Balkan Med J 2023;40:197-204

Allogeneic Hematopoietic Stem Cell Transplantation for Primary Myelofibrosis: A 20-year Experience in a Single Center

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Background: Allogeneic hematopoietic stem cell transplantation is a well-established approach for patients diagnosed with primary myelofibrosis and remains the only potentially curative treatment.

Aims: To present the overall outcome of patients with myelofibrosis treated with allogeneic hematopoietic stem cell transplantation.

Study Design: A retrospective cross-sectional study

Methods: This study is a retrospective analysis of 26 consecutive patients with primary myelofibrosis who underwent transplantation at our center between January 2002 and January 2022. Disease and transplant variables contributing to outcomes were analyzed.

Results: The median age at the time of transplantation was 52.5 (range, 32-63) years and the median time from diagnosis to allogeneic hematopoietic stem cell transplantation was 25 (range, 3.1-156.8) months. Myeloablative conditioning and reduced-intensity conditioning regimens were used in 8 (30.8%) and 18 (69.2%) transplantations, respectively. Neutrophil and platelet engraftment was achieved in 23 patients at a median follow-up of 21.2 months (range, 12 days to 234.8 months). Primary graft failure occurred in 1 of 23 patients (4.3%). Neutrophil and platelet engraftment occurred at a median of 16 (range, 12-39) days and 20 (range, 11-78) days, respectively. Acute graft-versus-host disease was seen in 11 of 22 patients engrafted allografts, of which 7 (31.8%) were grade 3-4 acute graft-versus-host disease. Eight patients (36.4%) developed chronic graft-versus-host disease, and three cases were extensive. Four patients (19%) relapsed after a median of 5.5 months, and three patients received donor lymphocyte infusion. The 3-year overall survival rate of the entire study population was 46.2%. The median overall survival was not reached in the myeloablative conditioning group; however, it was 11.9 months in the reduced-intensity conditioning group ($p = 0.3$). According to the donor graft source, the median overall survival was 0.73 months in mismatched unrelated graft recipients, 12 months in matched sibling donors, and not reached in matched unrelated graft recipients ($p = 0.03$). The 3-year progression-free survival rate of patients who survived > 100 days was 74.7%. The effect of JAK-2 status, graft source, conditioning regimen or dynamic international prognostic scoring system on progression-free survival was not statistically significant.

Conclusion: Given the poor prognosis of non-transplant recipients and the lack of non-transplant curative approaches, our results support the consideration of allogeneic hematopoietic stem cell transplantation for eligible patients with primary myelofibrosis.

INTRODUCTION

Primary myelofibrosis (PMF) is a rare myeloproliferative neoplasm presenting with fibrosis in the bone marrow and ineffective extramedullary hematopoiesis. The median age at diagnosis is 60 years, and despite treatment improvements, the median survival is 5-7 years according to risk scores.[1] Prominent heterogeneities in clinical presentation and outcomes highlight the requirement for a risk-adapted treatment strategy.[1] To target a specific therapeutic goal, disease risks can be balanced with the chosen approach.

Allogeneic hematopoietic stem cell transplantation (AHSCT), with a curative rate of 30-65% in the JAK inhibitor era, remains the only curative treatment for patients with PMF.[2] AHSCT is considered the

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Received: February 11, 2022 Accepted: March 08, 2023 Available Online Date: May 08, 2023 • DOI: 10.4274/balkanmedj.galenos.2023.2022-2-32

Available at www.balkanmedicaljournal.org

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Cite this article as:
Cengiz Seval G, Civriz Bozdağ S, Toprak SK, et al. Allogeneic Hematopoietic Stem Cell Transplantation for Primary Myelofibrosis: A 20-year Experience in a Single Center. *Balkan Med J.*;2023; 40(3):197-204.

"standard of care," with clinical evidence regarding intermediate and high-risk diseases. To determine these risks, several models have been developed.[3] The most used scoring systems include the international prognostic scoring system (IPSS),[4] dynamic IPSS (DIPSS),[5] refined DIPSS (DIPSS-plus),[6] and mutation-enhanced IPSS (MIPSS70).[7] IPSS and DIPSS are based primarily on clinical results, DIPSS-plus includes cytogenetics, and MIPSS70 contains driven mutations. A consensus has been established on the eligibility of the intermediate 2/high-risk group according to DIPSS for AHSCT, whereas the DIPSS-intermediate 1/low-risk group with high-risk mutations may be considered potential candidates for AHSCT.[8] Nevertheless, the optimal AHSCT platform and optimal conditioning regimen have not yet been defined in this setting.

