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ORIGINAL ARTICLE

Virus reactivation and low dose of CD34+ cell, rather than haploidentical transplantation, were associated with secondary poor graft function within the first 100 days after allogeneic stem cell transplantation

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Abstract

Secondary poor graft function (sPGF) is defined as secondary cytopenia after initial engraftment of allogeneic stem cell transplantation (allo-SCT). It has been shown to be associated with poor prognosis; however, there are very few reports on the incidence, risk factors, and outcomes of sPGF. Between January 2015 and December 2015, 564 patients, who received transplantation at Peking University People's Hospital, were retrospectively reviewed. Among the 490 patients who achieved initial engraftment of both neutrophils and platelets, 28 patients developed sPGF. The cumulative incidence of sPGF on day 100 was 5.7%. The median time of sPGF was 54.5 (34–91) days after transplantation. Low (< median) CD34+ cell dose ($p = 0.019$, HR 3.07 (95% CI, 1.207–7.813)), Epstein-Barr Virus (EBV) reactivation ($p = 0.009$, HR 3.648 (95%CI, 1.382–9.629)), and cytomegalovirus (CMV) reactivation ($p = 0.003$, HR 7.827 (95%CI, 2.002–30.602)) were identified as independent risk factors for sPGF. There was no significant difference in PGF incidence between the matched sibling donor (MSD) group and haploidentical donor (HID) group ($p = 0.44$). The overall survival of patients with sPGF at 1 year after transplantation was significantly poorer than that of patients with good graft function (GGF) (50.5% versus 87.2%, $p < 0.001$). In conclusion, sPGF developed in 5.7% patients after allo-SCT, especially in patients with CMV, EBV reactivation, or infusion with a low dose of CD34+ cells. The prognosis of sPGF is still poor owing to a lack of standard treatment.

Keywords Poor graft function · Cytomegalovirus · Graft-versus-host disease · Allogeneic stem cell transplantation

Introduction

Complete and robust hematopoietic reconstitution is very important to the success of allogeneic stem cell transplantation (allo-SCT) [1, 2]. The initial engraftment of neutrophils usually occurs in the first 2 weeks after allo-SCT, and the initial engraftment of platelets usually occurs 3–4 weeks after allo-SCT [3]. After the initial engraftment, some patients may develop secondary failure of hematopoietic recovery [4, 5], and this could be classified as either monolineage (most were isolated thrombocytopenia) or multiple lineage (secondary graft failure) [6]. Obviously, secondary cytopenia of multiple lineages is a severe situation, associated with a high risk of infection and bleeding. Among patients with multiple lineage secondary cytopenia, some patients suffered from loss of initial complete donor chimerism, defined as secondary graft rejection. However, other patients achieved complete donor chimerism and were defined as having secondary poor graft function (sPGF). Secondary graft rejection is very rare nowadays; however, sPGF is associated with graft versus host disease (GVHD), virus infection, or some unknown reasons [5–7]. Patients with sPGF were speculated to have poor

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prognosis owing to the increased risk of infection and hemorrhagic events. However, there are very few reports focused on sPGF. In this study, we describe the incidence, risk factors, and outcomes of sPGF.

Patients and methods

Patients

Patients who received transplantation from Peking University People's Hospital between January 2015 and December 2015 were retrospectively reviewed to check if they fulfilled the following conditions: (1) diagnosed with acute leukemia or myelodysplastic syndrome; (2) received allo-SCT from either matched sibling donor (MSD) or haploidentical related donor (HID). The Ethics Committee of the Peking University People's Hospital approved this study. All procedures performed in these studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committees and with the 1964 Helsinki declaration and its later amendments, or with some comparable ethical standards. All patients gave written informed consent.

