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Haploidentical hematopoietic stem cell transplantation using reduced-intensity conditioning for pediatric patients with familial hemophagocytic lymphohistiocytosis

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ABSTRACT

Importance: Allogeneic hematopoietic stem cell transplantation (HSCT) is considered to be the only curative treatment for familial hemophagocytic lymphohistiocytosis (FHLH). Treatment of pediatric FHLH with reduced-intensity conditioning (RIC)-based haploidentical donor (HID) HSCT has been rarely reported.

Objective: To investigate outcomes and adverse events in patients with FHLH who received HID-HSCT.

Methods: We conducted a retrospective study of five patients, including three with mutations in *PRF1* and two with *XIAP* deficiency. Four of the five donors were heterozygous for these mutations. The conditioning regimen included fludarabine, cyclophosphamide, and antithymocyte globulin, with or without low-dose irradiation. Unmanipulated mobilized bone marrow and peripheral blood stem cells were used as the grafts.

Results: All five patients were successfully engrafted. Four patients survived, and one patient died. All exhibited complete response (CR) after HSCT. All of the patients who survived exhibited CR to FHLH without severe regimen-related complications at a median of 29.5 months (range: 23–34 months) after HSCT. Four of the five patients had mixed donor chimerism. Three patients had 17% to 87% mixed donor chimerism but remained free of disease. Four patients received donor lymphocyte infusion (DLI), which improved the level of mixed donor chimerism. One patient experienced a decrease in donor chimerism to 1% and relapsed; Four patients developed acute graft-versus-host disease (GvHD) (grade I or II), and one patient developed grade IV GvHD.

Interpretation: HID-HSCT with RIC can be considered for treatment for patients with FHLH, but the conditions and DLI regimens need to be optimized for long-term use, and more prospective studies should be conducted.

KEYWORDS

Haploidentical, Hematopoietic stem cell transplantation, Hemophagocytic lymphohistiocytosis, Pediatric, Reduced intensity conditioning

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INTRODUCTION

Familial hemophagocytic lymphohistiocytosis (FHLH) is a collection of primary immune deficiencies that involves mutations in *PRF1*, *UNC13D*, *STX11*, or *STXBP2*.[1-4] Recently, mutation of X-linked inhibitor of apoptosis (*XIAP*) has also been identified as a cause of FHLH.[5] Allogeneic hematopoietic stem cell transplantation (HSCT) is considered to be the only curative treatment for FHLH. Human leukocyte antigen (HLA)-matched sibling donors or unrelated donors are the first choice for HSCT, but in China, there is usually no suitable donor available. Thus, haploidentical donors (HID) are commonly used. Treatment of pediatric FHLH with reduced-intensity conditioning (RIC)-based HID-HSCT has only been rarely reported. Here, we report a series of patients with FHLH who were treated in our center with a RIC regimen consisting of fludarabine (FLU), cyclophosphamide (CTX), and antithymocyte globulin (ATG), combined with low-dose total body irradiation (TBI) followed by allogeneic HID-HSCT without e*x vivo* T-cell depletion.

METHODS

Patients and donors

Patients who were included in this study fulfilled the HLH-2004 diagnostic criteria.[6] Consecutive patients with FHLH and with no HLA-matched sibling or unrelated donors, who were indicated for HID-HSCT, were included between December 2015 and November 2016 at Beijing Children's Hospital, National Center for Children's Health, China. All donors underwent gene and natural killer (NK) cell activity tests to exclude the possibility that they carried disease of FHLH. The patients' parents and the donors signed a consent form agreeing to the treatment and data collection.

Definition and criteria

Complete response (CR), partial response (PR) and active disease (AD) were defined according to the main symptoms and laboratory markers of HLH. CR should have normal markers including levels of soluble CD25, ferritin, and triglycerides, hemoglobin, neutrophil counts, platelet counts, and alanine aminotransferase. PR was defined as at least a 25% improvement in two or more markers. Patients who failed to PR were defined as AD.[7] The engraftment of white blood cells was defined as absolute neutrophil count > 0.5×10^9/L for three consecutive days and the engraftment of platelet was defined as platelet count > 20×10^9/L for seven consecutive days without infusion of platelets. Chimerism was assessed by fluorescence in situ hybridization and short tandem repeat analysis via polymerase chain reaction (PCR). Complete donor chimerism was defined as having > 95% donor-derived cells, while having < 95% of donor-derived cells was considered mixed donor chimerism. Acute and chronic GvHD were diagnosed and graded by attending physician, according to defined criteria.[8,9]

Pre-HSCT treatment

All patients received first-line immunochemotherapy regimens based on HLH-2004 protocol including glucocorticoid, etoposide, and ciclosporin (CsA) as initial therapy for 2–4 weeks. Maintenance treatment was started later for patients with CR or PR.[6] For refractory/recurrent patients with AD, salvage therapy (DEP: doxorubicin + etoposide + methylprednisolone) was provided as a second-line chemotherapy regimen.[10]