Despite advances in the AHSCT procedure, the post-transplant period can be complex regarding the aggressive, fibrotic, and proinflammatory marrow niche, massive splenomegaly, and an increased risk of poor graft function (PoGF).[9] Despite the improvement in transplant outcomes, higher relapse and non-relapse mortality (NRM) are still observed. Relapse rates may range from 15% to 25%, and relapse management varies greatly.[10-12]

In this study, we retrospectively evaluated the safety and outcomes of AHSCT in patients diagnosed with PMF at our transplant center.

MATERIALS AND METHODS

This retrospective single-center analysis included patients who were diagnosed with PMF and underwent AHSCT between January 2002 and January 2022. The characteristics and treatment outcomes of the patients were evaluated from our institutional database. The institutional ethic committee approved this study (09/05/2022- I05-261-22).

Myelofibrosis was graded according to the European Consensus criteria in pathology specimens.[13] Prognostic risk factors were assessed according to the DIPSS score as previously described at the time of AHSCT.[5] According to the European LeukemiaNet consensus report and the International Working Group-Myeloproliferative Neoplasms Research and Treatment (IWG-MRT), transfusion dependence was described as administration of > 6 units of erythrocytes within 12 weeks for a hemoglobin level < 8.5 g/dl in the absence of treatment-induced anemia or hemorrhage.[14] Peripheral blood JAK-2 mutation status (JAK-2V617F) testing has been performed with polymerase chain reaction testing upon diagnosis, before the AHSCT, on days +28, +60, +90, and +365, and when clinical needs developed.

The performance status and comorbidities of the patients determined the intensity of the conditioning regimen. The reduced-intensity conditioning (RIC) regimens included 180 mg/m^2 fludarabine (FLU) and 6.4 mg/kg intravenous (IV) administration of busulfan (BU, 180 mg/m^2 FLU, and 140 mg/m^2 IV administration of melphalan [MEL]). The conditioning regimen for myeloablative conditioning (MAC) included 12.8 mg/kg IV BU and 120 mg/kg IV cyclophosphamide. Recipients of an unrelated donor (URD) AHSCT routinely received Jurkat cell line-reactive ATG (ATG Fresenius®) in doses of 10 mg/kg/day (HLA-matched) or 15 mg/kg/day (HLA mismatched) IV on days -3, -2, and -1. A calcineurin inhibitor and short-term methotrexate or mycophenolate mofetil were given as graft-versus-host disease (GVHD) prophylaxis. Antiviral, antifungal, and antimicrobial prophylaxis was administered according to our institutional transplant guidelines.

Neutrophil and platelet engraftment was identified as two consecutive days with absolute neutrophil counts > 0.5 x 10^9/l and platelet counts > 20 x 10^9/l without transfusion. Donor chimerism was evaluated on days +28, +60, +90, and +365 and when clinical needs developed. The transformation to acute myeloid leukemia was defined as the presence of > 20% blasts in the bone marrow. Post-transplant bone marrow biopsies were performed on days +28, +90, and +365 and whenever clinically indicated using the IWG-MRT consensus criteria.[14] Relapse/progression was described as observed progression or recurrence of histologically documented fibrosis. Modified Glucksberg and the National Institutes of Health consensus criteria were used to grade acute and chronic GVHD.[15,16] Ruxolitinib (RUXO) failure was defined as a < 10% reduction in the spleen volume according to computed tomography or magnetic resonance imaging, which corresponds to a < 30% reduction in the spleen size by palpation from baseline following an initial response for ≥ 3 months of RUXO treatment.[17] All these patients were withdrawn with RUXO before the conditioning regimen and treated with prophylactic steroids for 5 days.

Statistical Analysis

Fisher's exact test or the χ^2 test was used to compare the categorical variables. Continuous variables were compared using the Kruskal-Wallis test for independent samples. NRM was identified as the duration between transplantation and death due to any cause before relapse/progression. Progression-free survival (PFS) was estimated as the time from AHSCT to disease progression, relapse, or death, whichever occurs first. The time from AHSCT until death from any cause was defined as the overall survival (OS). Cases still alive were censored at the time of the last follow-up.

