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Incidence, Risk Factors, and Outcomes of Primary Poor Graft Function after Allogeneic Hematopoietic Stem Cell Transplantation

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A B S T R A C T

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a curative therapy for both malignant and non-malignant hematologic disorders. However, primary poor graft function (PGF) is a serious early complication of allo-HSCT that leads to a poor outcome. Little is known about the characteristics, incidence, and risk factors of primary PGF occurring after allo-HSCT. Here we performed a 1:4 ratio nested case-control study in 830 patients who underwent allo-HSCT between April 2013 and November 2018 at our center. Twenty-four patients (14 males and 10 females; average age, 35.79 years; range, 17 to 53 years) developed primary PGF. On univariate and multivariate analyses, a $CD34^+$ cell dose $<5 \times 10^6$/kg ($P = .003$), a serum ferritin (SF) level >2000 ng/mL ($P = .008$), and splenomegaly ($P = .039$) were identified as 3 independent risk factors for primary PGF. After a median follow-up of 7.5 months (range, 1 to 48 months), only 5 patients (20.8%) survived. The survival rate of patients with primary PGF was significantly lower than that of patients with good graft function (GGF) (1-year overall survival, 25.0% versus 90.6%; $P < .001$). Cox regression analysis suggested that PGF and high SF level were strongly associated with rapid death in these patients. In conclusion, allo-HSCT recipients with a low $CD34^+$ cell dose in their graft and exhibiting a high SF level and splenomegaly should be monitored for the development of primary PGF after allo-HSCT, and effective therapies need to be explored.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a curative treatment that has benefited millions of patients with blood diseases over the last several decades. However, there are several long-standing problems, including poor graft function (PGF), a rare complication that is both fatal and intractable. According to recent studies, PGF was defined as at least bilinear severe cytopenia (persistent thrombocytopenia $\leq 20 \times 10^9$/L and hemoglobin ≤70 g/L, and/or neutropenia $\leq .5 \times 10^9$/L) after engraftment with hypocellular bone marrow and full donor chimerism, without severe graft-versus-host disease (GVHD) or disease relapse [1,2]. In contrast to PGF, graft rejection is defined as mixed chimerism or complete recipient chimerism, and it was excluded in patients with PGF. Other potential causes of pancytopenia after allo-HSCT, such as active infectious diseases or drug-induced myelosuppression, were excluded as well [3]. In concordance with graft rejection having a very poor prognosis [4], PGF has a high mortality rate due to infection and bleeding, especially in those patients who never achieve initial engraftment (primary PGF). Previous studies have suggested that cell dose, donor type, blood mismatch, GVHD, and cytomegalovirus infection are closely related to PGF. Despite the several treatment approaches available for PGF, not all patients achieve satisfactory results, and the prognosis of these patients remains poor. Compared with patients with secondary PGF, patients with primary PGF have a much lower hematologic recovery rate [5]. The exact pathogenesis of primary PGF requires further exploration. Monitoring high-risk patients and early prevention are also important.

We performed a nested case-control study in our center to examine the risk factors and outcomes in patients with primary PGF, with the goal of identifying patients at high risk for primary PGF and eventually leading to the development of better prevention and treatment strategies.

METHODS

Patients

A total of 830 patients who underwent allo-HSCT at our center between April 2013 and November 2018 were retrospectively reviewed. Twenty-four

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of these patients met the criteria for a diagnosis of primary PGF. Written informed consent was obtained from each patient or a relative before transplantation. The study protocol was in compliance with the Declaration of Helsinki and was approved by the Ethics Review Committee of the First Affiliated Hospital of Zhejiang University. The authors had full access to the data and assumes responsibility for its authenticity.

Control Selection

Once a patient with primary PGF was identified, 4 patients with good graft function (GGF) of the same sex and age (± 5 years) who underwent allo-HSCT in the same month were selected as matched controls.

Definitions

The definition of PGF has been described previously. PGF can be divided into 2 types: primary PGF, in which initial engraftment did not occur, and secondary PGF, occurring after initial engraftment. GGF was defined as engraftment of both neutrophils and platelets and hemoglobin concentration >70 g/L without transfusion support beyond day 28 post-transplantation [2].

In healthy adults, the normal spleen typically measures 12 cm along its craniocaudal length, 7 cm in anteroposterior width, and 3 to 4 cm in thickness [6]. Before receiving a pretransplantation conditioning regimen, each patient underwent a splenic ultrasound examination. Splenomegaly was defined as splenic thickness >4 cm or craniocaudal length >12 cm.

Disease status relies mainly on bone marrow aspiration/biopsy and minimal residual disease. For patients with aplastic anemia (AA), nonremission was defined as no response after treatment or disease progression to severe AA (SAA).

The patients were classified into a high-risk group and a low-risk group. Using the International Prognostic Scoring System model, patients with myelodysplastic syndrome (MDS) and myeloproliferative neoplasm at or above intermediate risk-2 were included in the high-risk group. For patients with acute leukemia (AL), the criteria for a high-risk classification were based mainly on cytogenetics and molecular abnormalities. For acute myelogenous leukemia (AML), the high-risk group included patients with intermediate-risk and poor-risk status. For acute lymphoblastic leukemia (ALL), the high-risk group included patients with poor risk status, and high risk was also defined by the therapeutic reaction, central nervous system leukemia, and a leukocyte count $>30 \times 10^9$/L for B-lineage ALL or $>100 \times 10^9$/L for T-lineage ALL at diagnosis. In addition, hemophagocytic lymphohistiocytosis and SAA were also identified as high-risk diseases.

