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Original Research

The efficacy and safety of third-party umbilical blood/umbilical cord mesenchymal stem cell assisted related haploid hematopoietic stem cell transplantation in pediatric patients with acute leukemia: an observational study

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Abstract

Background: There is limited data on third-party umbilical cord blood (UCB) or mesenchymal stem cell (MSC) transplantation-assisted haploidentical hematopoietic stem cell transplantation (haplo-HSCT) in pediatric patients.

Objective: To evaluate the efficacy and safety of UCB and MSC transplantation-assisted haplo-HSCT in pediatric patients with acute leukemia (AL).

Design: Observational study.

Methods: Clinical data of 152 children with AL undergoing haplo-HSCT at the Children's Hospital of Soochow University between January 2020 and June 2022 were collected. The patients were divided into the haplo-HSCT + UCB group ($n = 76$), haplo-HSCT + MSC group ($n = 31$), and haplo-HSCT group ($n = 45$). Hematopoietic reconstruction time, complications within 30 days after transplantation, and survival and recurrence at 3 years after transplantation were compared among the groups.

Results: Multivariate analysis revealed that haplo-HSCT with MSC and human leukocyte antigen (HLA) matching ⩾6/10 were independent factors reducing engraftment syndrome (ES) incidence. There were no significant differences among the groups in the hematopoietic reconstruction time or incidence of complications within 30 days after transplantation ($p > 0.05$). Overall survival, relapse-free survival, cumulative incidence of relapse, cumulative incidence of hematological relapse, and 3-year transplant-related mortality were not significantly different ($p > 0.05$). The incidence of adverse reactions in the haplo-HSCT + UCB group was 97.3% within 4 h after UCB infusion, with a particularly high occurrence rate of 94.7% for hypertension. No transfusion-related adverse reactions occurred after the transfusion of umbilical cord MSC in the haplo-HSCT + MSC group.

Conclusion: MSC-assisted haplo-HSCT can reduce ES incidence after transplantation in pediatric patients with AL. UCB infusion is associated with a high incidence of reversible hypertension. However, no adverse reactions were observed in umbilical cord MSC transfusion.

Keywords: acute leukemia, effectiveness, haploidentical hematopoietic stem cell transplantation, safety, umbilical cord blood, umbilical cord mesenchymal stem cells

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Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is an effective treatment for various hematological diseases. Human leukocyte antigen (HLA)-matched siblings or unrelated donors are considered the best options for allo-HSCT; however, only a few patients have HLA-matched donors. Haploidentical hematopoietic stem cell transplantation (haplo-HSCT) has been increasingly used in clinical practice because of the easy donor availability. However, haplo-HSCT increases the incidence of graft-versus-host disease (GVHD) and nonrelapse mortality and reduces quality of life and long-term survival rates.[1] Recently, optimized haplo-HSCT protocols such as third-party umbilical cord blood (UCB)/mesenchymal stem cell (MSC)-assisted haplo-HSCT and posterior cyclophosphamide conditioning regimens have been proposed. Previous studies have demonstrated the efficacy of UCB-assisted haplo-HSCT in improving overall survival (OS) and reducing transplant-related mortality and cumulative relapse rates in adult patients.[1,2] Umbilical cord MSC-assisted haplo-HSCT can promote engraftment, attenuate acute GVHD (aGVHD), and prevent chronic GVHD (cGVHD) in adults.[3] However, the efficacy and safety of UCB/MSC-assisted haplo-HSCT in pediatric patients with acute leukemia (AL) require further investigation.

This study aimed to investigate the efficacy and safety of UCB/umbilical cord MSC-assisted haplo-HSCT for the treatment of pediatric AL and identify the preferred option for haplo-HSCT for the treatment of AL in children.

Methods

Study subjects

Data were collected from 166 patients with AL who underwent allo-HSCT at the Children's Hospital of Soochow University between January 2020 and June 2022. The following exclusion criteria applied: age ⩾18 years; ex vivo T cell depletion; engraftment failure; death within 30 days of transplantation; and nonfirst transplantation. A total of 14 patients were excluded (Please refer to Figure 1 in the Supplemental File for details). The study subjects were divided into three groups: a haplo-HSCT + UCB group ($n = 76$), a haplo-HSCT + MSC group ($n = 31$), and a haplo-HSCT group ($n = 45$).

Conditioning regimens for HSCT

The conditioning regimens included either FLAG (fludarabine + cytarabine + granulocyte colony-stimulating factor) or CLAG (cladribine + cytarabine + granulocyte colony-stimulating factor) combining with total body irradiation or busulfan plus cyclophosphamide as myeloablative conditioning. Rabbit antihuman thymocyte immunoglobulin (ATG) for in vivo T-cell depletion, as appropriate (Please refer to Table 1–3 in the Supplemental File for details).

Principles of UCB and umbilical cord MSC infusion

The UCB was stored in liquid nitrogen, thawed using a 37°C water bath prior to infusion, and infused into the recipient within 15–20 min after resuscitation. The average cell doses for UCB infusion were 3.28 (0.37–12.67) $\times 10^7$/kg for mononuclear cells (MNCs) and 0.99 (0.2–3.22) $\times 10^5$/kg for CD34+ cells. The bone marrow stem cells (BMSCs) and peripheral blood stem cells (PBSCs) were transfused 4 h after UCB infusion.