Kaplan-Meier estimates were used for the calculation of the PFS and OS and comparison of the groups with the log-rank test. To obtain adjusted hazard ratios, the role of continuous prognostic factors was evaluated with the Cox proportional hazards regression model. Tables of survival results at the time point present the number of patients at risk and their 95% confidence intervals (CI). 95% CI is also been reported for hazard ratios from Cox regression. All comparisons are presented with an associated p-value, and *p*-values < 0.05 are accepted as statistically significant. IBM SPSS Statistics version 26 (IBM Corp., Armonk, NY, USA) was used for all analyses and figure creation.

RESULTS

Twenty-six consecutive patients (8 women and 18 men) with a median age of 52.5 (range, 32-63) years underwent transplantation between January 2002 and January 2022 at our institution. Patient characteristics and transplantation variables are shown in Table 1. The median follow-up between diagnosis and AHSCT was 25 (range, 3.1-156.8) months.

Risk stratification with DIPSS at the time of AHSCT was intermediate 2 risk (n = 14, 53.8%) and high-risk (n = 12, 46.2%). The spleen size before AHSCT (via ultrasonography) was enlarged in 18 (69.2%) patients and not enlarged in 3 (11.5%), and 5 (19.2%) patients underwent splenectomy. The JAK-2 mutation status (JAK-2V617F) was analyzed in 22 of 26 patients and was positive in 7 (31.8%) patients. JAK-2 mutation was undetectable in all these patients following AHSCT. Eleven patients (42%) received RUXO treatment before AHSCT, and all of these were non-responsive to RUXO treatment.

MAC and RIC regimens were used in 8 (30.8%) and 18 (69.2%) (FLU-BU, n = 5; FLU-MEL, n = 13) patients, respectively. Of the 26 transplanted allografts, 18 (69.2%) were from HLA-matched sibling donors (MRD), 5 (19.2%) were from HLA-matched URD (MUD), and 3 (11.5%) were from 9/10 matched URD (mmUD). Peripheral stem cells were used in 88.5% of the recipients. The median count of infused $CD34^+$ cells was 6.9 x 10^6 cells/kg body weight (range, 4.2-8.6 x 10^6 cells/kg bodyweight).

In all patients, the median time from AHSCT was 21.2 months (range, 12 days to 234.8 months). Twenty-three patients achieved neutrophil and platelet engraftment. Primary graft failure (PGF) occurred in 1 of 23 patients (4.3%). This patient underwent splenectomy before AHSCT. Neutrophil and platelet engraftment occurred in a median of 16 (range, 12-39) days and 20 (range, 11-78) days, respectively. No statistical significance was found in either neutrophil or platelet engraftment time according to the types of conditioning regimens ($p > 0.5$). Secondary graft failure occurred in one patient who underwent a second AHSCT (sAHSCT) in another center. Of the 23 patients, complete (100%) donor chimerism was observed in all patients, but during follow-up, mixed chimerism (75-90% donor cells) was demonstrated in 4 (17.4%) patients, of which 3 (13%) patients had lost their grafts. PoGF occurred in 3 (13%) patients who had full donor engraftment and transfusion-dependent low blood counts. Among patients with splenomegaly, two had a primary isolated thrombocytopenia and one had severe anemia. Fibrosis was resolved completely in 4 (17.4%) patients, and at least one grade reduction of fibrosis could be achieved in 11 (47.8%) patients at post-transplant day +90. Of note, RUXO exposure before AHSCT was not found to be a risk factor for graft failure.

A relapse occurred in 4 (19%) patients within a median of 5.5 (range, 2.2-9.5) months, and three patients were treated with donor lymphocyte infusion (DLI). None of the patients who underwent splenectomy before AHSCT relapsed after a median post-transplant follow-up of 21.1 months. Two patients developed a transformation to acute myeloblastic leukemia at post-transplantation +4 and +10 months, and both patients achieved hematological remission after induction treatment. Prior RUXO exposure had a non-significant effect on the disease relapse risk (9.1% vs. 20%; $p = 0.6$) in a small patient cohort. Furthermore, five patients received DLI (median dose 4.3 x 10^6 $CD3^+$cells/kg), and GVHD was developed in two of them with an acceleration of donor chimerism in a short time.