Hematopoietic response (HR) was defined as a persistent neutrophil count $>.5 \times 10^9$/L, platelet count $>20 \times 10^9$/L, and hemoglobin concentration >70 g/L without transfusion or use of agents that promote hematopoiesis for at least 7 consecutive days.

Transplantation Conditioning Regimen

Among the 120 patients, 113 received a myeloablative conditioning regimen [6]. For patients with a matched sibling donor or a matched unrelated donor, the main myeloablative conditioning regimen was BuCy (busulfan 3.2 mg/kg/day i.v. on days -7 to -4 and cyclophosphamide 60 mg/kg/day i.v. on days -3 to -2). For those patients with CNS leukemia or a high leukocyte count at diagnosis or nonremisson or partial remission status before transplantation, the conditioning regimen included cytarabine (2 g/m^2/day i.v. on days -8 to -7), Bu (3.2 mg/kg/day i.v. on days -6 to -4), Cy (60 mg/kg/day i.v. on days -3 to -2), and methyl-N-(2-chloroethyl)-N-cyclohexyl-N-nitrosourea (Me-CCNU) (250 mg/m^2 orally on day -1). Rabbit antithymocyte globulin (ATG, Thymoglobulin; Genzyme, Cambridge, MA) was also administered to patients undergoing URD HSCT (4.5 to 6 mg/kg total dose). For recipients of HLA-haploidentical related donor HSCT, the conditioning regimen was Ara-BuCy-Me-CCNU-ATG, which included cytarabine (4 g/m^2/day i.v. on days -10 to -9), Bu (3.2 mg/kg/day i.v. on days -8 to -6), Cy (60 mg/kg/day i.v. on days -5 to -4), Me-CCNU (250 mg/m^2 orally on day -3), and anti-T lymphocyte globulin (ATG-F; Fresenius, Bad Homburg, Germany) (2.5 mg/kg/day i.v. on days -5 to -2) or rabbit ATG (1.5 mg/kg/day i.v. on days -5 to -2).

The other 7 patients received a Flu-Bu-ATG regimen (fludarabine 30 mg/m^2/day i.v. on days -10 to d -5, busulfan 3.2 mg/kg/day i.v. on days -6 to -5, ATG 5 mg/kg/day i.v. on days -4 to -1) for nonmyeloablative conditioning [7].

Chimerism Analysis

Chimerism was tested in each patient at 4 weeks, 8 weeks, 12 weeks, 6 months, and 12 months post-transplantation using DNA fingerprinting for short tandem repeats in blood samples and chromosome fluorescence in situ hybridization of bone marrow samples. Complete donor chimerism was defined as no recipient hematopoietic or lymphoid cells detected.

GVHD Prophylaxis

Consistent with our previous work [7,8], all patients who underwent allo-HSCT in our center received cyclosporin A, methotrexate, and low-dose mycophenolate mofetil for GVHD prophylaxis.

Treatment of PGF

Supportive treatment was provided to all patients with primary PGF, including blood transfusion, granulocyte/granulocyte-macrophage colony-stimulating factor, thrombopoietin, and i.v. immune globulin. For patients with cryopreserved stem cells or freshly granulocyte colony-stimulating factor-mobilized peripheral blood stem cells from the same donor, a second donor stem cell infusion was provided, not preceded by administration of any chemotherapy or a conditioning regimen. Some patients opted for mesenchymal stem cell (MSC) infusion, which was given at a dose of 1×10^6 cells/kg i.v. weekly for 2 weeks. If no hematopoietic response was observed by 4 weeks, a third round of MSC treatment was administered.

Statistical Analysis

All clinical data were analyzed using SPSS (SPSS, Chicago, IL) and Prism 5 (GraphPad Software, La Jolla, CA). Continuous variables were summarized as mean or median, and categorical variables were expressed as count and percentage. The chi-square test and Fisher's exact test were used to compare incidence in univariate analysis. Potential risk factors with $P < .10$ were analyzed by multivariate logistic regression. To examine whether these independent risk factors could accurately predict PGF, an receiver operating characteristic (ROC) curve analysis was performed. In addition, the Kaplan-Meier method and Cox regression were used successively to estimate survival curves. All statistical tests were 2-sided, and a *P*value <.05 was considered statistically significant.

RESULTS

Incidence and Characteristics

Of the 830 patients who underwent allo-HSCT between April 2013 and December 2018, 43 (5.18%) were diagnosed with PGF. Twenty-four patients (2.89%) met the criteria for primary PGF, and the other 19 had secondary PGF (Figure 1). The characteristics of the patients with primary PGF are presented in Table 1. The primary PGF group comprised 14 males and 10 females, of whom 16 were diagnosed with AL, 3 with MDS, 2 with AA, 2 with myeloproliferative neoplasm, and 1 with hemophagocytic lymphohistiocytosis. The average age of recipients and donors was 35.79 years (range, 17 to 53 years) and 36.67 years (range, 13 to 61 years), respectively. Of the 24 patients with primary PGF, 21 underwent HLA-haploidentical related donor HSCT, 2 underwent matched unrelated donor HSCT, and 1 underwent matched sibling donor HSCT.