Transplant methods

The principles of HLA typing, donor selection, donor stem cell harvesting, conditioning regimen, and prevention of GVHD and infection were the same as previous reports [3]. As for the conditioning regimen, all MSD transplant patients received uniform modified busulfan/cyclophosphamide (mBu/Cy) conditioning regimen, which consisted of hydroxyurea (80 mg kg^{-1}, on day − 10), cytarabine (2 g m^{-2} day^{-1} on day − 9), busulfan (3.2 mg kg^{-1} day^{-1} intravenously on days − 8 to − 6), cyclophosphamide (1.8 g m^{-2} day^{-1} on days − 5 to − 4), and semustine (250 mg/m^2 orally on day − 3). Further, all HID transplant patients received uniform modified busulfan/cyclophosphamide/antithymocyteglobulin (mBu/Cy/ATG) conditioning regimen, which consisted of cytarabine (4 g m^{-2} day^{-1} on days − 10 to − 9), busulfan (3.2 mg kg^{-1} day^{-1} intravenously on days − 8 to − 6), cyclophosphamide (1.8 g m^{-2} d^{-1} on days − 5 to − 4), semustine (250 mg/m^2 orally on day − 3), and anti-thymocyte globulin (2.5 mg kg^{-1} d^{-1}, rabbit [Sang Stat, Lyon, France] on days − 5 to − 2). Cyclosporine (CSP) plus short-term methotrexate (MTX) and mycophenolate mofetil (MMF) were administered to prevent graft-versus-host disease. On day + 1, MTX (15 mg/m^2) was administered intravenously, following 10 mg/m^2 was given on days + 3, + 6, and + 11 after transplantation. Four doses of MTX were given in the HID transplantation, while only the first three doses were given in MSD transplantation. All patients, either HID or MSD, received a combination of bone marrow and peripheral blood as the graft.

All patients received prophylactic ganciclovir (5 mg/kg twice daily i.v.) from day − 9 to day − 2. After transplantation, plasma CMV DNA test with real-time polymerase chain reaction (PCR) was used to monitor CMV reactivation (kits purchased from Sino-American Biotech, Beijing, China). CMV-positive patients ($> 1 \times 10^3$ copies/mL) usually received preemptive i.v. ganciclovir 5 mg/kg twice daily for 10–14 days or until CMV tests were negative. In patients with neutropenia, two doses of foscarnet 90 mg/kg i.v. were administered in place of ganciclovir. The plasma EBV-DNA was also monitored twice weekly by real-time PCR. Preemptive therapy with rituximab was prescribed after two consecutive EBV-DNA more than 10,000 copies/mL, or EBV $> 5 \times 10^2$ copies/mL with suspected EBV-related diseases.

Definitions

Engraftment of neutrophils was defined as the first of 3 consecutive days when the absolute neutrophil count achieved 0.5×10^9/L without granulocyte colony-stimulating factor stimulation (G-CSF). Engraftment of platelets was defined as the first of 7 consecutive days when the platelet count was $\geq 20 \times 10^9$/L, independent of platelet substitution. Good graft function (GGF) was defined as achievement of both sustained neutrophil and platelet engraftment, independent of transfusion.

Isolated cytopenia, defined as the presence of 1 cytopenic count, was mainly isolated thrombocytopenia. Isolated thrombocytopenia included delayed platelet engraftment (DPE) and secondary failure of platelet recovery (SFPR). DPE was defined as persistent severe thrombocytopenia ($< 20 \times 10^9$/l) > 35 days after transplantation [8]. Further, SFPR was defined as a decline of platelet counts to $< 20 \times 10^9$/l lasting at least 7 consecutive days or requiring platelet transfusion within 7 days after achieving primary platelet recovery [9, 10]. PGF was defined as persistent neutropenia ($\leq 0.5 \times 10^9$/L), thrombocytopenia (platelets $\leq 20 \times 10^9$/L), and/or hemoglobin ≤ 70 g/L for at least 3 consecutive days, transfusion-dependence, associated with hypoplastic-aplastic bone marrow (BM), and complete donor chimerism without concurrent active graft-versus-host disease (GVHD) or disease relapse. Primary PGF was defined as the failure to achieve initial engraftment by day 28 after transplantation. Secondary PGF was defined as the fulfillment of the criteria of PGF after initial engraftment of HSCT [1]. In addition, graft rejection was defined as never having achieved engraftment with mixed chimerism or complete recipient chimerism. Underlying disease was classified as high-risk or standard-risk. High-risk disease included acute leukemia either in non-remission or in the third or greater complete remission; standard-risk disease included all other diagnoses. Hematological recovery was defined as

neutrophils > 0.5×10^9/L, platelets > 20×10^9/L, and hemoglobin > 70 g/L, without transfusion or G-CSF.