Those who died before engraftment (n = 3) and had failed primary engraftment (n = 1) were not assessable for GVHD. Acute GVHD (aGVHD) was seen in 11 of 22 who had transplantation (50%), of which 7 (31.8%) had grade 3-4 aGVHD. aGVHD occurred similarly between patients who underwent splenectomy (40%) and others (42.9%) ($p = 1$), even if the patients with aGVHD who underwent splenectomy received related donor grafts. Only one patient who underwent splenectomy had grade IV aGVHD in the third month of transplantation. The incidence of aGVHD (36.4% vs. 46.7%; $p = 0.7$) was similar between the group with RUXO treatment and without RUXO treatment, whereas severe grade 3-4 aGVHD (83.3% vs. 40%; $p = 0.2$) occurred in the RUXO group. A similar risk of aGVHD for patients receiving related (7/17; 41.2%) and unrelated (4/9; 44.4%) donor grafts was observed ($p = 1$). Chronic GVHD (cGVHD) developed in ei8ght (36.4%) patients, of which three had extensive disease. Of those patients, 5 (62.5%) had MAC and 3 (16.7%) had RIC regimens before AHSCT. In the RUXO group, the incidence of cGVHD was significantly lower (9.1% vs. 46.7%; $p = 0.04$), and no extensive disease was observed. Prior splenectomy had no impact on cGVHD occurrence ($p = 1$). Both aGVHD and cGVHD decreased the relapse risk by 18.2% and 12.5%, respectively.

Among 21 patients who survived > 100 days, the transplant-related mortality (TRM) rate was 16.7% for the MAC group compared with

TABLE 1. Baseline Characteristics of the Patients with Primary Myelofibrosis (PMF).

Total number of patients	n=26
Gender (male/female)	8 (30.8%)/18 (69.2%)
Age (median year)	48.2 (range: 32-63)
Risk groups based on a DIPSS score at allo-HSCT (n)	
Intermediate-2	14 (53.8%)
High	12 (46.2%)
JAK2 mutation before transplantation	(n=22)
Negative	15 (68.2%)
Positive	7 (31.8)
Splenomegaly before transplantation (n)	**18 (69.2%)**
Transfusion dependency at transplant (n)	**22 (84.6%)**
Bone marrow fibrosis before transplant (n)	
Grade 4	14 (63.6%)
Grade 3	12 (46.2%)
HLA matching (n)	
10/10	23 (88.5%)
9/10	3 (11.5%)
Donor type (n)	
Related	18 (69.2%)
Unrelated	8 (30.8%)
Source of stem cells (n)	
Bone marrow	3 (11.5%)
Peripheral blood	23 (88.5%)
Conditioning regimen (n)	
FluMel	13 (50%)
FluBu	5 (19.2%)
BuCy	8 (30.8%)

FluMel, fludarabine and melphalan; FluBu, fludarabine and Busulfan; BuCy, busulfan and cyclophosphamide.

33.3% for the RIC group ($p = 0.6$). Transplant outcomes according to the intensity of the conditioning regimens are summarized in Table 2. The 3-year OS rate for all patients was 46.2%. In the MAC group, the median OS was not reached; however, it was 11.9 months (95% CI, 0.74-23.19) in the RIC group ($p = 0.3$) (Figure 1). JAK-2 mutations did not represent a significant prognostic relevant factor (median OS; presence vs. absence = 25 vs. 17.3 months; $p = 0.9$). No statistical significance in the death incidence was found between the splenectomy and non-splenectomy group (60% vs. 52.4%; $p = 1$); however, the median OS was improved in the non-splenectomized group (25 vs. 5 months; $p = 0.5$). When our patients were classified according to graft source, the estimated median OS was 0.73 months in mmUD, 12 months in MRD, and NR in MUD graft recipients ($p = 0.03$) (Figure 2). All four patients who had a relapse were transplanted from MRD, and the estimated 3-year PFS was 67%. When stratified by DIPSS groups, as expected, the median OS was longer but not statistically significant in patients who have intermediate 2 disease (NR vs. 5.1 months, $p = 0.1$) (Figure 3). The median OS was shortened among patients with aGVHD: 6.6 vs. 25 months, $p = 0.9$. The probability of 3-year PFS for patients who survived > 100 days was 74.7%. JAK-2 status, DIPSS, graft source, or conditioning regimen had no significant effect on the PFS.

