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ORIGINAL ARTICLE

Haploidentical transplantation for pediatric patients with acquired severe aplastic anemia

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Techniques for haploidentical hematopoietic stem cell transplantation (haplo-HSCT) to treat severe aplastic anemia (SAA) have recently improved, but no protocol has been evaluated in a large number of pediatric patients. Fifty-two children with SAA received haplo-HSCT in our center. The treatment protocol used G-CSF-primed bone marrow with G-CSF-mobilized PBSCs without *in vitro* T-cell depletion. The conditioning regimen included busulfan/cyclophosphamide and antithymocyte globulin. Fifty-one patients achieved primary engraftment; one child died of regimen-related toxicity on the day +1. Secondary graft failure occurred in three patients. The cumulative incidences of aGVHD grade II–IV and grade III–IV were 39.2 ± 0.5 and 13.7 ± 0.2%, respectively. The cumulative incidence of cGVHD was 34.2 ± 0.5%. The 3-year overall and failure-free survival rates were 84.5 ± 5.0 and 82.7 ± 5.2%, respectively, with a median follow-up time of 744.5 days (100–3294) for surviving patients. The Eastern Cooperative Oncology Group score was the only predictor of overall and failure-free survival rates. Clinical outcomes were similar between the upfront and salvage group. This result suggests that both newly diagnosed and refractory pediatric SAA patients benefit from haplo-HSCT, especially when patients are in good general condition. Therefore, haplo-HSCT might be an alternative therapy for pediatric SAA patients without HLA-matched sibling donors.

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INTRODUCTION

Severe aplastic anemia (SAA) is a life-threatening marrow syndrome defined as pancytopenia with a hypocellular bone marrow.[1] An estimated 6–9 million cases of SAA occur each year in Asia, three times as many as in Europe and America.[2]

SAA outcomes have significantly improved in recent decades, thanks to hematopoietic stem cell transplantation (HSCT) and immunosuppressive treatments (IST), including antithymocyte globulin (ATG) and cyclosporine A (CsA). HLA-matched sibling donor (MSD) HSCT is the first treatment choice for SAA patients younger than 18 years of age.[2] Data from different countries show that long-term overall survival (OS) is above 90%[3–5] in MSD cases. However, more than 70% of pediatric patients lack the opportunity for MSD,[6] particularly in China where family sizes are shrinking. IST is also an effective treatment for SAA, but response rates at 6 months are only 55–60%, 5–6% patients have clonal evolution within 10 years and relapse rates can reach 15%.[7] Another treatment recommended by Associazione Italiana Ematologia Oncologia Pediatrica guidelines is matched unrelated donor HSCT.[2] Treatment outcomes for matched unrelated donor HSCT in children are as good as MSD HSCT,[8] but difficulties finding a matched unrelated donor and preparing for an HSCT can cost precious time during which complications may occur. Meanwhile, almost every patient has a haploidentical donor and haploidentical HSCT (haplo-HSCT) can be prepared within several weeks. Despite recent progress in haplo-HSCT, the OS is about 64.6–79.7%, still worse than MSD HSCT.[3,9,10] However, these studies included small sample sizes (fewer than 20 patients) of both children and adults. Furthermore, these studies either mixed upfront and salvage therapy or only included salvage therapy. No study including a sufficient number of patients has evaluated a protocol focused on pediatric patients.

PATIENTS AND METHODS

Patients

Fifty-two children (under 18 years) with acquired SAA/VSAA who underwent haplo-HSCT at the Institute of Hematology, Peking University were enrolled in this study between February 2007 and November 2015. All patients were diagnosed with SAA/VSAA as defined by the International Aplastic Anemia Study Group.[11] Patients had no active infection or severe diseases of vital organs before transplantation. Informed consent was obtained from all patients or their guardians and donors. This protocol was approved by the Institutional Review Board of Peking University. Twenty-nine patients were included in the clinical trial registered as ChiCTR-ONC-12002107 at www.chictr.org.cn. Seven cases were previously reported.[10]

Patients and their donors were haploidentical. Donor selection was based on HLA compatibility for HLA-A, -B and -DRB1 (-C also included in 26 patients) by high-resolution techniques. Additional selection factors included age (younger preferred), father or mother (father preferred), non-inherited maternal antigens mismatch, and health status (healthier preferred).