Statistical analysis

Incidence of secondary PGF was calculated among patients who acquired initial neutrophil and platelet engraftment, with death before 100 days as competing risk, using the R statistical software, version 3.1.0 (R Foundation for Statistical Computing, Vienna, Austria). To analyze the risk factors, the GGF patients were treated as the control group. Variables included are as follows: patient sex, patient age, underlying disease, disease status (standard-risk versus high-risk), donor type (MSD versus HID), donor-

Table 1 Patient characteristics

Variable	Patients with initial engraftment ($n = 490$)	sPGF ($n = 28$)	GGF ($n = 462$)	p value
Gender, male (%)	301 (61.4%)	13 (46.4%)	288 (62.3%)	0.110
Age (years), median (range)	29 (2–65)	35.5 (11–62)	28 (2–65)	0.231
disease				0.499
AML	231 (47.1%)	11 (39.3%)	220 (47.6%)	
ALL	195 (39.8%)	11 (39.3%)	184 (39.8%)	
MDS	64 (13.1%)	6 (21.4%)	58 (12.6%)	
Status				0.386
SR	466 (95.1%)	28 (100%)	438 (94.8%)	
HR	24 (4.9%)	0 (0)	24 (5.2%)	
Donor type				0.647
Matched sibling	116 (23.7%)	5 (17.9%)	111 (24.0%)	
Haploidentical donor	374 (76.3%)	23 (82.1%)	351 (76.0%)	
Donor relation				0.666
Parents	210 (42.9%)	11 (39.3%)	199 (43.1%)	
children	57 (11.6%)	5 (17.9%)	52 (11.3%)	
sibling	215 (43.9%)	12 (42.8%)	203 (43.9%)	
lateral	8 (1.6%)	0 (0)	8 (1.7%)	
Donor gender, male (%)	340 (69.4%)	20 (71.4%)	320 (69.3%)	1.000
Donor age (years), median (range)	40 (8–66)	41.5 (13–60)	40 (8–66)	0.820
D-R blood type				0.616
Match	242 (49.4%)	12 (42.9%)	230 (49.8%)	
Major mismatch	114 (23.2%)	8 (28.6%)	106 (22.9%)	
Minor mismatch	104 (21.2%)	5 (17.9%)	99 (21.4%)	
Major + minor	30 (6.1%)	3 (10.7%)	27 (5.8%)	
MNC ($\times 10^8$/kg), median (range)	8.17 (5.32–15.60)	8.26 (6.04–13-.33)	8.16 (5.32–15-.60)	0.846
CD34+ cells ($\times 10^6$/kg), median (range)	2.64 (0.21–10.85)	1.97 (0.21–4.-54)	2.68 (0.42–10-.85)	0.034
ANC engraft (days), median (range)	13 (3–28)	13 (8–24)	13 (9–28)	0.951
PLT engraft (days), median (range)	14 (7–57)	14.5 (10–43)	14 (7–57)	0.905
CMV reactivation	247 (50.4%)	25 (89.3%)	222 (48.1%)	< 0.001
Grades 2–4 aGVHD	123 (25.1%)	12 (42.9%)	111 (24.0%)	0.019
EBV reactivation	48 (9.8%)	8 (28.6%)	40 (8.7%)	0.002
Follow-up time for survivor (days), median (range)	337 (71–602)	325 (99–504)	337 (71–602)	1.000

sPGF, secondary poor graft function; *GGF*, good graft function; *AML*, acute myeloid leukemia; *ALL*, acute lymphoblastic leukemia; *MDS*, myelodysplastic syndrome; *D-R blood type*, donor-recipient blood type; *MNC*, mononuclear cell; *ANC*, absolute neutrophil count; *PLT*, platelet; *aGVHD*, acute graft versus host disease; *CMV*, cytomegalovirus; *EBV*, Epstein-Barr Virus; *SR*, standard risk; *HR*, high risk