In this study, 21 (80.8%) patients were dependent on transfusion before AHSCT. During the post-transplant follow-up, 12 (46.2%) patients became transfusion-independent with normal blood count, and 6 (23.1%) patients remained dependent on transfusion post-transplant. Overall, 61.5% experienced CMV reactivation after AHSCT. In total, 14 of the 26 patients died. Three patients died within a median of 21 days following AHSCT during the aplasia period because of a severe and uncontrolled infection. In addition, nine patients died beyond post-transplant day +100, and the causes of death were GVHD in 3 (aGVHD, n = 2, cGVHD, n = 1), infection in 4, and relapsed disease in 2. Twelve patients were alive and free of GVHD at the time of our analysis.

TABLE 2. Transplant Outcomes According to the Intensity of the Conditioning Regimens.

	MAC (n = 8)	RIC (n = 18)
Neutrophil engraftment, median	19 (range, 12-28) days	16.5 (range, 12-33) days
Platelet engraftment, median	30 (range, 13-68) days	18.5 (range, 11-78) days
GvHD		
Acute	37.5% (n = 3)	44.4% (n = 8)
Chronic	62.5% (n = 5)	16.7% (n = 3)
Overall survival	NR	11.9 (95% CI, 0.74-23.19) months
Relapse	12.5% (n = 1)	16.7% (n = 3)
Non-relapse mortality	25% (n = 2)	27.8% (n = 5)
Transplant-related mortality-30 days	25% (n = 2)	5.6% (n = 1)

GvHD, graft-versus-host disease; MAC, myeloablative conditioning; PFS, progression-free survival; RIC, reduced-intensity conditioning; CI, confidence interval

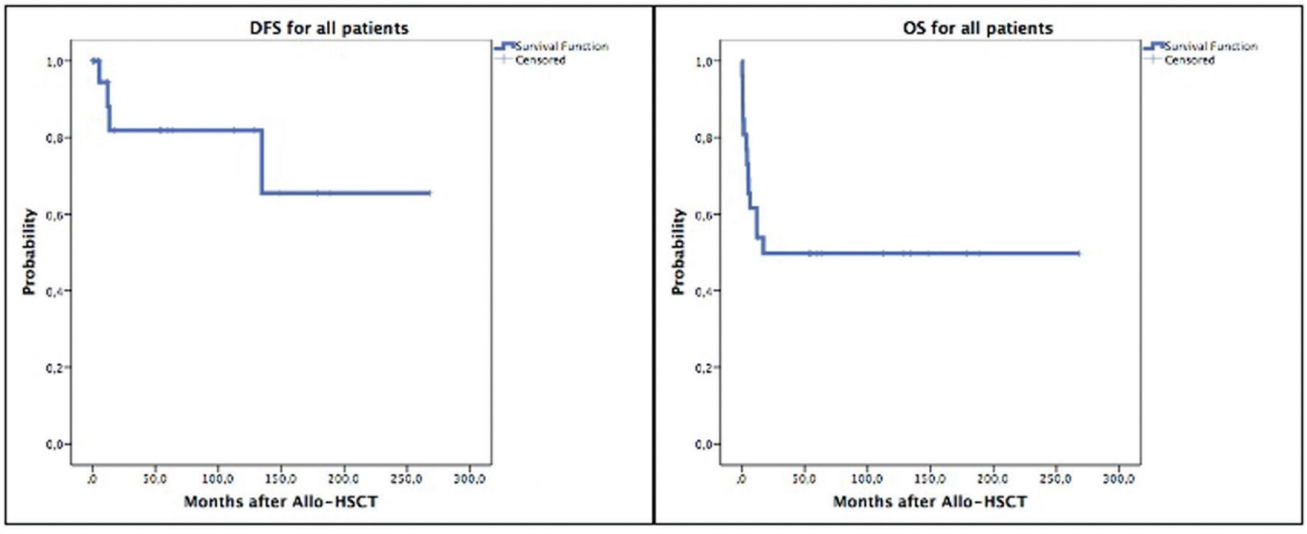

FIG. 1. Disease-free (a) and overall survival (b) for all patients.

DISCUSSION

AHSCT is the only proven curative treatment option for eligible patients with PMF.[2] In this study, we summarized the outcomes of individuals who were transplanted in a single center. Even though the number of patients in our analysis appears limited, it gives significant information for the treatment of PMF, a rare indication for AHSCT and only experienced bone marrow transplantation centers consider it for transplant.