Conditioning regimen

All patients received the following IV conditioning regimen: busulfan (0.8 mg/kg every 6 h on days −7 and −6, total dose 6.4 mg/kg), cyclophosphamide (50 mg/kg once daily on days −5 to −2, total dose 200 mg/kg), and rabbit ATG (SangStat, Lyon, France; 2.5 mg/kg once daily on days −5 to −2, total dose 10 mg/kg).

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Stem cell harvest

All donors received 5 μg/kg recombinant human granulocyte CSF (rhG-CSF) once daily for 5–6 consecutive days starting on day −3. BM cells were collected on day 01. The target volume was 10–12 ml/kg of donor weight or the target mononuclear cell count is $2–4\times10^8$/kg of recipient weight. PBSCs were harvested on day 02 or day 03 to achieve a target mononuclear cell of $6–8\times10^8$/kg of recipient weight from BM and PBSCs. All patients received the BM transfusion on day 01, the PBSC transfusion on day 02 (and day 03 if necessary). For patients with major ABO blood group incompatibility with their donors, hydroxyethyl starch was used for red blood cell sedimentation from BM cells.

GVHD prophylaxis and treatment

All patients received immunosuppressive agents including CsA, mycophenolate mofetil and short-time methotrexate to prevent GVHD.[12–14] Patients received IV CsA (1.5 mg/kg) every 12 h from day −9 until the patient's gastroenteric function returned to normal, then CsA could be given orally. Patients received mycophenolate mofetil (250–500 mg every 12 h) from day −9 to day +30, and 250 mg once or every 12 h from day +30 to day +60 based on body weight. Methotrexate was administered IV at 15 mg/m^2 on day +1 and 10 mg/m^2 on days +3, +6, and +11. CsA concentrations were monitored and kept at 200–250 ng/ml for 12 months after HSCT, then CsA was gradually reduced for the next 2–3 months. Acute GVHD was treated as described by Huang *et al.*[12–14]

Prevention of other complications and supportive care

All patients received medicated baths and skin preparation before entering the laminar airflow clean ward, where they remained until neutrophil recovery. Nonabsorbable oral antibiotics (generally gentamycin for children) were taken for gastrointestinal decontamination from day −9 to myeloid recovery. Infection prevention measures were as described by Huang *et al.*[12–14]

Patients received red blood cell transfusions when their hemoglobin levels were below 70 g/L or platelet transfusions if their platelet levels dropped below 20×10^9/L. All patients received G-CSF (5 μg/kg once daily) from day +6 until myeloid recovery. All blood products were irradiated with 2500 cGy before infusion. Human Ig (400 mg/kg) was administered IV on days +1, +11, +21 and +31.

Definitions and post-transplantation assessment

The day of myeloid engraftment was defined as the first of three consecutive days with an ANC $\geqslant0.5\times10^9$/L, and the day of platelet engraftment was defined as the first day with a platelet count $\geqslant20\times10^9$/L for consecutive 7 days without platelet transfusion. Hematopoietic chimerism was evaluated by PCR amplification of STRs for all patients using peripheral blood samples and buccal mucosa and by FISH for sex-mismatched pairs using BM samples. Full donor chimerism was defined as >95% donor hematopoietic cell and mixed chimerism was defined as 5–95% donor cells. After HSCT, BM samples were drawn on months +1, +2, +3, +6, +12, +24 and +36 for FISH, myelogram and biopsy. Lymphocyte counts, cellular immune reconstitution (CD3+, CD4+ and CD19+ cells from peripheral blood) and humoral immune reconstitution (IgA, IgG and IgM) were monitored when BM samples were drawn.

The primary end point is myeloid engraftment

Graft failure involves rejection and poor graft function. Primary rejection was defined as failure to achieve myeloid engraftment until day +28 post HSCT. Secondary rejection was defined as graft loss after initial engraftment, that is, complete or partial recovery of donor-origin hematopoiesis followed by recurrent pancytopenia with a markedly hypocellular BM in the absence of moderate to severe acute GVHD.[15,16] Poor graft function was defined as ANC $<0.5\times10^9$/L and platelet counts $<20\times10^9$/L (with or without hemoglobin decline) lasting for at least 2 weeks with full donor chimerism. Incidents caused by infection, GVHD or drugs that could recover without donor lymphocyte infusion were not included.

Acute and chronic GVHD was defined and graded as previously described.[17,18] The regimen-related toxicity (RRT) defined as toxicity due to the preparative regimen was evaluated as previously reported.[19] Death, engraftment failure and relapse were defined as treatment failures. Failure-free survival (FFS) was defined as survival with response. Death without disease progression was defined as transplantation-related mortality (TRM).