recipient relation (parent versus children versus sibling), donor-recipient ABO blood type, donor sex, donor age, infused MNC, infused CD34+ cell, acute GVHD (grades 0–I versus grades II–IV), CMV reactivation, and EBV reactivation. Continuous data were compared using the non-independent sample *t* test. Categorical variables were compared using χ^2 or Fisher exact test. Variables with *p* values < 0.20 in univariate models were entered into Cox regression models, and two-sided tests were considered to be significant at *p* values of 0.05. Multivariate Cox proportional model was used for identifying risk factors with the SPSS package software (SPSS, Chicago, IL, USA). Survival curves were plotted using the Kaplan-Meier method, and they were compared using the two-tailed log-rank tests.

Results

Characteristics of patients who achieved initial engraftment

In total, 564 patients were retrospectively reviewed, and the initial engraftment of neutrophils and platelets was achieved in 490 patients. The basic characteristics of the 490 patients are summarized in Table 1. In brief, there are 116 (23.7%) from MSD and 375 (76.3%) from HID. The median age of patients was 29 (2–65) years. There were 301 (61.4%) males. Further, there were 231 (47.1%) acute myeloid leukemia (AML), 195 (39.8%) acute lymphoblastic leukemia (ALL), and 64 (13.1%) myelodysplastic syndrome (MDS) patients. Twenty-four (4.9%) patients were defined as high-risk. The median engraftment time of neutrophils and platelets was 13 (8–28) and 14 (7–57) days, respectively. The 100-day cumulative incidence of CMV and EBV reactivation was 50.4% and 10.2%, respectively. The 100-day cumulative incidence of grade 2–4 or 3–4 GVHD was 24.3% and 7.6%, respectively.

Incidence and risk factors of secondary PGF

Among the 490 patients who achieved initial engraftment of both neutrophils and platelets, 28 patients developed sPGF (Table 2). The cumulative incidence of sPGF on day 100 was 5.7% (Fig. 1).

As for risk factors of secondary PGF, a low (< median) dose of CD34+ cells ($p = 0.034$), CMV reactivation ($p < 0.001$), EBV reactivation ($p = 0.002$), and grade 2–4 acute GVHD ($p = 0.019$) were associated with a higher risk of sPGF in the univariate analysis (Table 2). In the final multivariate analysis, low (< median) dose of CD34+ cells ($p = 0.019$, HR 3.07 (95%CI, 1.207–7.813)), EBV reactivation ($p = 0.009$, HR 3.648 (95%CI, 1.382–9.629)), and CMV reactivation ($p = 0.003$, HR 7.827 (95%CI, 2.002–30.602)) were identified as independent risk factors of sPGF (Table 3).

Table 2 Patients with secondary PGF

Variables	Summary
Onset time (days after transplant), median (range)	54.5 (34–91)
Treatment	
supportive treatment	26 (92.9%)
Second transplantation	2 (7.1%)
Response	15(53.6%)
Recovery time (days after diagnosis), median (range)	61 (11–146)
Survival	50.5%

There was no significant difference of PGF incidence in the MSD group and HID patients (6.2% versus 4.4%, $p = 0.49$) (Fig. 1). However, CMV reactivation ($p = 0.004$, HR 43.03 (95%CI, 3.256–568.664)) was the only factor identified in the MSD transplant group (Table 3). In the HID subgroup, CMV reactivation ($p = 0.047$, HR 4.538 (95%CI, 1.021–20.165)), EBV reactivation ($p = 0.006$, HR 4.101 (95%CI, 1.504–11.183)), and low (< median) CD34+ cell count ($p = 0.049$, HR 2.803 (95%CI, 1.006–7.810)) were associated with sPGF (Table 3).