Splenomegaly is one of the most prominent physical findings in PMF as evidenced by extramedullary hematopoiesis and prominent symptoms.[18] Splenomegaly may cause the sequestration of donor stem cells and deferred hematologic recovery; however, its effect on relapse incidence and survival is uncertainç.[19,20] Splenectomy can effectively improve symptoms but results in numerous complications, morbidity, and mortality.[8] Perioperative mortality ranged from 5% to 10%; nevertheless, improvement of event-free survival and OS was demonstrated among patients with pre-transplant splenectomy in a recent study.[21] Of the 26 patients in our cohort, 23 (88.5%) had splenomegaly and five patients underwent splenectomy before AHSCT. During the follow-up, bone marrow fibrosis regressed completely in four patients, and at least one grade reduction of fibrosis could be achieved in 11 patients. In addition, 12 (46.2%) patients became transfusion-independent with a normal blood count. Based on the results of the European Society for Blood and Marrow Transplantation (EBMT) registry study, 74% of the cohort had a complete histo-hematologic response, whereas partial histo-hematologic response and treatment failure could be achieved in 5% and 19% of the patients, respectively.[22]

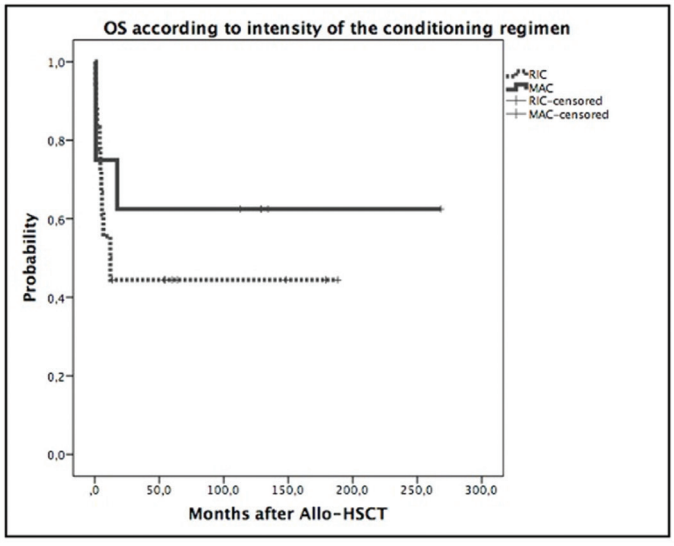

FIG. 2. Overall survival according to the donor-type (matched related donor [MRD] vs. matched unrelated donor [MUD] vs. mismatched unrelated donor [mmUD]).

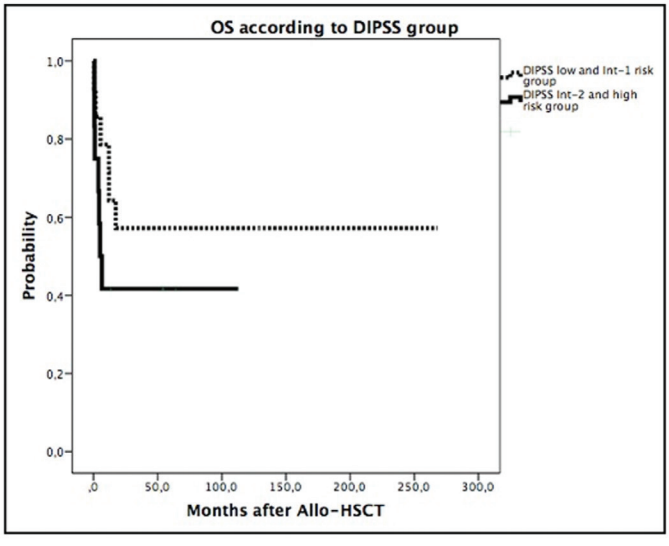

FIG. 3. Overall survival according to the dynamic international prognostic scoring system.