Functional status was evaluated by the Eastern Cooperative Oncology Group (ECOG) Performance Status score ranging from 0 (asymptomatic) to 5 (death) that has been adopted by the World Health Organization.[20]

Upfront therapy is when patients received IST for 0–4 months prior to HSCT. Salvage therapy is when patients received IST for longer than 4 months or had experienced HSCT failure before haplo-HSCT.

Statistics

The last follow-up for all surviving patients occurred on 28 February 2016. OS and FFS were estimated using the Kaplan–Meier method as implemented in SPSS. The cumulative incidence (CI) of engraftment and acute and chronic GVHD was evaluated by the competing risk model in the R package 'cmprsk'. The patient who did not achieve primary engraftment was excluded when calculating the GVHD. Statistical analyses were conducted using SPSS 19.0 and R version 3.2.2.

RESULTS

Patient characteristics

Fifty-two SAA/VSAA pediatric patients (27 male) with a median age of 9 (2–17) years were enrolled in this study. Twenty-nine patients received haplo-HSCT as salvage treatment. Among these patients, one received MSD HSCT previously, 15 received ATG treatment for at least one course, and 13 patients previously received CsA+ stanozolol or testosterone ± steroid treatments lasting for at least 4 months. The median time from diagnosis to transplantation was 7.5 (1–91) months. Details are shown in Table 1.

Engraftment

Fifty-one patients achieved myeloid recovery after haplo-HSCT. One patient died of toxicity during the conditioning regimen on day +1, but no primary graft failure occurred. CI of engraftment was 96.2 ± 0.1%. The median time of myeloid engraftment was 12 (10–22) days.

Three patients had secondary graft failure. One patient experienced secondary rejection on day +20 and salvage therapy using a combination of unrelated umbilical cord blood and a second HSCT from original haplo-donor failed. This patient died on day +84. Another patient experienced mixed chimerism and rejection on day +25 after having achieved primary engraftment on day +11. This patient recovered after a second HSCT from the original haplo-donor combined with umbilical cord blood, and achieved myeloid engraftment on day +45 after the second transplantation. The third patient developed late poor graft function caused by severe GVHD, CMV antigenemia and drugs used in the treatment on day +110. This patient was treated with G-CSF, but died on day +145.

Fifty patients (96.2%) reached the criteria of WBC $\geqslant1.5\times10^9$ for consecutive 3 days on the median time of 13 (10–21) days.

Forty-seven patients (90.4%) achieved platelet engraftment with a median time of 14 (7–180) days. The CI was 90.4 ± 0.2%.

GVHD

Among the 51 patients who achieved primary engraftment, aGVHD was observed in 38 patients (74.5%), 18 cases (35.3%) experienced grade I, 13 cases (25.5%) experienced grade II, 5 cases (9.8%) experienced grade III and 2 cases (3.9%) experienced grade IV. CI of aGVHD grades II–IV and grades III–IV was 39.2 ± 0.5% and 13.7 ± 0.2%, respectively.

Sixteen of the 42 patients who survived more than 100 days after haplo-HSCT (38.1%) developed cGVHD. CI of cGVHD was 34.2 ± 0.5%. In 13 patients (81.3%) the GVHD involved the skin, 1 patient (6.3%) the liver and 1 patient (6.3%) the skin and gastrointestinal tract. The patient with extensive cGVHD (liver,

Table 1. Patient characteristics

Disease status at transplantation	
SAA	32
VSAA	20
ECOG	
0	3
1	28
2	16
3	5
HLA-mismatched loci	
0	1
1	2
-B	1
-DRB1	1
2	13
-A, -B	6
-A, -DRB1	4
-B, -DRB1	3
3	
-A, -B, -DRB1	36
HLA-mismatched loci including -C	26
1 (-DRB1)	1
2	6
-A, -B	1
-A, -C	1
-B, -C	1
-A, -DRB1	1
-B, -DRB1	1
-C, -DRB1	1
3 (-A, -B, -C)	1
4 (-A, -B, -C, -DRB1)	18
Serum ferritin (28)	1738 ng/mL (259.1–7434)
Transfusion before haplo-HSCT	
Red cells (42)	19U (0–120 U)
Platelets (42)	15U (2–120 U)
Cells infused (×108/kg)	
MNC	8.9750 (7.11–18.57)
CD3+ T cells	2.1809 (0.2609–4.2793)
CD4+ T cells	1.1784 (0.1309–2.51)
CD8+ T cells	0.7542 (0.1113–22.5116)
CD34+ cells	3.2850 (1.0371–17.57)
CD4/CD8 ratio	
BM	1.16 (0–2.75)
PBSC	1.61 (0.64–5.71)
Total	1.50 (0.09–5.15)
Follow-up for surviving patients (days)	632.5 (18–3182)