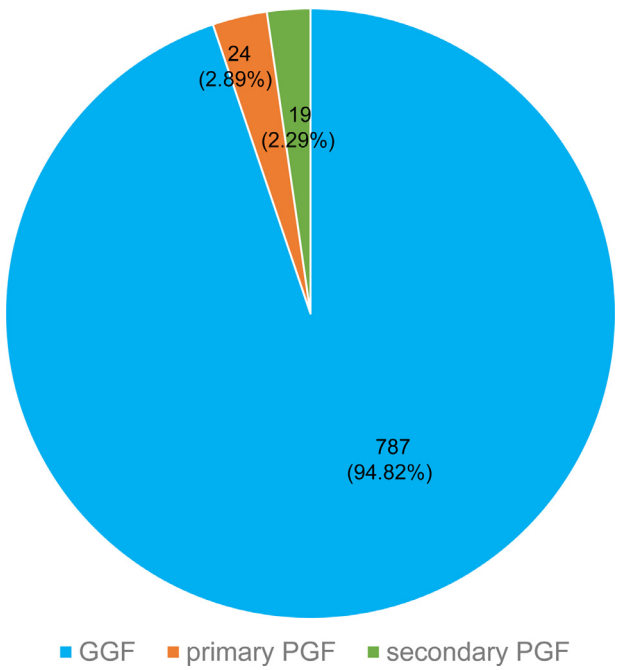

Figure 1. Groups and numbers of patients.

Table 1
Characteristics of Patients with Primary PGF

ID	Age, yr	Sex	Disease	Splenomegaly	Disease State	Risk Stratification	Donor Type	SF, ng/mL	MNCs ($\times 10^8$)	$CD34^+$ ($\times 10^6$)	CMV
1	40	M	MPN	Yes	CR	HR	HRD	2248	11.2	4.7	No
2	45	F	AL	No	PR	HR	MUD	522.3	10.09	6.03	No
3	25	M	AL	Yes	CR	HR	HRD	2471	25.29	3.63	Yes
4	38	F	MDS	No	CR	HR	HRD	4684	7.35	3.51	No
5	42	F	AL	No	CR	HR	MSD	348	9.55	0.7	No
6	39	M	MDS	No	NR	HR	HRD	4674	7.12	2.91	No
7	49	M	AL	Yes	NR	HR	HRD	1164	16.75	7.28	Yes
8	34	M	AL	Yes	CR	HR	HRD	2254	9.26	1.91	Yes
9	25	F	MDS	No	CR	HR	MUD	663.5	6.1	2.2	No
10	29	M	AL	Yes	NR	HR	HRD	6750	15.7	4.4	No
11	32	M	AL	Yes	CR	HR	HRD	530	4.2	3	No
12	39	F	AL	Yes	CR	HR	HRD	737.6	22.7	4.46	Yes
13	42	M	AL	No	CR	HR	HRD	2691	13.68	7.27	No
14	21	M	AA	No	NR	HR	HRD	3919	6.01	1.21	Yes
15	30	F	AL	No	CR	HR	HRD	5055	11.17	5.2	No
16	34	F	AL	No	CR	LR	HRD	114.5	9.15	4.31	No
17	43	F	MPN	Yes	PR	HR	HRD	114.6	27.1	18.6	No
18	38	F	AL	No	CR	LR	HRD	23.1	6.52	1.19	No
19	43	F	AL	No	CR	HR	HRD	402.5	15.07	5.89	No
20	53	M	AL	No	CR	HR	HRD	2992	19.5	4.6	No
21	29	M	AL	No	CR	LR	HRD	1921	19.221	4.424	No
22	26	M	HLH	Yes	/	HR	HRD	243.2	7.88	2.55	No
23	46	M	AL	No	CR	LR	HRD	5318	10.8	3.8	Yes
24	17	M	AA	No	NR	HR	HRD	8460	6.398	2.28	Yes

MNCs indicates mononuclear cells; CMV, cytomegalovirus; M, male; F, female; MPN, myeloproliferative neoplasm; CR, complete remission; PR, partial remission; NR, Nonremission; HR, high risk; LR, low risk; HRD, HLA-haploidentical related donor; MUD, matched unrelated donor; MSD, matched sibling donor; HLH, hemophagocytic lymphohistiocytosis.

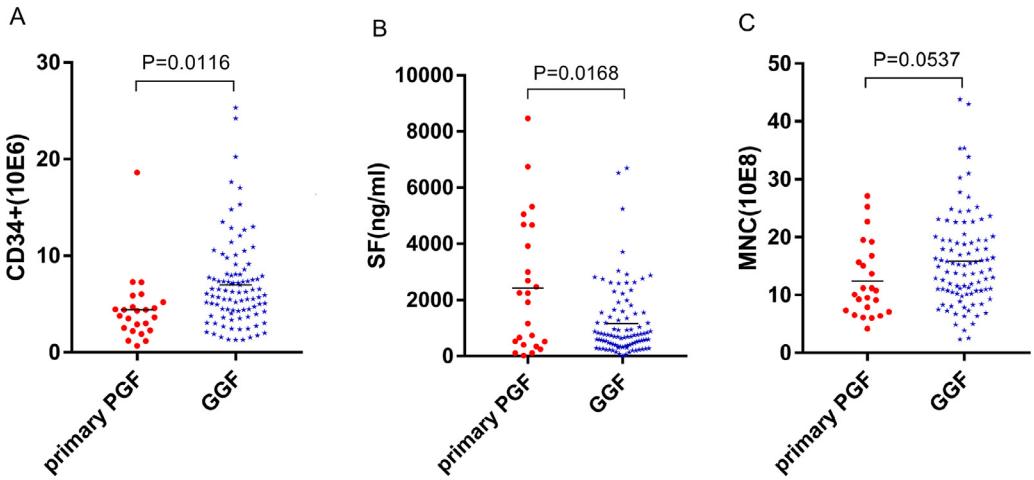

Figure 2. (A) Mean CD34$^+$ cell dose infused in the primary PGF group and GGF group. (B) Mean SF in the primary PGF group and GGF group. (C) Mean mononuclear cell dose infused in the primary PGF group and GGF group.

