Hazard ratio*	*95% CI*	*p*
Age at SCT modeled as a continuous variable	1.00	0.97-1.04	0.915
Primary diagnosis			
Idiopathic myelofibrosis	1		
Secondary myelofibrosis	0.97	0.37-2.54	0.957
Dupriez score			
0	1		
1	0.65	0.22-1.96	0.447
2	0.86	0.27-2.71	0.800
Splenectomy before SCT			
No	1		
Yes	1.05	0.52-2.12	0.888
Spenomegaly before SCT			
No	1		
Yes	1.08	0.53-2.23	0.829
Circulating blasts at SCT			
No	1		
Yes	1.20	0.55-2.63	0.650
Abnormal karyotype at SCT			
No	1		
Yes	1.38	0.46-4.11	0.561
RCB transfusions before SCT			
No	1		
Yes	0.91	0.43-1.91	0.803
Hemoglobin levels at SCT modeled as a continuous variable	0.997	0.98-1.02	0.805
Leukocyte count at SCT modeled as a continuous variable	1.00	0.99-1.01	0.869
Year of SCT[a]			
<1995	1		
1996-2000	0.41	0.16-1.05	0.063
>2001	0.30	0.13-0.69	0.004
Time from diagnosis to SCT, modeled as a continuous variable	1.006	1.001-1.010	0.012
Conditioning regimen			
Myeloablative	1		
Reduced intensity	0.93	0.49-1.75	0.816
Donor[a]			
Sibling matched	1		
Unrelated or 'mismatched'	1.81	0.90-3.66	0.097
Cell source			
Bone marrow	1		
Peripheral blood	0.70	0.37-1.32	0.268
Grade II-IV acute GVHD[b]			
No	1		
Yes	1.013	0.93-1.10	0.774
Chronic GVHD[b]			
No	1		
Yes	1.05	0.93-1.19	0.421

SCT: stem cell transplantation; RBC: red blood cell; GVHD: graft-versus-host disease. [a]Variables significant at p≤0.10. [b]Acute and chronic GVHD were treated as time-dependent variables.

Patients' outcome: relapse, relapse-free survival and overall survival

Overall, 23 patients relapsed at a median time of 9.5 months after transplantation and 17 patients died because of relapse. The cumulative incidence of relapse was 41% (95% CI, 1.2-2.2) at 2 years after transplantation. The estimated 1-year, 3-year, and 5-year RFS rates were 53%, 35% and 28%, respectively. Prognostic factors that were significantly ($p \geq 0.10$) associated with RFS in the univariate proportional hazard model were: transplant time, interval between diagnosis and transplantation, and source of stem cells (Table 5). In the univariate analysis, transplantation after 2001 favorably affected RFS ($p=0.038$). There was a trend for the use of peripheral blood as compared to bone marrow to have a favorable impact on RFS ($p=0.077$). The final survival model did not show any significant prognostic factor for RFS.

Four patients received donor lymphocyte infusions because of relapse (3 patients) or mixed chimerism (1 patient). All but one had been transplanted with RIC regimens based on thiotepa plus cyclophosphamide. Only one patient had a transient response, two other patients died of overt recurrence, and the patient with mixed chimerism died of GVHD. Five patients had a second

Table 4. Multivariate analysis of transplant-related mortality (TRM) data and overall survival data.

Factor	*TRM*		
Transplant time	HR	95%CI	p
<1995	1		
1996-2000	0.37	0.14-0.96	0.041
>2001	0.24	0.10-0.58	0.001
Time from diagnosis to SCT modeled as a continuous variable	1.01	1.001-1.011	0.007
Donor			
Matched sibling	1		
Unrelated or mismatched	2.49	1.19-5.23	0.016

SCT: stem cell transplantation.

transplant because of relapse: two received a myeloablative regimen and the other three were given a RIC regimen. All were reinfused with stem cells from the same sibling donor of the original graft. One patient died of transplant-related causes, but the other four are still alive and in remission. The estimated 1-, 3- and 5-year OS rates after HSCT were 59%, 42% and 31%, respectively (Figure 5). The 5-year median survival time was 24 months. Prognostic factors that were significantly ($p \leq 0.10$) associated with survival in the univariate proportional hazard model were: transplant time, interval between diagnosis and transplantation, and source of stem cells (Table 6). In the univariate analysis, transplantation after 2001 significantly reduced the hazard of mortality ($p=0.022$), whereas there was a trend for the use of peripheral blood as compared to bone marrow to have a favorable impact on OS ($p=0.070$). However, there was a borderline significant trend towards shorter OS for patients whose transplant was performed a long time after diagnosis ($p=0.058$). The final survival model did not show any significant prognostic factor for OS. At the last follow-up, with a median survival follow-up of 34 months (range, 14-234), 39 patients were alive and free of disease after the first SCT and another 4 patients, previ-

Table 5. Univariate analysis of relapse-free survival data.