Abbreviations: ATG = antithymocyte globulin; CsA = cyclosporine A; ECOG = Eastern Cooperative Oncology Group Performance Status; SAA = severe aplastic anemia; VSAA = very severe aplastic anemia.

skin and gastrointestinal tract) on day +540 was treated with corticosteroid, CsA and mycophenolate mofetil, and the GVHD was well controlled.

Among 35 patients whose follow-up time was >15 months, only 7 patients (20.0%) remained on immunosuppression after month +15 because of cGVHD. Three patients have stopped taking the immunosuppression on months +18, +25 and +17, respectively. The other four remained on immunosuppression until the end point of follow-up. The follow-up times were 18, 20, 24 and 26 months, respectively.

Infection and immune reconstitution

Of 52 SAA children, 11 (21.2%) had bacterial infections, 6 (11.5%) had fungal infections and 41 (78.8%) had virus infections. CMV antigenemia occurred in 36 patients (69.2%). EBV antigenemia occurred in three patients (5.8%). Only one patient developed post-transplant lymphoproliferative disorder and recovered after rituximab treatment.

The median absolute lymphocyte count on day +30 was 390 (50–3080)/μL. Median IgA, IgG and IgM values were 0.4 (0.1–0.95), 9.4 (3.5–41.3) and 0.327 (0.096–1.19) g/L, respectively. Other data of immune reconstitution after haplo-HSCT are shown in Table 2. For the only successful second haplo-HSCT case, the absolute lymphocyte count on day +30 and +60 were 100 and 300/μL. The CD3+ T cells, CD4+ T cells, CD+19 T cells, IgA, IgG and IgM on day +60 were 15.99×10^6/L, 30.99×10^6/L, 0.03×10^6/L, 0.57 g/L, 7.1 g/L and 0.850 g/L, respectively.

Regimen-related toxicity

All patients received the conditioning regimen on schedule, and 31 patients did not experience RRT. Grades I and II RRT occurred in 17 and 3 patients, respectively. One patient suffered Grade IV RRT, and died of acute myocardial, renal and gastrointestinal injury on day +1. Sixteen cases had gastrointestinal involvement, two patients experienced liver injury and one patient had hemorrhagic cystitis.

Survival and TRM

Forty-four (84.3%) patients survived to the final follow-up date. Three-year OS was 84.5 ± 5.0% and FFS was 82.7 ± 5.2% with a median follow-up time of 744.5 (100–3294) days for surviving patients (Figures 1a and b). TRM at day +100 and year +1 was 13.5 ± 4.7% and 15.5 ± 5.0%, respectively. Causes of death included RRT (one case), GVHD (three cases), graft failure (two cases), cerebral fungal infection (one case) and cerebral hemorrhage (one case). Forty-three of all 44 surviving patients achieved hematologic CR. One patient required transfusions after the second transplantation until the end of follow-up period.

Kaplan–Meier analysis revealed that the ECOG score before transplantation was the only predictor for OS and FFS ($P = 0.004$ and 0.014, respectively) (Figures 2a and b). Other factors are shown in Table 3.

Donors

Among all 52 donors, 36 were fathers, 14 were mothers and two were siblings. Clinical outcomes were similar for father and mother donors: myeloid engraftment time ($P = 0.973$), platelet engraftment time ($P = 0.779$), aGVHD grade II–IV ($P = 0.475$), cGVHD ($P = 0.578$), bacterial infection ($P = 0.520$), fungal infection ($P = 0.102$), virus infection ($P = 0.520$), OS ($P = 0.490$) and FFS ($P = 0.720$).

Thirty-six patients had three HLA-mismatched loci and16 patients 0–2 HLA-mismatched loci for all the patients whose HLA compatibility was for HLA-A, -B and -DRB1. No significant difference was found between these two groups: myeloid engraftment time ($P = 0.385$), platelet engraftment time ($P = 0.657$), aGVHD grade II–IV ($P = 0.664$), cGVHD ($P = 0.093$), OS ($P = 0.233$) and FFS ($P = 0.347$).