Characteristics and outcomes of patients with sPGF

The characteristics of the 28 patients with sPGF are summarized in Table 2. The median time of secondary PGF was 54.5 (34–91) days after transplantation. Most patients (26/28, 92.9%) received supportive treatment (including transfusion, cell-stimulating factors, and anti-infection prophylaxis), and two patients received second transplantation. Among them, 53.6% (15/28) recovered at a median of 61 (11–146) days after sPGF diagnosis. The overall survival of patients with sPGF was 50.5% at 1 year after transplantation, significantly poorer than patients with GGF (50.5% versus 87.2%, $p < 0.001$, Fig. 2a). Furthermore, patients who recovered from sPGF had significantly better survival than those without recovery (86.7% versus 7.7%, $p < 0.001$, Fig. 2b).

Discussion

Although it is well known that sPGF is associated with poor outcomes after transplantation, there are few studies on the incidence, risk factors, and clinical characteristics of sPGF, especially in the setting of haploidentical stem cell transplantation. Our study described the incidence of sPGF, identified several risk factors, and confirmed the poor outcomes in a large cohort of patients that received mainly haploidentical stem cell transplantation.

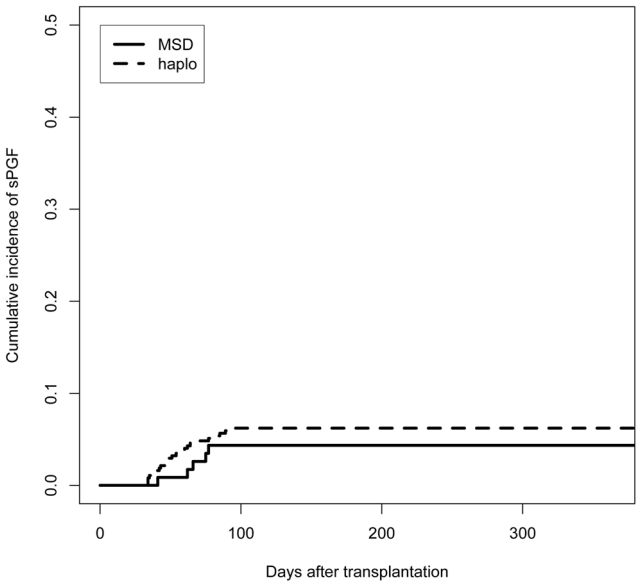

Fig. 1 Cumulative incidence of secondary PGF

A Korean center [6] has reported 12.7% (21 out of 165) patients suffered subsequent failure of trilineage reconstitution after transplantation from HLA-matched sibling donors or unrelated donors. Another study from Fred Hutchinson Cancer Research Center [5] reported that 7% patients developed subsequent cytopenia involving all three lines among patients who achieved neutrophil engraftment at day 28 after transplantation. In our study, sPGF occurred in 5.7% patients who achieved initial engraftment. However, it is difficult to compare results from different studies due to several reasons. First, the definition used in each study was very different. Second, the number of clinically important factors analyzed, the transplant protocol, and supportive care strategies were highly heterogeneous in different studies. Nevertheless, all studies suggested that sPGF is not rare after allo-HSCT, and all demonstrated poor prognosis compared with those patients with fast reconstitution.

Until now, there has not been a study in the setting of haploidentical SCT. Very interestingly, we found that transplant from a haploidentical donor was not associated with a higher incidence of sPGF, which is very different to primary graft function [1]. Although primary (early) PGF and secondary (late) PGF have the same performance in the bone marrow microenvironment [11], there are still clues demonstrating the difference between primary PGF and sPGF, such as the risk factors and prognosis. The most significant factors associated with sPGF were virus reactivation (mainly CMV), regardless of MSD or HID transplantation. It has been reported in previous studies [5, 6, 12]; however, the mechanism remains controversial. Several possible hypothesis have been postulated, include direct impairment of CMV [13] or indirect impairment from anti-virus drugs [5]. CMV has the ability of infecting and inhibiting the function of stromal cells and hematopoietic progenitors. In addition, drugs for prevention or treatment of CMV such as gancilovir and forscarnet have direct impacts of bone marrow suppression. It suggests that options for CMV prevention or management might be very helpful in reducing the incidence of sPGF. It has been described that patients can develop sPGF when complicated by GVHD, and the possible mechanism may include the bone marrow niche as a target of GVHD [14–16]. However, GVHD was not identified as a risk factor of sPGF in our study. The possible reason may be that severe GVHD is always complicated with virus reactivation.