To the best of our knowledge, patient-specific factors were as important as disease-specific parameters in predicting transplantation outcomes. Several studies have revealed a correlation between the DIPSS risk category and post-transplantation mortality.[23,24] Eligible patients with higher-risk myelofibrosis (e.g., DIPSS-intermediate 2 or high-risk) should be considered for AHSCT,[8] and the survival outcomes were found in the entire study population compared with the favorable natural history of PMF, with a median OS of 4 and 1.5 years for intermediate-2 and high-risk DIPSS cases, respectively. Consistent with previous trials, the estimated 5-year OS rates were 51.1% in intermediate-2 and 33.3% in high-risk patient groups by DIPSS. On the contrary, a systemic meta-analysis (including 43 studies with 8739 patients with myelofibrosis) was recently published, presenting the proven role of AHSCT in identifying disease, baseline patients, and transplant characteristics with prognostic effects on outcomes. Subgroup analysis did not show any difference between the ratios of the DIPSS-intermediate 2/high-risk group.[25] Based on these results, the poor outcomes among the higher group can be abrogated by AHSCT. However, the sample sizes of each subgroup in included studies are limited and heterogeneous in this meta-analysis. A large recent registry evaluation in the Center for International Blood and Marrow Transplant Research (CIBMTR) (n = 551) presented an OS advantage with AHSCT among patients with intermediate-1 DIPSS, albeit at the cost of early NRM.[23]

The significant influence of the donor-type on the survival of the whole patient group was demonstrated in various trials.[26-28] EBMT[27] and CIBMTR[28] registries have shown the unfavorable effect of URD on AHSCT outcomes. The CIBMTR group evaluated the outcomes of the 233 AHSCT and found a donor source as an independent TRM risk factor, with a relative risk of death: MUD with 3.92 and mmUD with 9.37, when compared with MRD. As a result, the 5-year OS rates were 56%, 48%, and 34% for MRD, MUD, and mmUD, respectively.[29] Similar results were demonstrated in other studies; otherwise, contrasting data also had been reported.[22,29-32] Our results recapitulate the published trials demonstrating significantly worse outcomes in mmUD graft recipients compared with matched donor graft recipients, with a median OS of 0.73 months vs. not reached (p = 0.01). ATG was

administered as part of GVHD prophylaxis to all URD graft recipients. On that account, different donor types developed similar aGVHD or cGVHD rates in our cohort.

No large, prospective, and randomized trial has compared the conditioning intensity in a transplant setting. Conditioning regimens appear to be one of the major factors related to transplantation outcomes. Because of the wider use of RIC, the number of transplant-eligible patients increased, and the NRM reduced in older and frailer patients.[8] The first prospective EBMT multicenter phase II trial of RIC AHSCT demonstrated low PGF rates and rapid hematologic recovery.[22] OS outcomes with RIC and MAC were reported similarly in a meta-analysis[25] and the largest retrospective study conducted by EBMT.[12] Based on the results of this study, younger and fitter candidates should be considered for MAC to improve GVHD-free and relapse-free survival.[12] Stewart et al.[33] reported 3-year NRM rates of 41% and 32% for the MAC and RIC groups, respectively. Even though our study is limited to showing the effect of the conditioning regimen on survival, 1- and 3-year OS rates were 50% and 38.9% in the RIC group and 87.5% and 75% in the MAC group, with 5-year OS > 50% in the MAC group.

Graft failure is one of the well-known factors contributing to increased mortality after AHSCT. PGF is defined as the lack of engraftment of donor stem cells and is characterized by cytopenia, with mixed or no donor chimerism.[34] PGF must be defined to differentiate it from PoGF or cytopenia with full donor chimerism.[35] The incidence of PGF is between 2% and 24%.[8] Of 103 patients, 2 developed PGF in a prospective EBMT study. Therefore, PoGF was seen in 11% of patients who received additional stem cell boast.[12] The effect of the donor source on the incidence of PGF was shown in the CIBMTR study, which was rare with HLA identical donor grafts (9%) when compared with family mismatched (27%) and unrelated (20%) donor grafts.[26] In the current series, PGF occurred in up to 4.3% of patients. PoGF occurred in 3 (13%) patients with splenomegaly and no spleen response after AHSCT. Our results highlight the effect of the spleen size on graft failure risk in the PMF setting.