HLA-C was detected in 26 patients and their donors. Eight patients had 1–3 HLA-mismatched loci and other 18 patients had 4 HLA-mismatched loci. There was also no significant difference between these two groups: myeloid engraftment time ($P = 0.170$), platelet engraftment time ($P = 0.414$), aGVHD grade II–IV ($P = 0.142$), cGVHD ($P = 0.458$), OS ($P = 0.962$) and FFS ($P = 0.808$).

Similar outcomes between upfront therapy and salvage therapy

Among all 52 cases, 23 cases received haplo-HSCT as upfront therapy and 29 as salvage therapy. There were no significant differences in baseline data (age, recipient gender, donor gender,

Table 2. Data of immune reconstitution

	30 days	*60 days*	*90 days*	*180 days*	*365 days*	*730 days*
Cell ($\times 10^6$/L)						
ALC	390 (50–3080, 41)	1295 (150–3610, 34)	1480 (160–6640, 31)	1860 (360–4220, 30)	2390 (1100–5210, 27)	3440 (2270–3640, 3)
CD3+	15.41 (0.26–674.622, 39)	66.192 (1.1492–887.285, 33)	462.981 (8.736–4406.968, 31)	593.75 (64.98–1874.102, 30)	956.8 (179.5–3057.228, 27)	974.896 (955.897–1445.444, 3)
CD4+	4.588 (0.02–129.636, 39)	47.31 (1.326–218.12, 33)	61.88 (0.96–1124.152, 31)	114.018 (1.08–594.664, 30)	249.487 (44.11–804.375, 27)	384.02 (305.769–452.704, 3)
CD19+	0.9 (0.025-9.32, 39)	3.78 (0.442–19.494, 33)	9.52 (0–97.614, 31)	32.979 (0.576–355.68, 30)	110.88 (3.85–498.96, 27)	187.136 (21.112–198.398, 3)
Humoral (g/L)						
IgA	0.4 (0.1–0.95, 33)	0.23 (0.0647–0.52, 27)	0.26 (0.0067–1.58, 28)	0.523 (0.0796–1.21, 29)	0.78 (0.29–2.34, 25)	1.265 (1.16–1.37, 2)
IgG	9.4 (3.5–41.3, 33)	8.4 (1–19.7, 27)	7 (2.1–15.1, 28)	8.1 (1.9–20.3, 29)	10.1 (2.82–19.5, 25)	14.15 (10.6–17.7, 2)
IgM	0.327 (0.096–1.19, 33)	0.24 (0.057–0.976, 27)	0.284 (0.075–1.28, 28)	0.504 (0.104–2.4, 29)	0.743 (0.275–2.04, 25)	1.149 (0.998–1.3, 2)

Abbreviation: ALC = absolute lymphocyte count.

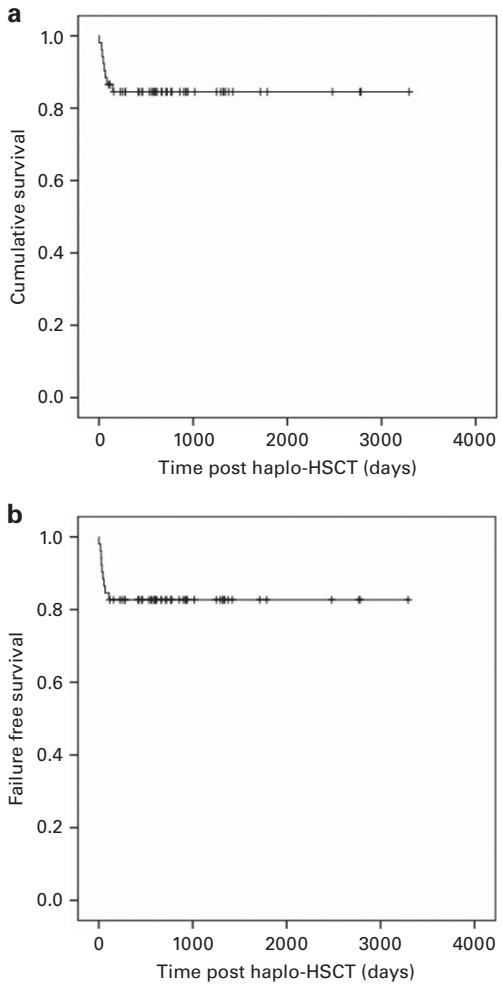

Figure 1. Kaplan–Meier estimates of (**a**) overall survival and (**b**) failure-free survival in children with SAA/VSAA after haplo-HSCT.