HID-HSCT regimen

Reduced intensity non-myeloablative conditioning, consisting of FLU, CTX, and ATG, with or without TBI, was used as follows: FLU (150 mg/m^2, over 5 days on days –10 to –6), CTX (200 mg/kg, over 4 days on days –5 to –2), and ATG (2.5 mg/kg/d, over 4 days on days –5 to –2), with low-dose TBI (3 Gy, on day –11). Patient 5 was too young to receive TBI, so we used low-dose busulfan (4.8 mg/kg/d, over 2 days on days –7 to –6) instead of TBI. All of the transplant recipients received prophylactic CsA, mycophenolate mofetil (MMF), and methotrexate (MTX) for acute GvHD. CsA (2.5 mg/kg, q12h, i.v.) was administered from day –1, and the trough concentration was adjusted to 150–250 ng/mL; after the concentration was stable, it was administered orally. From day +1, MMF was administered orally every 12 h at a dose of 600 mg/m^2/d, which was gradually tapered off to 300 mg/m^2/d by day +60 and then discontinued. Following graft infusion, 15 mg/m^2 MTX was administered i.v. on day +1, with an additional dose of 10 mg/m^2 on days +3, +6, and +11. Bone marrow and peripheral blood stem cells were used as a combined graft source for all patients. The residual stem cells were cryopreserved for donor lymphocyte infusion (DLI).

Supportive care

Patients were monitored twice weekly for cytomegalovirus (CMV) and Epstein-Barr virus antigenemia via PCR. Different antibiotics were administered depending on the infection site and pathogen. Voriconazole or caspofungin was used for antifungal prophylaxis. Sulfamethoxazole was used for Pneumocystis jirovecii pneumonia prophylaxis. All patients received intravenous immunoglobulin (200 mg/kg twice a week) prophylaxis for infection post-transplantation.

Post-HSCT monitoring and treatment

Whole blood total donor chimerism was monitored weekly from the time of engraftment. When the donor chimerism fell rapidly or was below 50%, DLI was performed at the attending physician's discretion. The DLI dose usually started at 1×10^6 CD3$^+$ cells/kg, and the timing of the

doses was adjusted based on the patient's response to the DLI. Lymphocytes for DLI were frozen aliquots collected from the donor at the time of the original harvest or collected peripherally from the donor before DLI. FHLH remission was assessed at months +1, +3, +6, and then once a year.

RESULTS

Patient characteristics before transplantation

Five patients with FHLH (three with mutations in *PRF1* and two with mutations in *XIAP*) were included in this study. Four of the five donors had heterozygous mutations. All of the donors had normal NK cell activity. Mobilized bone marrow and peripheral blood stem cells were used as the grafts. The median age at HSCT was 2.8 years (range: 1.4–4.6 years). Four patients had AD before HSCT. The patient characteristics before HSCT are listed in Table 1.

Engraftment

All five patients were successfully engrafted, with neutrophil recovery at a median of 12 days (range: 11–13 days) and platelet recovery at a median of 17 days (range: 12–24 days). Mixed donor chimerism occurred in four of five patients after engraftment. Three patients exhibited mixed donor chimerism ranging from 17% to 87% but remained free of disease. Four patients received DLI, with three patients receiving two or more DLI doses. Three of the four patients who received DLI showed significant improvement in donor chimerism, with two patients eventually reaching full donor chimerism (> 95%). Patient 4 exhibited less than 1% mixed donor chimerism and relapsed. This patient did not experience any significant improvement in donor chimerism after three subsequent rounds of DLI. After two stem cell boosts (7.9×10^6 $CD34^+$ cells/kg) were administered, the donor chimerism increased to 99%, and the patient achieved second CR (CR2) (Table 2).

TABLE 1 Characteristics of patients before transplantation

Patient	Gene	Patients' mutation	Donor	Donors' mutation	Age at onset (years)	Status of the HLH	HLH treatment before HSCT (time)
1	*PRF1*	c.1090_1091delCT (p.L364Efs*93) c.1349C>T (p.T450M)	Father heterozygous	c.1349C>T (p.T450M)	1.4	AD	HLH2004 (1 year) DEP×1 (2 months)
2	*PRF1*	c.1349G>A (p.T450M) c.218C>T (p.C73Y)	Father heterozygous	c.1349G>A (p.T450M)	3.2	AD	HLH2004 (3 weeks) DEP×1 (1 month)
3	*PRF1*	c.133G>A (p.G45R) c.116C>A (p.P39H)	Father heterozygous	c.133G>A (p.G45R)	4.1	AD	HLH2004 (2 months) DEP×3 (3 months)
4	*XIAP*	c.1253_1256 delAGAA (p.Q418Qfs*23)	Father	N/A	2.2	AD	HLH2004 (3 months) DEP×2 (2 months)
5	*XIAP*	c.589C>T (p.Q197X)	Sibling heterozygous	c.589C>T (p.Q197X)	0.7	CR	HLH2004 (8 months)