The prognosis of sPGF is very poor. Current methods include growth factor support [17–19], infusion of donor peripheral blood to harvest and boost CD34+ cells [20–22], second allogeneic stem cell transplantation, or infusion of mesenchymal cells [23]. However, effective treatment options are lacking. One important reason is that the mechanism of sPGF is complex and not very clear. Our recent works suggest that an impaired bone marrow (BM) microenvironment is associated with PGF, mainly through reduced numbers of endothelial progenitor cells (EPCs) [11, 24–27]. Further, we found that atorvastatin, a lipid-lowering drug, could reverse this pathway in vitro [25].

Table 3 Risk factors associated with secondary PGF according to donor type

	Overall cohort			Haploidentical donor			Matched sibling donor		
	p	HR	95%CI	p	HR	95%CI	*p*	HR	95%CI
CD34 cell (< median)	0.019	3.070	1.207–7.813	0.049	2.803	1.006–7.810	0.565		
CMV reactivation	0.003	7.827	2.002–30.602	0.047	4.538	1.021–20.165	0.004	43.03	3.256–568.664
EBV reactivation	0.009	3.648	1.382–9.629	0.006	4.101	1.504–11.183	0.992		
Grades 2–4 aGVHD	0.215			0.121			0.386		
MDS versus other	0.143			0.179			0.317		
HID versus MSD	0.141			–			–		

CMV, cytomegalovirus; *EBV*, Epstein-Barr Virus; *aGVHD*, acute graft versus host disease; *MDS*, myelodysplastic syndrome; *HID*, haploidentical donor; *MSD*, matched sibling donor

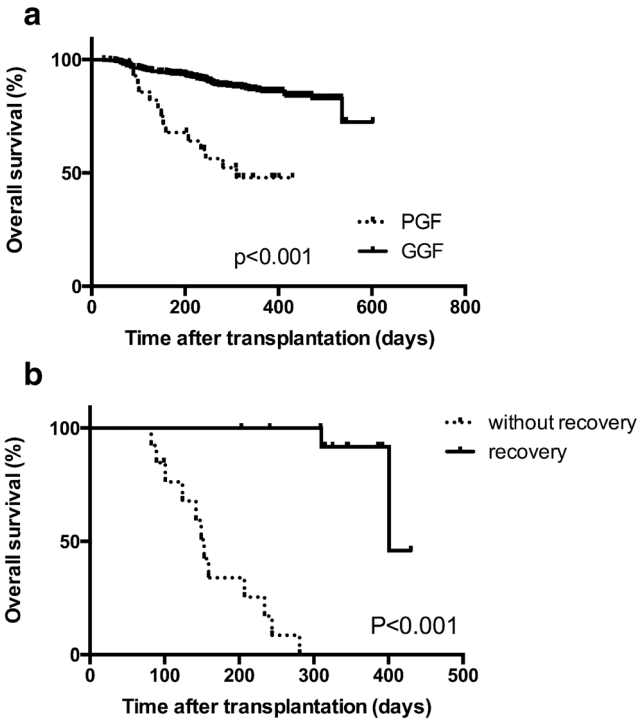

Fig. 2 Response and survival of secondary PGF (**a** survival compared with GGF; **b** response)

These data might provide a promising pathway to manage the complications of PGF after stem cell transplantation. In addition, the combination of methods targeting different pathways (such as promoting/supplementing hematopoietic progenitor cells and repairing the microenvironment) might be potential options; however, this hypothesis requires confirmation using clinical trials.

In summary, sPGF can develop in 5.7% of patients after allo-SCT, especially in patients with CMV or EBV reactivation or infusion with a low dose of CD34+ cells. However, the prognosis of sPGF is still poor due to lack of standard treatment.

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Compliance with ethical standards

The Ethics Committee of the Peking University People's Hospital approved this study. All procedures performed in these studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committees and with the 1964 Helsinki declaration and its later amendments, or with some comparable ethical standards. All patients gave written informed consent.

Conflict of interest The authors declare that they have no conflicts of interest.

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