Compared with the GGF group, the primary PGF group had a much lower mean CD34$^+$ cell dose (4.42×10^6/kg versus 6.99×10^6/kg; $P = .0116$) (Figure 2A). The mean serum ferritin (SF) level was almost twice as high in the primary PGF group compared with the GGF group (2429.16 ng/mL versus 1158.69 ng/mL; $P = .0168$) (Figure 2B). Of the 120 patients analyzed, 116 (96.7%) received peripheral blood stem cells, and the other 4 (including 3 with AA) received stem cells from both the bone marrow and peripheral blood. The mean interval from diagnosis to transplantation was 17.04 months for the primary PGF group and 12.46 months for the GGF group. The average number of chemotherapy courses was similar in the 2 groups (5.50 in the primary PGF group versus 5.41 in the GGF group), and the mean mononuclear cell count was high in both groups (12.41×10^8/kg in the primary PGF group versus 15.90×10^8/kg in the GGF group; $P = .0537$).

Risk Factors for Primary PGF

In univariate analysis, disease, time from diagnosis to transplantation, splenomegaly, disease states, risk stratification, SF level, cytomegalovirus infection, and CD34$^+$ cell dose in the graft were identified as risk factors ($P < .05$) (Table 2). There were no significant between-group differences in patient/donor age, number of chemotherapy courses, donor type, blood mismatch, sex mismatch, myelofibrosis, conditioning regimen, aGVHD grade, and Epstein-Barr virus infection. On multivariate logistic analysis, 3 independent risk factors were identified: CD34$^+$ cell dose $<5 \times 10^6$/kg ($P = .003$; odds ratio [OR], 5.089; 95% confidence interval (CI), 1.745 to 14.841), SF level >2000 ng/mL ($P = .008$; OR, 4.147; 95% CI, 1.452 to 11.845), and splenomegaly ($P = .039$; OR, 3.306; 95% CI, 1.062 to 10.289) (Table 3). We also performed an ROC curve analysis on these 3 independent risk factors and found a predictive value of .789 (Figure 3A). For the AL and MDS subgroup, low CD34$^+$ cell dose ($P = .003$; OR, 5.635; 95% CI, 1.800 to 17.635) and high SF level ($P = .031$; OR, 3.408; 95% CI, 1.120 to 10.376) were 2 independent risk factors for primary PGF, and the predictive value of the ROC curve analysis was .762 (Figure 3B).

Treatment and Outcomes of Primary PGF

All patients with primary PGF received supportive treatment and other salvage treatments; 9 (37.5%) achieved HR at a median interval of 48 days (range, 40 to 95 days). The cell count dynamics of these 9 patients are presented in Figure 4. Seven patients (29.2%) had a response totally attributed to supportive treatments. For salvage treatments, 6 patients received a second donor stem cell infusion; however, HR was observed in only 1 patient at 14 days after the second infusion. Three patients received MSC treatment, and 1 patient achieved a rapid HR at 11 days after infusion. After a median follow-up of 7.5 months (range, 1 to 48 months), only 5 patients (20.8%) patients survived until the time of this report. Twelve patients died of multiple organ failure due to severe infection, 4 patients died from disease relapse, 1 patient died of intracranial hemorrhage, and 2 patients died of gastrointestinal hemorrhage (Figure 5). Compared with the GGF group, what is striking to us is that patients with primary PGF experienced significantly poor overall survival (1-year overall survival [OS], 25.0% versus 90.6%; $P < .0001$) (Figure 6A). For the patients with AL and MDS, results from each subgroup also showed that patients with primary PGF had a much poorer outcomes compared with patients with GGF (Figure 6B and C). Furthermore, using a Cox regression model, we identified PGF ($P < .001$; OR, 13.214; 95% CI, 5.551 to 31.459) and high SF level ($P = .044$; OR, 2.290; 95% CI, 1.021 to 5.136) as 2 independent factors related to poor OS for all patients (Table 4).

DISCUSSION

PGF is a life-threatening post-HSCT complication. The incidence of PGF for hematologic malignancies ranged from 5% to 27% in previous reports [5,9]. In our center, the incidence of PGF was 5.18%, and that of primary PGF was 2.89%. Our patients with primary PGF had a dismal prognosis (1-year OS, 25.0%), indicating an urgent need to explore the risk factors for primary PGF and search for effective prevention strategies.

In previous studies, a higher donor cell dose was associated with a decreased risk of graft failure [10,11]. Moreover, CD34$^+$ cells, which consist of hematopoietic stem cells and progenitor cells, are crucial for hematopoietic and immune reconstitution. Notably, based on the definition used here, the incidence of primary PGF was significantly lower in our study compared with that reported by Sun et al [1] (2.89% versus 5.6%; $P = .015$). This discrepancy may be explained by the significantly higher median CD34$^+$ cell dose in our study (5.5×10^6/kg versus 2.21×10^6/kg), indicating that a higher CD34$^+$ cell dose is important to reduce the risk of developing primary PGF.