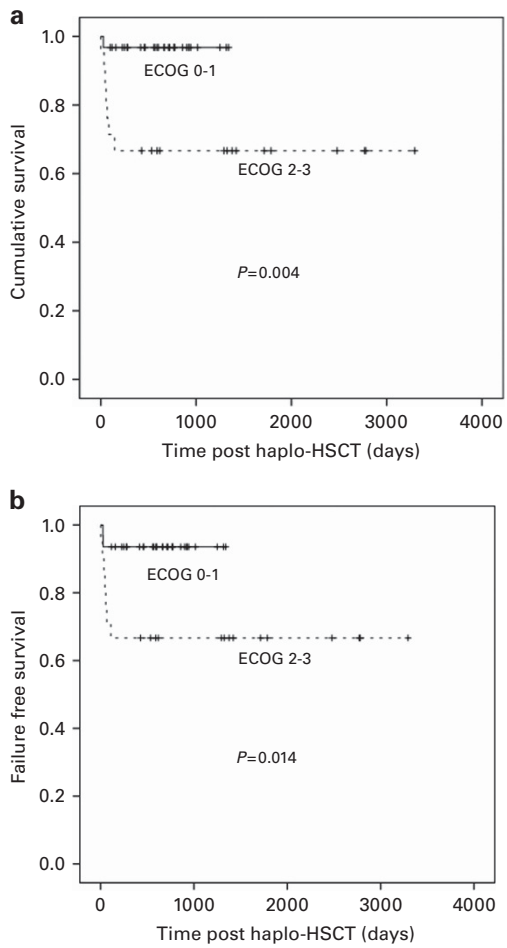

Figure 2. Kaplan–Meier estimates of (**a**) overall survival and (**b**) failure-free survival after haplo-HSCT according to ECOG in pediatric SAA/VSAA patients.

Table 3. Effects of different factors on OS and FFS of pediatric SAA/VSAA patients

Factors	N	*OS (%)*	P-*value*	*FFS (%)*	P-*value*
Patients age at transplantation (year)			0.409		0.645
⩾9	27	88.9 ± 6.0		85.2 ± 6.8	
<9	25	80.0 ± 8.0		80.0 ± 8.0	
Patient gender			0.526		0.309
Female	25	88.0 ± 6.5		88.0 ± 6.5	
Male	27	81.3 ± 7.5		77.8 ± 8.0	
Donor gender			0.440		0.658
Female	14	78.6 ± 11.0		78.6 ± 11.0	
Male	38	86.7 ± 5.6		84.2 ± 5.9	
Donor–recipient relationship			0.490		0.720
Mother–child	14	78.6 ± 11.0		78.6 ± 11.0	
Father–child	36	85.9 ± 5.8		83.3 ± 6.2	
Donor–recipient sex match			0.337		0.210
Female–female/male–male	31	80.5 ± 7.1		77.4 ± 7.5	
Female-male/male–female	21	90.5 ± 6.4		90.5 ± 6.4	
ATG before transplantation			0.825		0.656
Yes	15	86.7 ± 8.8		86.7 ± 8.8	
No	37	83.6 ± 6.1		81.1 ± 6.4	
Disease status at transplantation			0.937		0.754
SAA	32	84.1 ± 6.5		81.3 ± 6.9	
VSAA	20	85.0 ± 8.0		85.0 ± 8.0	
ECOG			**0.004**		**0.014**
0–1	31	96.8 ± 3.2		93.5 ± 4.4	
2–3	21	66.7 ± 10.3		66.7 ± 10.3	
Diagnosis-to-transplantation interval (month)			0.460		0.802
⩾7.5	26	80.6 ± 7.8		80.8 ± 7.7	
<7.5	26	88.5 ± 6.3		84.6 ± 7.1	
Diagnosis-to-transplantation interval (month)			0.152		0.304
⩾24	15	72.7 ± 11.7		73.3 ± 11.4	
<24	37	89.2 ± 5.1		86.5 ± 5.6	
Haplo-HSCT status			0.698		0.899
Upfront therapy	23	87.0 ± 7.0		82.6 ± 7.9	
Non-upfront therapy	29	82.6 ± 7.1		82.8 ± 7.0	
HLA-mismatched loci			0.233		0.347
0–2	16	75.0 ± 10.8		75.0 ± 10.8	
3	36	88.9 ± 5.2		86.1 ± 5.8	
Serum ferritin (ng/mL)			0.258		0.167
⩾1738	14	92.9 ± 6.9		92.9 ± 6.9	
<1738	14	78.6 ± 11.0		71.4 ± 12.1	
Regimen-related toxicity			0.854		0.679
0	31	83.7 ± 6.7		80.6 ± 7.1	
1–4	21	85.7 ± 7.6		85.7 ± 7.6	
MNC ($\times10^8$/kg) infused			0.964		0.634
⩾8.975	26	84.6 ± 7.1		80.8 ± 7.7	
<8.975	26	84.4 ± 7.2		84.6 ± 7.1	
CD3+ T-cell counts ($\times10^8$/kg) infused			0.907		0.655
⩾2.1809	27	85.2 ± 6.8		85.2 ± 6.8	
<2.1809	25	83.8 ± 7.4		80.0 ± 8.0	
CD4+ T-cell counts ($\times10^8$/kg) infused			1.000		0.666
⩾1.1784	26	84.6 ± 7.1		80.8 ± 7.7	
<1.1784	26	84.4 ± 7.2		84.6 ± 7.1	
CD8+ T-cell counts ($\times10^8$/kg) infused			0.962		0.737
⩾0.7542	26	84.6 ± 7.1		84.6 ± 7.1	
<0.7542	26	84.6 ± 7.1		80.8 ± 7.7	
CD34+ T-cell counts ($\times10^8$/kg) infused			0.986		0.747
⩾3.285	27	85.2 ± 6.8		81.5 ± 7.5	
<3.285	25	84.0 ± 7.3		84.0 ± 7.3	
Red cells transfusion (U) pre-HSCT			0.655		0.623
⩾19	21	90.5 ± 6.4		90.5 ± 6.4	
<19	21	85.7 ± 7.6		85.7 ± 7.6	
Platelets transfusion (U) pre-HSCT			0.202		0.457
⩾15	22	81.8 ± 8.2		81.8 ± 8.2	
<15	20	95.0 ± 4.9		90.0 ± 6.7	