HLH, hemophagocytic lymphohistiocytosis; HSCT, hematopoietic stem cell transplantation; HLH2004, HLH-2004 protocol; DEP, second-line chemotherapy regimen;CR, complete response; AD, active disease

TABLE 2 Outcomes of mixed donor chimerism and DLIs after HSCT

Patient	Initial chimerism (%)	Peak chimerism after DLI (%)	Last chimerism (%)	Maximum cells count of DLI ($CD3^+$cells/kg)	Times of DLI	GvHD after DLI	Notes
1	21.0	62.0	>95.0	2.7×10^7	2	Ⅱ (skin) Chronic (skin)	
2	51.8	92.8	>95.0	1.0×10^7	2	Ⅰ (skin) Chronic (skin, liver)	
3	25.2	41.4	73.2	1.0×10^6	1	Ⅰ (skin) Chronic (skin)	MCSC boost
4	0.5	3.9	>95.0	1.0×10^7	3	Ⅰ (bowel)	$CD34^+$ PBSC boosts (7.9×10^6 cells/kg) on day +55,+75
5	>95.0	>95.0	>95.0	N/A	N/A		

MCSC, mesenchyma stem cell; PBSC, peripheral blood stem cell; N/A, not available; DLI, donor lymphocyte infusion; GvHD, acute graft-versus-host disease; HSCT, hematopoietic stem cell transplantation.

GvHD

Four patients developed acute GvHD after DLI. In most of the patients, the GvHD was mild, with three patients having grade I or II skin GvHD. Two patients developed skin or liver chronic GvHD (cGvHD) after DLI. Patient 3 received a mesenchymal stem cell boost (1.0×10^6 cells/kg) to treat cGvHD and to improve the level of engraftment. Patient 4 developed grade IV severe intestinal tract GvHD after having received two stem cell boosts (Table 2).

Other complications

Two patients developed CMV infections, which were eliminated by treatment with ganciclovir and anti-CMV immunoglobin. One patient developed autoimmune hemolytic anemia on day +150 post-HSCT. Methylprednisolone and rituximab were administered immediately, and the patient's condition gradually stabilized over the course of two weeks. Unfortunately, patient 4 suffered hemorrhage caused by sternal bone marrow puncture at +28 days after HSCT, leading to acute pericardial tamponade and shock.

Survival and outcomes

All patients achieved CR at a median of 27 months (range: 7–34 months) after HSCT. Four patients survived for 23–34 months after transplantation, whereas one patient died 7 months after transplantation. This patient died from intracranial hemorrhage and fungal septicemia. All of the survivors exhibited CR from HLH with no severe regimen-related complications at a median of 29.5 months (range: 23–34 months) after HSCT (Table 3).

DISCUSSION

FHLH is a genetically determined disorder. Immunochemotherapy-based treatments can result in remission, but even after a CR, relapse may still occur. HSCT is considered to be the only curative treatment for FHLH. Aricò et al[11] reported an estimated 5-year survival rate of 66% for patients undergoing HSCT, as opposed to 10% for patients who do not receive HSCT. However, a recent study by Marsh et al[12] reported that patients who received RIC had a 3-year probability of survival of 92%, as compared to 43% in patients who received myeloablative conditioning (MAC). Similar studies of RIC regimens consisting of alemtuzumab, FLU, and melphalan have reported survival rates of 75%–84%.[13,14] Because alemtuzumab is not available in our center, we used a regimen including FLU, CTX, and ATG combined with low-dose TBI. While the total number of patients receiving RIC in our study was small (n = 5), the survival rates seem similar to those reported in the United States. Long-term observation is required to confirm these findings.

Selection of the optimal donor is important. HSCT outcomes for patients with HLH are comparable, whether a matched unrelated donor or a matched sibling donor is used.[15] However, there is a shortage of HLA-matched unrelated donors and sibling donors in China, which is an issue for clinicians. We are therefore required to select haploidentical donors. The optimal criteria for selecting HID for transplantation are controversial. Data from Wang et al[16] suggest that young, male, non-inherited maternal antigens (NIMAs)-mismatched donors are good candidates. On this basis, potential donors were also tested for NK cell activity in our study.

A high incidence of mixed donor chimerism was observed in patients who received RIC treatment regimens in our study: four of the five patients exhibited mixed donor chimerism after engraftment. One possible explanation for this observation is that the patients were on corticosteroids for a long time, and the corticosteroid dose had to be reduced until after HSCT. Similar observations have been reported in other studies; for example, Marsh et al[12] observed a greater incidence of mixed donor chimerism in patients who received RIC (65%) compared with the MAC group (18%).