aGVHD is the most important factor in determining transplantation success. EBMT conducted a retrospective study to evaluate 2916 patients with myelofibrosis.[27] According to their results, grade II-IV aGVHD was significantly associated with increased NRM, whereas grade I-II aGVHD had no significant effect. They also reported increased grade II-IV aGVHD risk associated with URD, and regarding conditioning intensity, as expected, severe aGVHD was reduced with MAC regimens.[27] McLornan et al.[12] analyzed 2,224 patients from the EBMT registry, who underwent transplantation between 2000 and 2014, and inconsistent with prior results, they could not demonstrate the effect of the conditioning regimen on aGVHD and cGVHD rates. According to our single-institution results, of the 42 patients with myelofibrosis, 38% developed grade III-IV aGVHD, and an increased aGVHD risk was observed among URD graft recipients, with no statistically significant differences.[30] Conflicting results have been established in the influence of donor-type and conditioning intensity on aGVHD risk in myelofibrosis, as in the aforementioned trials. aGVHD and cGVHD incidences have been sought in 36 and 32 trials, including 5,334 and 4,962 patients with myelofibrosis who underwent AHSCT, respectively.[24] Patients with aGVHD accounted for 44% of all the included population, in which 15.2% had grade III-IV GVHD. cGVHD developed in 46.5% of the recipients, and extensive or moderate/severe cGVHD occurred in 26.1%.[25] Two multicenter studies from Italy[36] and CIBMTR[28] demonstrated no association between cGVHD and OS in myelofibrosis. In a retrospective EBMT registry study, a lower relapse risk was observed in cGVHD, which did not translate into prolonged OS regarding the increased NRM mediated by extensive cGVHD.[27] Based on the results of the present study, any grade of aGVHD and cGVHD occurred in 50% and 36.4% of the patients, respectively, bearing in mind that AHSCT may be associated with significant complications among patients with PMF. Notably, the aGVHD group had an increased NRM rate (36.4%) with 18.2% relapse risk and an unfavorable effect on median OS (6.6 vs. 25 months; $p = 0.9$).

Despite advances in AHSCT conditions and the significantly prolonged survival, post-transplant primary disease relapse remains the major cause of death and warrants any improved prognostication and prevention. In a retrospective study conducted with 1,055 patients diagnosed with myelofibrosis from the EBMT registry, the main cause of death (41-61%) was a disease relapse for all established periods (2-5 and 5-10 years).[37] Unfortunately, no standardized re-treatment for post-transplant relapse was proven. Based on limited published data, RUXO, DLI, and sAHSCT may be considered according to the patient's age, fragility, molecular or hematologic relapse, and GVHD occurrence.[8] Klyuchnikov et al.[38] reported a complete response to DLI in 39% of patients who relapsed after reduced-intensity allografting. Based on the results of a recent real-life retrospective evaluation including patients with myelofibrosis from the EBMT registry, who relapsed after AHSCT, 23% of the patients received DLI, 25% underwent sAHSCT alone, and 13% were treated with DLI and sAHSCT. The median OS periods from the time of relapse for the patients receiving DLI alone, DLI followed by a second AHSCT, and second AHSCT were 76, 54, and 27 months, respectively.[10] In our cohort, DLI was given in five patients, which increased the donor-type chimerism, and GVHD occurred in three patients within a median of 26 days after DLI.

Limitations to our conclusion include the small sample size, retrospective analyses, and incomplete data for molecular profiles; thus, we could not calculate accurate and molecular prognostic scoring systems. However, the quality of the evidence is limited by the randomized clinical trials in the field and the heterogeneity of patients and transplant characteristics across the included studies. Consistent with this observation, our study presents one of the largest single-center experiences, including only PMF. In addition, given the poor prognosis of patients not receiving transplants and in the absence of curative non-transplantation therapies, our results support the consideration of AHSCT for eligible patients with PMF. The timing of transplantation, role of splenectomy before AHSCT, and intensity of conditioning regimens remain relatively high and need to be analyzed in prospective, randomized trials to improve outcomes.

Ethics Committee Approval: The Ankara University School of Medicine Ethic Committee Approved this study (09/05/2022- I05-261-22).

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authorship Contributions: Concept- G.C.S., S.C.B., M.O.; Design- G.C.S., S.C.B., M.O.; Data Collection and Processing- G.C.S., S.C.B., S.K.T., M.K.Y., P.T., O.A., T.D., G.G., M.B., O.I., M.O.; Analysis or Interpretation- G.C.S., S.C.B., S.K.T., M.K.Y., P.T., O.A., T.D., G.G., M.B., O.I., M.O.; Literature Search- G.C.S., S.C.B.; Writing- G.C.S., S.C.B., M.O.

Conflict of Interest: No conflict of interest was declared by the authors.

Funding: The authors declared that this study received no financial support.

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