It has been recently recognized that patients with PGF treated with a boost of CD34$^+$-selected stem cells without

Table 2
Univariate Analysis of Risk Factors for Primary PGF

Risk Factor	Primary PGF Group (N = 24), n (%)	GGF Group (N = 96), n (%)	*P* Value
Disease			
AL	16 (66.7)	81 (84.4)	.003
MDS	3 (12.5)	7 (7.3)	
AA	2 (8.3)	1 (1.0)	
NHL	0 (0)	7 (7.3)	
MPN	2 (8.3)	0 (0)	
HLH	1 (4.2)	0 (0)	
Age, yr			
≤35	9 (37.5)	48 (50.0)	.273
>35	15 (62.5)	48 (50.0)	
Time from diagnosis to transplantation, mo			
≤6	5 (20.8)	35 (36.5)	.039
≤12	8 (33.3)	41 (42.7)	
>12	11 (45.8)	20 (20.8)	
Myelofibrosis			
No	21 (87.5)	91 (94.8)	.197
Yes	3 (12.5)	5 (5.2)	
Splenomegaly			
No	15 (62.5)	82 (85.4)	.018
Yes	9 (37.5)	14 (14.6)	
Disease states at HSCT			
CR	17 (70.8)	87 (90.6)	.018
NR or PR	7 (29.2)	9 (9.4)	
Risk stratification			
Low risk	4 (16.7)	38 (39.6)	.035
High risk	20 (83.3)	58 (60.4)	
Chemotherapy courses before HSCT			
≤5	13 (54.2)	64 (66.7)	.253
>5	11 (45.8)	32 (33.3)	
Conditioning regimen			
Myeloablative	21 (87.5)	92 (95.8)	.142
Nonmyeloablative	3 (12.5)	4 (4.2)	
Donor type			
HRD	21 (87.5)	67 (69.8)	.098
MSD	1 (4.2)	21 (21.9)	
URD	2 (8.3)	8 (8.3)	
Donor age, yr			
≤35	14 (58.3)	44 (47.3)	.336
>35	10 (41.7)	49 (52.7)	
Blood mismatch			
Identical	16 (66.7)	51 (53.1)	.232
Mismatch	8 (33.3)	45 (46.9)	
Sex mismatch			
Identical	10 (41.7)	54 (56.3)	.254
Mismatch	14 (58.3)	42 (43.8)	
SF level, ng/mL			
≤2000	12 (50.0)	79 (82.3)	.001
>2000	12 (50.0)	17 (17.7)	
$CD34^+$ cell dose, $\times 10^6$/kg			
<5	18 (75.0)	33 (34.4)	<.001
≥5	6 (25.0)	63 (65.6)	
aGVHD			
No	20 (83.3)	65 (67.7)	.638
Grade I	3 (12.5)	20 (20.8)	
Grade II	1 (4.2)	9 (9.4)	
Grade III	0 (0)	2 (2.1)	
CMV infection in 30 days			
No	17 (70.8)	87 (90.6)	.018
Yes	7 (29.2)	9 (9.4)	
Epstein-Barr virus infection in 30 days			
No	15 (62.5)	74 (77.1)	.144
Yes	9 (37.5)	22 (22.9)	

NHL, Non-Hodgkin lymphoma.

Table 3
Multivariate Logistic Analysis of Risk Factors for Primary PGF

Potential Risk Factors (P <.10)	All Allo-HSCT Recipients		AL and MDS Subgroup	
	P value	OR	*P* value	OR
Disease	.096			
Time from diagnosis to HSCT, mo	.279		.256	
Splenomegaly	**.039**	**3.306**	.111	
Disease state at HSCT	.324		.341	
Risk stratification	.226		.294	
Donor type	.763		.195	
SF level, ng/mL	**.008**	**4.417**	**.031**	**3.408**
$CD34^+$ cell dose, $\times 10^6$/kg	**.003**	**5.089**	**.003**	**5.635**
CMV infection in 30 d	.642		.657	

Significant values are in bold type.

further conditioning have a good chance of hematopoietic recovery and with a low risk of GVHD. In the patients with PGF who had a hematopoietic response, the median $CD34^+$ dose was higher than that in the patients who did not show a response. In a recent study, a $CD34^+$ cell dose $>3.25 \times 10^6$/kg was related to a better improvement in neutrophil count [12]; however, no multivariate analysis found a cutoff value of $CD34^+$ cells correlated with hematopoietic recovery. We suspected that two aspects may affect the result. First, there was no unified standard of hematopoietic recovery. Second, a large proportion of second PGF in some research may interfere with the result. When we exclude reasearch mainly about Primary PGF, and define hematopoietic recovery as neutrophils $>1.0 \pm 0.5 \times 10^9$/L, hemoglobin $>80 \pm 5$ g/L, and platelets $>25 \pm 5 \times 10^9$/L, there is a tendency for that higher doses of CD34+-selected cells to lead to better outcomes [12–15]. In summary, a higher $CD34^+$ cell dose could reduce the incidence of primary PGF and improve the outcomes of patients with primary PGF.

Iron is an essential element for hematopoiesis and the excess part is mainly stored in the liver, spleen, bone marrow, muscle, as well as blood. SF, a form of iron storage, widely involved in metabolic activities, also has been used as a biomarker of iron overload. Several factors, such as tumor cell proliferation, long-term blood transfusion, ineffective erythropoiesis, and inflammation, could lead to iron overload. Iron overload not only causes liver and heart damage, but also has an inhibitory effect on the bone marrow microenvironment and medullary hematopoiesis. In normal circumstances, hepcidin expressed by the liver regulates intestinal iron absorption and iron release from the store to meet the demands of