Factor	*Hazard ratio*	*95% CI*	*p*
Age at SCT			
modeled as a continuous variable	0.99	0.97-1.023	0.901
Primary diagnosis			
Idiopathic myelofibrosis	1		
Secondary myelofibrosis	0.91	0.43-1.93	0.797
Dupriez score			
0	1		
1	0.78	0.32-1.89	0.584
2	0.85	0.34-2.16	0.735
Splenectomy before SCT			
No	1		
Yes	0.99	0.58-1.70	0.966
Spenomegaly before SCT			
No	1		
Yes	1.10	0.63-1.90	0.740
Circulating blasts at SCT			
No	1		
Yes	1.29	0.70-2.37	0.412
Abnormal karyotype at SCT			
No	1		
Yes	1.35	0.59-3.09	0.474
RBC transfusions before SCT			
No	1		
Yes	0.88	0.49-1.57	0.669
Hemoglobin levels at SCT			
modeled as a continuous variable	0.99	0.98-1.01	0.844
Leukocyte count at SCT	1.00	0.99-1.01	0.442
modeled as a continuous variable			
Year of SCT[a]			
<1995	1		
1996-2000	0.50	0.22-1.15	0.101
>2001	0.46	0.22-0.96	0.038
Time from diagnosis to SCT,[a]	1.00	0.99-1.01	0.074
modeled as a continuous variable			
Conditioning regimen			
Myeloablative	1		
Reduced intensity	0.88	0.53-1.45	0.617
Donor			
Sibling matched	1		
Unrelated or *mismatched*	1.38	0.76-2.51	0.294
Cell source[a]			
Bone marrow	1		
Peripheral blood	0.63	0.38-1.05	0.077
Grade II-IV acute GVHD[b]			
No	1		
Yes	0.98	0.93-1.03	0.351
Chronic GVHD[b]			
No	1		
yes	1.02	0.97-1.08	0.449

SCT: stem cells transplantation; RBC: red blood cell; GVHD: graft-versus-host disease. [a]Variables significant at p ≤0.10. [b]Acute and chronic GVHD were treated as time-dependent variables.

Table 6. Univariate analysis of overall survival data.

Factor	*Hazard ratio*	*95% CI*	*p*
Age at SCT			
modeled as a continuous variable	0.99	0.97-1.02	0.657
Primary diagnosis			
Idiopathic myelofibrosis	1		
Secondary myelofibrosis	0.69	0.29-1.64	0.406
Dupriez score			
0	1		
1	0.62	0.25-1.51	0.290
2	0.79	0.31-2.04	0.626
Splenectomy before SCT			
No	1		
Yes	0.95	0.53-1.70	0.856
Spenomegaly before SCT			
No	1		
Yes	0.98	0.55-1.75	0.954
Circulating blasts at SCT			
No	1		
Yes	1.55	0.81-2.97	0.184
Abnormal karyotype at SCT			
No	1		
Yes	1.60	0.61-4.18	0.338
RBC transfusions before SCT			
No	1		
Yes	0.99	0.52-1.86	0.963
Hemoglobin levels at SCT			
modeled as a continuous variable	0.99	0.98-1.01	0.509
Leukocyte count at SCT modeled	1.00	0.99-1.01	0.561
as a continuous variable			
Year of SCT[a]			
<1995	1		
1996-2000	0.45	0.19-1.04	0.062
>2001	0.42	0.20-0.88	0.022
Time from diagnosis to SCT,[a]	1.004	0.99-1.01	0.058
modeled as a continuous variable			
Conditioning regimen			
Myeloablative	1		
Reduced intensity	0.78	0.45-1.34	0.363
Donor			
Sibling matched	1		
Unrelated or *mismatched*	1.57	0.84-2.94	0.159
Cell source[a]			
Bone marrow	1		
Peripheral blood	0.61	0.35-1.04	0.070
Grade II-IV acute GVHD[b]			
No	1		
Yes	0.98	0.94-1.02	0.247
Chronic GVHD[b]			
No	1		
yes	0.99.52	0.94-1.04	0.700

SCT: stem cells transplantation; RBC: red blood cell; GVHD: graft-versus-host disease. [a]Variables significant at p ≤0.10. [b]Acute and chronic GVHD were treated as time-dependent variables.

Table 7. Summary of the results of previous reports and of the present study with regard to allogeneic stem cell transplantation (SCT) in myelofibrosis.