Abbreviations: ECOG = Eastern Cooperative Oncology Group Performance Status; MNC, mononuclear cell; SAA = severe aplastic anemia; VSAA = very severe aplastic anemia. Significant P-values are in bold type.

disease status at transplantation, disease duration and ECOG). No significant differences in clinical outcomes between the two groups were observed: myeloid engraftment time ($P = 0.175$), aGVHD grade II–IV ($P = 0.699$), cGVHD ($P = 0.916$), OS ($P = 0.698$) and FFS ($P = 0.899$) (Figures 3a and b).

DISCUSSION

A new technique for HLA-mismatched allogeneic HSCT using G-CSF-primed bone marrow with G-CSF-mobilized PBSCs without *in vitro* T-cell depletion known as the GIAC protocol has been explored at the Institute of Hematology, Peking University People's Hospital.[21–24] Nineteen SAA patients treated with the GIAC protocol experienced positive treatment outcomes, suggesting that haplo-HSCT might be feasible for SAA patients without MSD.[10]

When MSD HSCT was administered in 65 pediatric SAA patients, the FFS was 87.7, 36.9% patients experienced aGVHD grade II–IV, 20% experienced aGVHD grade III–IV and extensive cGVHD occurred in 7.7% of patients.[25] The FFS of our upfront therapy group using the GIAC protocol was similar at 82.6 ± 7.9%. Although the CI of cGVHD was higher, only one extensive cGVHD occurred and all cGVHD cases were well controlled.

For SAA children without matched siblings or unrelated donors, IST should be administered rather than haplo-HSCT according to current guidelines.[1,2] In a recent European study, 167 SAA patients aged 0–12 who received IST as frontline therapy experienced OS at 87%, but FFS was only 33%.[26] Another study included 63 pediatric SAA patients without MSD who were treated with rabbit ATG and CsA as firstline IST.[27] Thirty-four of these patients (64.9%) went into remission, but only 24 (38.0%) went into CR. The 10-year OS and FFS were 67 and 57%. Another study from Japan found that the FFS for SAA patients younger than 17 years who were treated with firstline IST was only 56%.[28] These global studies have shown that clinical outcomes of IST, including ATG, were unsatisfactory because of clonal evolution and high relapse rates. A recent finding suggests that the OS of haplo-HSCT in pediatric SAA is comparable to IST.[29] Long-term FFS of the upfront therapy group in our trial was much better than that of IST mentioned above.