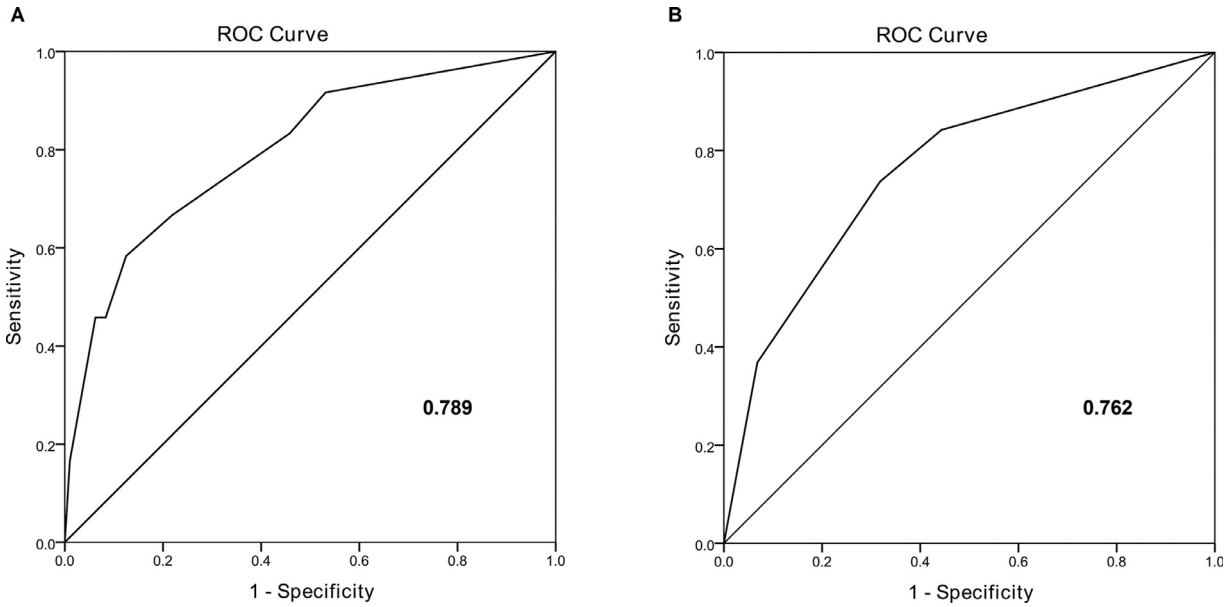

Figure 3. ROC curve analysis of (A) all patients and (B) AL and MDS subgroup.

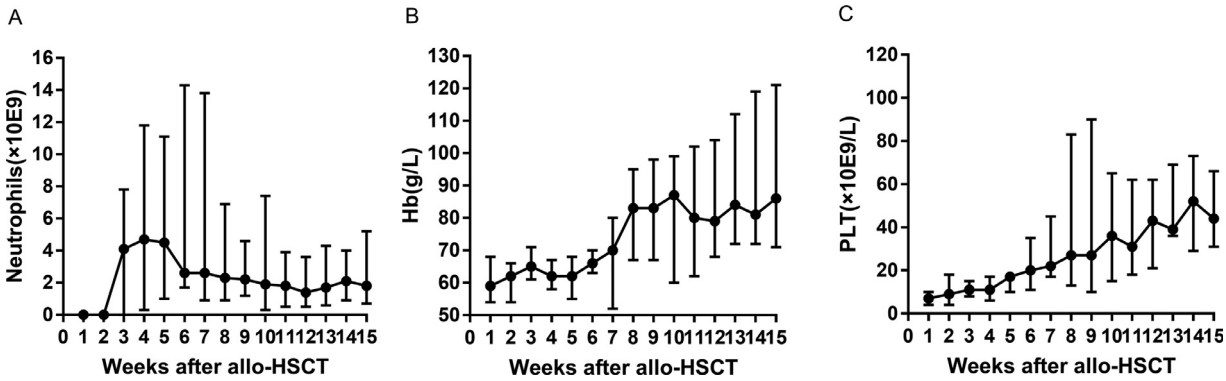

Figure 4. Median neutrophil count (A), hemoglobin concentration (B), and platelet count (C) after allo-HSCT in 9 responding patients with primary PGF.

erythropoiesis. Nevertheless, hepcidin production is increased due to the iron overload, leading to decreases in both iron absorption and release [16]. As a result, those patients with iron overload barely get enough hematopoietic material. On the other hand, iron overload has been shown to have a suppressive effect on the erythroblast differentiation of human $CD34^+$ cells. It also increases the level of the intracellular reactive oxygen species (ROS) through the Haber-Weiss and Fenton reactions, which can promote the apoptosis of immature erythroblasts by suppressing *BCL2* gene expression [17,18]. Kong et al [19] recently reported that elevated ROS level is associated with an increased DNA damage and apoptosis, resulting in exhaustion of $CD34^+$ cells in the bone marrow for patients with PGF. These studies support the strong association of high SF level with primary PGF.

In line with other 2 studies [20,21], we found that high SF level was related to poor OS. Iron is known to be an important nutrient for bacteria and fungi, and excessive iron may promote the reproduction of them [22]. Several studies have indicated that patients with a high SF level are more likely to experience severe infection and/or organ failure after transplantation [23–27], which is fatal to patients with primary PGF patients, half of whom died of serious infection in our study. Moreover, iron overload is associated with defective chemotaxis and phagocytosis of neutrophils and macrophages, as well as with decreased bactericidal activity, resulting in decreased immune function [28]. Fortunately, patients with MDS treated with deferasirox were found to have an increased hemoglobin level and a decreased transfusion requirement [29–33]. Iron chelation therapy also led to a hematopoietic response in some patients with AA [34]. An Italian study consisting of 7 patients with AL and 1 patient with AA with incomplete hematopoietic reconstitution also confirmed the beneficial effect of deferasirox in improving hematopoiesis [35]. Even a patient with SAA who was diagnosed with PGF after transplantation achieved complete hematopoietic recovery, which was clearly attributed to the iron chelation [36]. Of note, hematopoietic improvement from this treatment occurred several months later, and it might be difficult for some patients to wait such a long time. Here we had reason to believe that iron overload not only inhibits hematopoiesis, but also makes patients more susceptible to infection, which may explain the dismal 1-year OS in our patients with SF >2000 ng/mL. Thus, iron chelation therapy might become an effective prevention strategy, but more prospective studies are needed to verify this.