	Guardiola[7]	*Deeg*[8]	*Kerbau*[9]	*Daly*[11]	*Ditschkowski*[12]	*Rondelli*[13]	*Kroger*[14]	*Synder*[15]	*Merup*[17]	*Present GITMO series*
N. of patients	55	56	104	25	20	21	21	9	27	100
Median age, years	42 (4-53)	43 (10-66)	49 (18-70)	48 (46-50)	45 (22-57)	54 (27-68)	53 (32-63)	54 (46-68)	40 (5-63)	49 (21-68)
Conditioning	myelo	myelo	90 myelo 10 RIC	myelo	myelo	RIC	RIC	RIC	17 myelo 10 RIC	49 myelo 51 RIC
Donor rel/unrel	49/6	36/26	59/45	15/10	13/2	19/2	8/13	2/7	20/7	82/18
Median follow-up of survivors	36 months	32 months	63 months	35 months	13 months	31 months	22 months	32 months	50 months	34 mo
TRM (%)	27% (at 1 y)	32% (at 3 y)	34% (at 5y)	48% (at 1 y)	40%	9%	16% (at 1 y)	44%	29%	43% (at 3 y)
OS (%)	47% (at 5 y)	58% (at 3 y)	61% (at 5y)	41% (at 2 y)	38% (at 3 y)	78% (at 2 y)	84% (at 3 y)	56%	70%	42% (at 3 y)
Graft failure (%)	9%	5%	10%	9%	n.v.	5%	0 %	11%	7%	12%

rel: HLA matched sibling; unrel: unrelated or HLA mismatched; myelo: myeloablative regimens; RIC: reduced-intensity regimens; y: years; TRM: transplant-related mortality; OS: overall survival.

ously described, were in remission after a second SCT. Among the survivors who had not undergone splenectomy, the spleen was of normal size in all patients. The median white cell count was 5.2×10^9/L (range, 3.7-15.0×10^9), median hemoglobin level was 13.7 g/dL (11.7-16.3) and the median platelet count was 250×10^9/L (range, 105-422×10^9).

Discussion

This retrospective study included all patients with myelofibrosis transplanted in Italy in a 20-year period. Because it is based on a population living in a specific geographic area, this study differs from all the other studies reported in the literature, which are mostly single center studies from well-known large transplants centres or collaborative international studies between large transplant centers. The heterogeneity of the patients' clinical features, the large number of the participating centers, and the long period of patient enrolment may explain the overall outcome in our survey with a 3-year TRM rate of 43% and a 3-year OS rate of 42%, which are poorer than those of previous studies (Table 7). That said, the clinical characteristics of our patients are similar to those of other groups, with most patients having high-risk disease, 90% with a high or intermediate Dupriez score, and 40% with transfusion-dependent anemia. The median age of 49 years is younger than that of non-selected patients with myelofibrosis,[1] but is very similar to that of other reported groups of transplanted patients.[7-9,11-12] Thirteen percent of our patients were over 60 years of age, unlike most previously reported series.[7,11,12]

Among the clinical factors related to disease, time between diagnosis and transplantation was the only

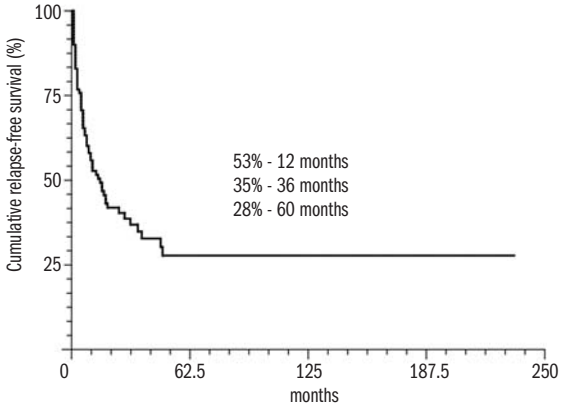

Figure 3. Kaplan-Meier estimate of relapse-free survival.

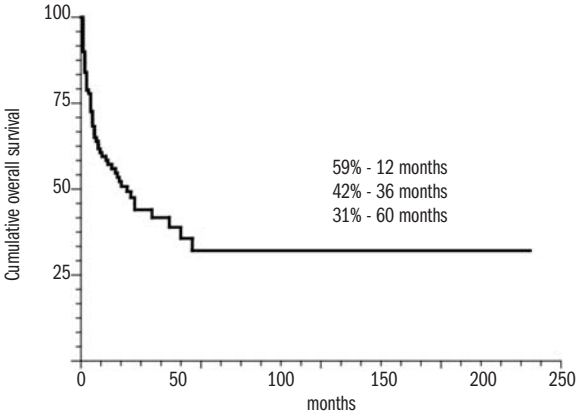

Figure 4. Kaplan-Meier estimate of overall survival.

significant factor for outcome in univariate analysis, and was statistically predictive of TRM but not of OS in the multivariate regression model. Other disease features, such as Dupriez score, abnormal karyotype,[8,9] circulating blasts, and marrow fibrosis,[12] which had emerged as unfavorable prognostic factors in previous studies, were not associated with a worse outcome in the present series. However, these unfavorable clinical features are strictly associated with duration of the disease, since a long interval between diagnosis and transplantation allows the development of severe hematologic and cytogenetic abnormalities. In the first publication of the Seattle group by Deeg,[8] in which the median time between diagnosis and transplantation was 33 months, a low Dupriez score was observed in 25% of patients; in the enlarged series reported by Kerbauy,[9] the median duration of the disease decreased to 15 months and a low Dupriez score was reported in 44% of the population.