IST is sometimes preferred over HSCT for patients without matched donors because of the high risk of TRM in haplo-HSCT. However, the 1-year TRM in our study was acceptable at only

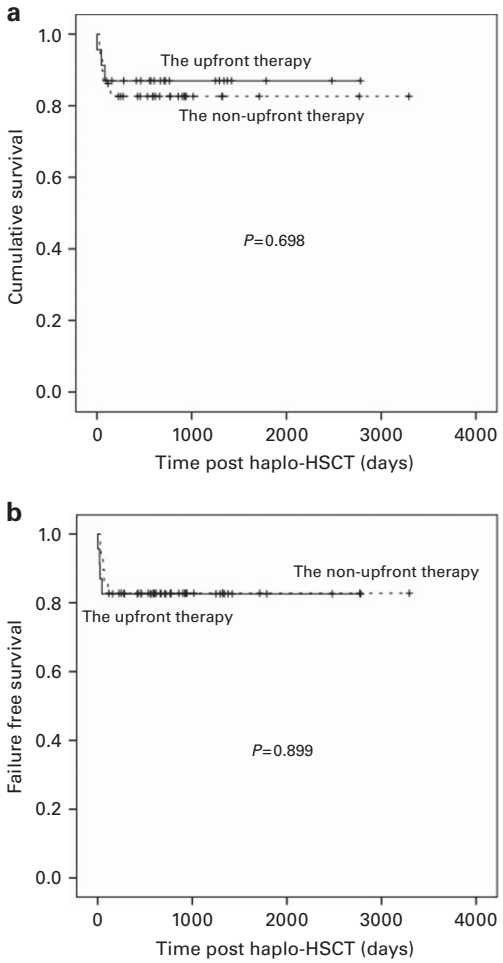

Figure 3. Kaplan–Meier estimates of (**a**) overall survival and (**b**) failure-free survival after haplo-HSCT according to haplo-HSCT status in pediatric SAA/VSAA patients.

15.5 ± 5.0%. Furthermore, many problems may occur when delaying HSCT. For example, hemorrhage may occur because of low platelet counts, low WBC increases the risk of refractory infections and iron overload may be caused by extensive transfusions. Events such as these may influence the patient's general condition and decrease the ECOG score before HSCT. A reliable ECOG score is important, as it was the only predictor of OS/FFS in our study. More tragically, some patients may miss their opportunity for HSCT or die while waiting for the treatment.

In previous studies of haplo-HSCT for hematological malignancies,[30] father donors were associated with better outcomes than mothers. We found no significant difference in clinical outcome between father and mother donors in these SAA pediatric patients. Larger SAA trials are needed to further investigate this result.

Studies of acute leukemia show that the CI of aGVHD and cGVHD was not associated with the extent of HLA disparity.[14] We also found no difference in clinical outcomes (engraftment, aGVHD, cGVHD, OS and FFS) not only between patients in the 0–2 loci mismatched group and in the three loci mismatched group but also between 1–3 loci mismatched group and 4 loci mismatched group (for 26 patients who received HLA-C detected).

OS for patients who waited ⩾2 years after diagnosis to receive haplo-HSCT was similar to OS for patients who received the treatment more quickly after diagnosis. This finding suggests that SAA patients can still benefit from haplo-HSCT years after diagnosis and thus should be considered for treatment as soon as possible if they have no haplo-HSCT contraindications.

There were also several limitations in this study. This was a single center study. The data of serum ferritin and transfusions were not complete, especially only 28 ferritin values before HSCT were available. And this study did not include any patients with poor general condition who were excluded from haplo-HSCT previously.

Nevertheless, both the upfront therapy group and the salvage therapy group experienced excellent clinical outcomes (OS, FFS and GVHD), showing that both haplo-HSCT strategies are beneficial. Our findings suggest that haplo-HSCT can be considered as an alternative therapy for newly diagnosed and refractory pediatric SAA patients if MSD is not available. Further studies with larger sample sizes are required to validate these findings and secure mechanistic insight into factors influencing clinical outcomes after haplo-HSCT.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

XJ Huang designed the research; LP Xu and XJ Huang analyzed the data and wrote the manuscript; and all authors, provided patient data and gave final approval for the manuscript.

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