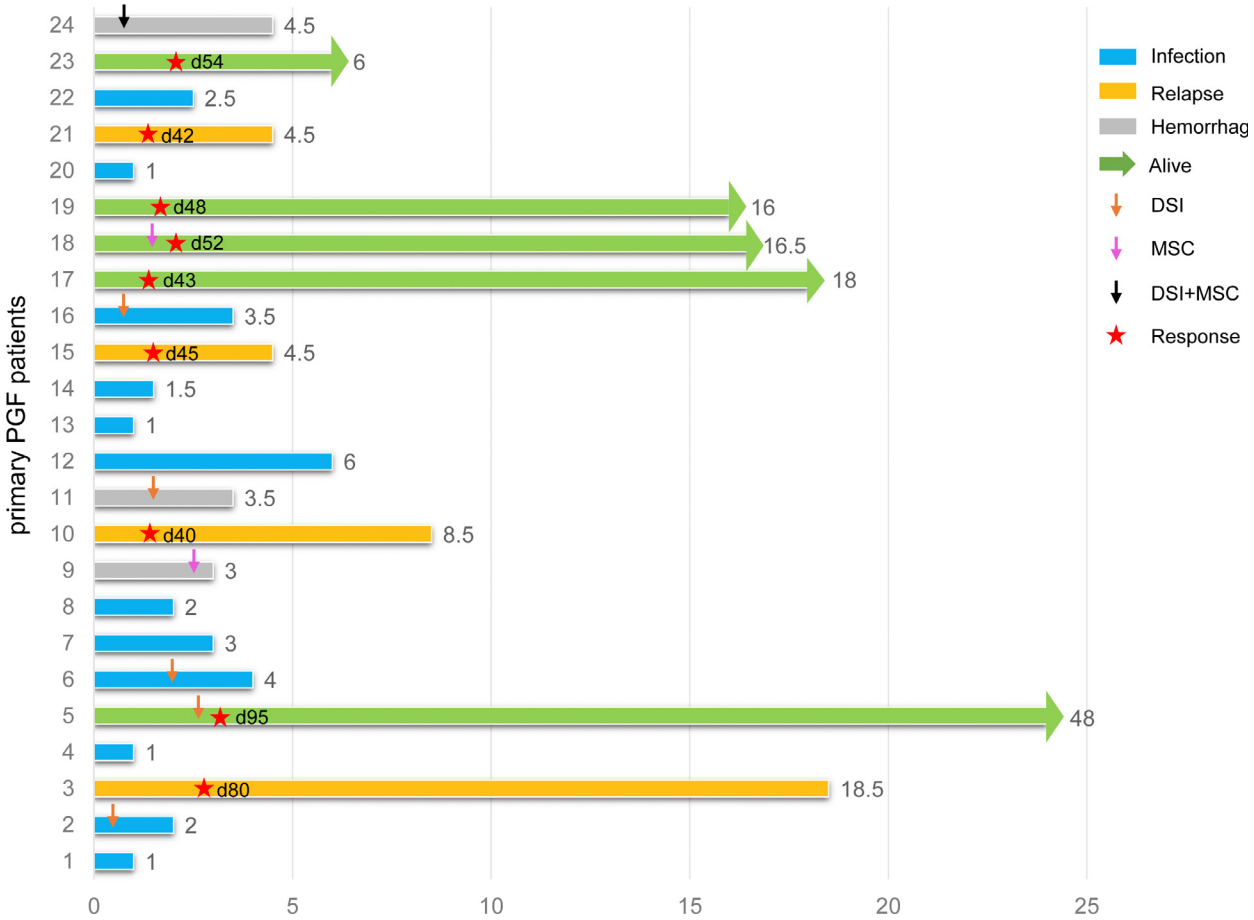

Figure 5. Treatment and outcomes in patients with primary PGF.

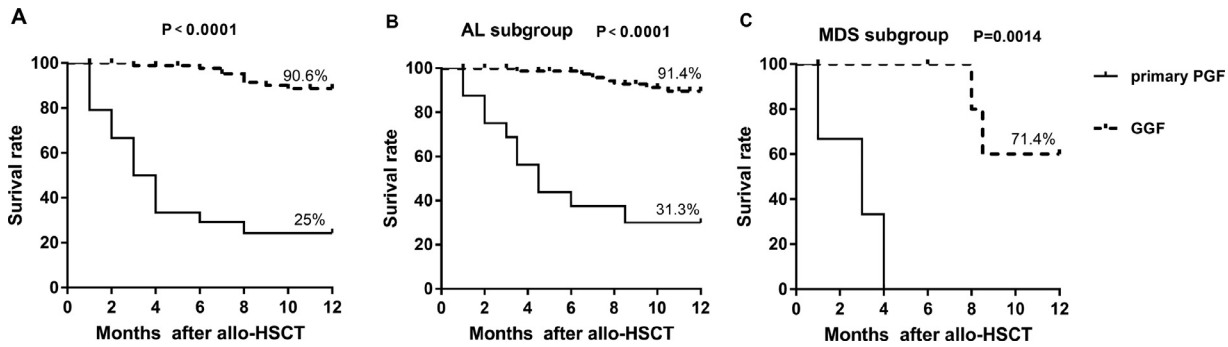

Figure 6. One-year OS was significantly poorer in the primary PGF group compared with the GGF group in all patients (A), the AL subgroup (B), and the MDS subgroup (C).