DLI is potentially useful for the treatment of mixed donor chimerism. In our study, four patients with mixed donor chimerism received DLI and exhibited significant improvement. A single-center retrospective review showed that 56% of patients experienced at least a 20% improvement in donor chimerism within 6 weeks of DLI, with 37% patients reaching full donor chimerism with long-term improvement.[17] However, GvHD after DLI cannot be ignored. One patient in our study developed severe grade IV GvHD after receiving three DLIs and two stem cell boosts. Unfortunately, there are currently limited data upon which to base decisions regarding instigation of DLI and DLI dose.

Although mixed donor chimerism occurred, most patients achieved CR from FHLH without relapse. A multicenter retrospective study of 103 patients with hereditary HLH showed that > 20%–30% donor chimerism is protective against relapse.[18] These findings are greatly encouraging, and suggest that future efforts should be directed to reducing the toxicity of conditioning regimens and eliminating unnecessary DLI procedures.

In conclusion, our report of five patients with FHLH from a single center suggests that HID-HSCT with a RIC regimen is a curative therapeutic option for patients with FHLH. However, there are controversial issues of mixed chimerism and need for additional allogeneic hematopoietic cell products including DLI which is often complicated by GvHD. Further studies are needed to optimize this FLU, CTX, ATG, and low-dose TBI conditioning regimen and improve patient outcomes.

TABLE 3 Regimens and outcomes of transplantation

Patient	Age at HSCT (years)/ gender	Regimens of HSCT				Engraftment				Complications	HLH status	F/U time (month)
		Condition regimen (mg/m^2 or mg/kg)	Donor	Graft Source	GvHD prophylaxis	Cells BM (cells/kg) TNC, CD34$^+$	Cells PBSC (cells/kg) TNC, CD34$^+$	Engraftment (day) NEUT, PLT	Donor chimerism MIN (%), MAX (%)			
1	2.5 Female	FLU 150, CY 200 ATG 12.5, TBI 3GY	Father haplo	BM PB	CSA+MTX+MMF	8.5×10^8, 1.2×10^6	15.1×10^8, 7.4×10^6	+12, +24	15.7, >95.0	aGvHD Ⅱ cGvHD (skin)	Alive CR	34
2	3.5 Male	FLU 150, CY 200 ATG 12.5, TBI 3GY	Father haplo	BM PB	CSA+MTX+MMF	13.0×10^8, 2.0×10^6	9.8×10^8, 7.7×10^6	+12, +20	51.8, >95.0	aGvHD Ⅰ cGvHD (skin, liver) EBV, CMV infection	Alive CR	32
3	4.6 Female	FLU 150, CY 200 ATG 12.5, TBI 3GY	Father haplo	BM PB	CSA+MTX+MMF	10.0×10^8, 3.2×10^6	4.0×10^8, 6.9×10^6	+11, +12	25.2, 73.2	aGvHD Ⅰ cGvHD (skin) AIHA	Alive CR	27
4	2.8 Male	FLU 150, CY 200 ATG 12.5, TBI 3GY	Father haplo	BM PB	CSA+MTX+MMF	9.4×10^8, 1.2×10^6	9.6×10^8, 6.9×10^6	+12, +15	1.0, >95.0	Accident† aGvHD Ⅳ (bowel) Intracranial hemorrhage	Dead CR2	7
5	1.4 Male	FLU 140, CY 200 ATG 10, BU9.6‡	Sibling haplo	BM PB	CSA+MTX+MMF	9.6×10^8, 0.7×10^6	10.9×10^8, 9.1×10^6	+13, +17	>95.0	aGvHD Ⅰ CMV infection	Alive CR	23

HLH, hemophagocytic lymphohistiocytosis; HSCT, hematopoietic stem cell transplantation; haplo, haploidentical; BM, bone marrow; PB, peripheral blood; PBSC, peripheral blood stem cell; TNC, total nucleated cell; NEUT, neutrophil; PLT, platelet; MIN, minimum; MAX, maximum; aGvHD, acute graft-versus-host disease; cGvHD, chronic graft-versus-host disease; FLU, fludarabine; CY, cyclophosphamide; ATG, anti-T lymphocyte globulin; TBI, total body irradiation; CSA, cyclosporine A; MMF, mycophenolate mofetil; MTX, methotrexate; EBV, Epstein-Barr virus; CMV, cytomegalovirus; AIHA, atuoimmunehemolytic anemia; CR, complete response; F/U, follow-up; BU, busulfan; CR2, second complete response.

†The patient complicated hemorrhage inducing acute pericardial tamponade and shock caused by sternal bone marrow puncture at +28 days after HSCT.

‡The patient was too young to accept TBI, so we used low dose BU instead of TBI.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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