We found a progressive improvement of outcomes during the 20-year period analyzed. The most impressive result was the significant reduction in TRM rate after 1996, which emerged from the multivariate analysis. However, a trend towards improvements in both OS and RFS was observed in the periods 1996-2000 and 2001-2006. Of the transplant-related variables, transplants from unrelated or mismatched sibling donors were associated with a higher TRM, whereas there was a trend for the use of peripheral blood instead of bone marrow to have a favorable impact on both RFS and OS. There was only a minority of unrelated or mismatched transplants in previous reports, and the outcomes observed were very different, varying from a high TRM described by Snyder[15] and a high risk of graft failure observed by Deeg[8] to outcomes equivalent to those of transplants from matched sibling donors in the most recent report of the Seattle group.[9] Our negative results could be, in part, due to the lack of a homogeneous policy of HLA matching of unrelated donors.

In our study, we did not observe an advantage in terms of decreased TRM and prolongation of OS after RIC transplants in comparison with myeloablative transplants. Previous studies[13,14] reported encouraging TRM rates below 15% and OS rates of around 80% in small series of 20-25 patients transplanted with different RIC regimens, but there was no comparison with other preparative regimens. It has to be underlined that in our series, conditioning regimens were heterogeneous, including truly myeloablative regimens as well as different kinds of RIC regimens. The distinction between the two categories was sometimes quite difficult; for example, thiotepa–cyclophosphamide was classified as myeloablative or as RIC on the basis of the drug doses delivered to the patient. The great heterogeneity of drugs and doses received by the patients could partly explain the failure to detect any difference in outcomes after these two procedures. However, in our series RIC regimens tended to be associated with a higher rate of engraftment failure in comparison with myeloablative transplants. Moreover, it could be hypothesized that the reduction of TRM observed after 1996 was not due to the introduction of RIC transplants, but rather to a general improvement of supportive, anti-infectious and immunosuppressive procedures and treatments.

We reported the first series of second transplants for myelofibrosis (8 cases). Second transplants were successful in four out of five cases when they were performed because of relapse, whereas they did not achieve hematologic reconstitution when they followed a previously failed graft.

This study has several limitations. It is retrospective, like all the previous published studies; it is multicenter, with the participation of numerous transplant centers, the majority of which included one or two patients; and it covers a 20-year period, during which policies and strategies for transplantation in myelofibrosis have changed. Moreover, the study does not report information on biological factors that have recently been shown to have an important role in assessing risk before transplantation or on minimal residual disease in the follow-up, such as histopathological evaluation of the degree of marrow fibrosis,[7,12] percentage of circulating CD34-positive cells,[20] and molecular assessment of *JAK2*-V617F mutations.[21]

Despite these limitations, our results confirm that allogeneic HSCT may be an attractive treatment approach for patients with high-risk myelofibrosis. The outcome of such patients has improved significantly since 1996 due to the reduction of TRM. Since the TRM rate increases as the duration of disease before transplant is prolonged, the biological factors mentioned above should to be taken into consideration in the initial history of the disease to plan early transplantation in selected patients. Future prospective trials should address the issue of the choice of conditioning regimen, the source of stem cells, and the type of donor. Some suggestions emerging from our results are that peripheral blood should be preferred to bone marrow as the source of stem cells and the need for *full* HLA matching (10 out of 10 identical loci) for unrelated donors, which may overcome the increase in TRM observed in our series in comparison with the outcome following grafts from matched sibling donors. RIC transplants tended to be associated with a higher rate of graft failure than myeloablative procedures, but the impact of this type of conditioning on outcome should be evaluated further in prospective trials.

Authorship and Disclosures

FP: conception and design of the study, literature search, collection of data, writing the article; AB: design of the study, interpretation of data, collaboration in writing the article; AS: collection and interpretation of the data; MI, FS: statistical analysis; BB: collection of the data and organization of database; MTvL, API, PDB, FP, PP, GV, PI: collection of clinical data; RF, AB: interpretation of the data and revision of the final version of the article.The authors reported no potential conflicts of interest.

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