Splenomegaly was identified as another independent risk factor in our study. Previous studies suggested that enlarged spleen is related to the low rate of engraftment [37,38], which probably resulted from 2 factors. On one hand, the infused $CD34^+$ cells could be sequestered by the spleen which leads to a delayed homing and hypocellular bone marrow [39]; on the other hand, the newly generated blood cells could easily get trapped and destroyed by the enlarged spleen [40]. Splenectomy or spleen irradiation might be an option, but these treatments remain controversial. Akpek et al [41] found that patients with an enlarged spleen tend to take longer to achieve hematopoietic recovery. Although splenectomy before HSCT obviously promoted faster recovery of neutrophils and platelets, OS was not improved. In addition, the effect of delayed engraftment caused by splenomegaly could be counteracted by allo-HSCT with peripheral blood stem cells with a $CD34^+$ cell dose $>5.7 \times 10^6$/kg [41]. Interestingly, a similar study found the opposite, that pretransplantation splenectomy could improve OS and event-free survival for patients with myelofibrosis (MF). Considering the risk of surgery, some patients with extensive splenomegaly might benefit from this procedure [42]. Alternatively, splenic irradiation can shrink the spleen, but this effect lasts for only 2.2 months on average [43]. No favorable outcomes were observed in these studies, likely related from the heterogeneous doses, indications, and timing of SI and severe toxicity, such as myelosuppression

Table 4
Univariate and Multivariate Analysis for 1-Year OS

Variable	Total Number	One-Year OS, % (n)	*P* Value^{K-M}	*P* Value^{COX}
Group				
Primary PGF	24	25.0 (6)	<**.001**	<**.001**
GGF	96	90.6 (87)		
Age, yr				
≤35	57	78.9 (45)	.686	
>35	63	76.2 (48)		
Time from diagnosis to allo-HSCT, mo				
≤6	40	82.5 (33)	.592	
≤12	49	75.5 (37)		
>12	31	74.2 (23)		
Splenomegaly				
No	97	80.4 (78)	.097	.535
Yes	23	65.2 (15)		
Disease status at HSCT				
CR	104	79.8 (83)	.044	.593
NR or PR	16	62.5 (10)		
Risk stratification				
Low risk	42	90.5 (38)	.012	.216
High risk	78	70.5 (55)		
Courses of chemotherapy pre-HSCT				
≤5	77	79.2 (61)	.627	
>5	43	74.4 (32)		
Conditioning				
Myeloablative	113	77.9 (88)	.492	
Nonmyeloablative	7	71.4 (5)		
Donor type				
HRD	88	75.0 (66)	.069	.328
MSD	22	86.4 (19)		
URD	10	80.0 (8)		
Donor age, yr				
≤35	58	77.6 (45)	.889	
>35	59	76.3 (45)		
Blood mismatch				
Identical	67	76.1 (51)	.731	
Mismatch	53	79.2 (42)		
Sex mismatch				
Identical	64	76.6 (49)	.830	
Mismatch	56	78.6 (44)		
SF level, ng/mL				
≤2000	91	85.7 (78)	<**.001**	**.029**
>2000	29	51.7 (15)		
$CD34^+$ cell dose, 10^6/kg				
<5	51	66.7 (34)	.009	.989
≥5	69	85.5 (59)		
aGVHD				
No	85	76.5 (65)	.870	
Grade I	23	78.3 (18)		
Grade II	10	80.0 (8)		
Grade III	2	100 (2)		
CMV infection in 30 days				
No	104	80.8 (84)	.015	.809
Yes	16	56.3 (9)		

Significant values are in bold type.
P value^{K-M}, *P* value of Kaplan-Meier method; *P* value^{COX}, *P* value of Cox regression analysis.

[41,43]. Whether there is a suitable protocol for SI remains to be explored. A recent study noted that in patients with advanced myelofibrosis, administration of ruxolitinib before allo-HSCT was associated with a reduction in spleen size, and no patient experienced graft failure [44]. Therefore, ruxolitinib might have value in treating primary PGF.

MSCs have the capability of self-renewal and multilineage differentiation [45]. MSC infusion also has been reported to

promote hematopoiesis. In a pilot study, 2 of 6 patients with poor hematopoietic recovery achieved rapid hematopoietic recovery (on days 12 and 21) after receiving MSCs at a dose of 1×10^6/kg [46]. Liu et al [47] reported that 20 patients with PGF (including 7 with primary PGF) received MSC infusion (1×10^6/kg) 1 to 3 times at a 28-day interval, and 17 of the patients attained hematopoietic response (including 5 of those with primary PGF). In these 17 patients, the median time to achieve partial response and complete response were 16 days and 39 days, respectively, for neutrophils and 24 days and 42 days, respectively, for platelets. Compared with iron chelation therapy, hematopoietic recovery was significantly quicker with MSC infusion. A recent study demonstrated that patients with PGF have reduced and dysfunctional MSCs and significantly lower hematopoiesis-supporting ability compared with patients with GGF. They also found that increased ROS and p53 levels in patients with PGF are associated with impaired MSCs [48]. Based on the foregoing work, MSC infusion may be helpful to repair the impaired marrow microenvironment, also a promising therapy for PGF.

In conclusion, this study demonstrates that low $CD34^+$ cell count, high SF level, and splenomegaly are strongly associated with primary PGF. Because some patients with primary PGF did not achieve satisfactory results, monitoring high-risk patients and strategies aimed at early prevention, such as increasing the $CD34^+$ cell dose and decreasing the SF level, may prevent the occurrence of primary PGF, eventually improving patient survival. Effective therapies remain to be developed and confirmed.

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