Umbilical cord MSCs are derived from the umbilical cord and undergo rigorous isolation, extraction, and cultivation to obtain third-generation cells. MSCs 1×10^6/kg were infused at +1 day after transplantation, and 0.5–1.0 mg/kg of promethazine hydrochloride was given orally before infusion to prevent allergy.

Hematological reconstruction monitoring and engraftment evaluation

Hematological changes were monitored daily after HSCT. Upon neutrophil engraftment, short tandem repeats of DNA from the bone marrow (BM) or peripheral blood (PB) of the patients were regularly monitored as a quantitative test of donor cell chimerism.

GVHD prophylaxis

All patients were treated with cyclosporine A/tacrolimus, mycophenolate mofetil (MMF), and methotrexate to prevent GVHD (Please refer to Tables 1–3 in the Supplemental File for details).

Clinical definitions

The first of three consecutive days with an absolute neutrophil count $>0.5 \times 10^9$/L was defined as the time of neutrophil engraftment. In the absence of platelet transfusion, the first of seven consecutive days with a platelet count $> 20 \times 10^9$/L was defined as the time of platelet engraftment. Platelets were not implanted +30 days after transplantation; this was defined as delayed platelet engraftment.[4]

The engraftment syndrome (ES) refers to the clinical criteria proposed by Maiolino et al.[5] The diagnosis of aGVHD was based on the MAGIC classification criteria.[6] cGVHD was diagnosed based on the criteria proposed by Shulman et al.[7] Diagnosis and grading of hemorrhagic cystitis (HC) were made with reference to the roller criteria.[8] Veno-occlusive disease (VOD) was defined according to the revised diagnostic criteria of the European Society for Blood and Marrow Transplantation in 2018.[9] Thrombotic microangiopathy (TMA) was diagnosed based on the transplant-associated thrombotic microangiopathy diagnostic criteria proposed by Jodele et al.[10]

There is no standard diagnostic for capillary leak syndrome (CLS) after transplantation, so in this study, CLS was diagnosed mainly by serum albumin below 25 g/L or a progressive albumin level decrease, suggesting exudative changes in the pulmonary interstitium, accompanied by systemic edema, polyserositis, increase in body mass, oliguria, and hypoxemia.

Viral infections after transplantation are managed aggressively in our research center, and antiviral drugs are often administered as soon as the viral copy number in whole blood exceeds the detection limit. Accordingly, in this study, cytomegalovirus (CMV) DNA copy number $>4 \times 10^2$ copies/ml in whole blood was defined as CMV viremia, Epstein–Barr virus (EBV) DNA copy number $>4 \times 10^2$ copies/ml in whole blood was defined as EBV viremia and whole-blood humanparvovirus B19 (VB19) DNA positivity was defined as VB19 virus infection.

There is currently no clear definition of adverse reactions after UCB or MSC transfusion in children both at the national and international levels. This study adopted the following definitions for the adverse effects of UCB or MSC infusion. The mean blood pressure in the quiet state before the start of conditioning chemotherapy was defined as basal blood pressure. An increase in systolic blood pressure in the quiet state of >20 mmHg from the basal systolic blood pressure and/or an increase in diastolic blood pressure in the quiet state of >10 mmHg from the basal diastolic blood pressure from the beginning of UCB infusion to the time of the donor's BMSCs/PBSCs infusion was defined as an elevation in blood pressure. The mean heart rate in the quiet state before the start of conditioning chemotherapy was defined as the basal heart rate, and an elevation of the heart rate in the quiet state by >30% from the basal heart rate after the start of the UCB or MSC infusion and before the donor BMSCs/PBSCs infusion was defined as an increase in the heart rate, and a drop of >30% was defined as a decrease in the heart rate. Allergic, gastrointestinal, neurological, and urinary adverse reactions were defined and graded according to the *Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0*, published by the United States Department of Health and Human Services in 2017.

OS was defined as the duration from transplantation to death or last follow-up. Relapse-free survival (RFS) was defined as the duration from transplantation to death or relapse (including hematological and molecular biological relapses). The cumulative incidence of relapse (CIR) was defined as the time from transplantation to death or relapse (including hematological and molecular biological relapses). The cumulative incidence of hematological relapse was defined as the time from the date of transplantation to death or hematological relapse. Transplant-related mortality (TRM) was defined as the time from the date of transplantation to the time of nonrelapse (including hematological and molecular biological relapses).

Statistical analysis

The statistical analyses were performed using SPSS 26.0 software (IBM, Armonk, NY, USA). Normality and homogeneity of variance were assessed for continuous variables. If the assumptions were met, analysis of variance (ANOVA) was applied; otherwise, the Mann–Whitney *U* test was used for independent sample comparisons, and the Kruskal–Wallis H test was used for multiple group comparisons. The chi-square test was employed for categorical variables that met the

requirements, whereas the rank-sum test was used for variables that did not meet the assumptions. OS, RFS, CIR, cumulative hematological relapse rate, and TRM were calculated using the Kaplan–Meier method. The log-rank test was used for univariate analysis of relevant risk factors, and factors with a p-value $\leqslant 0.1$ were included in the Cox proportional hazards model for multivariate analysis. Statistical significance was set at $p < 0.05$. significant.

Results

Clinical characteristics

This study included 152 pediatric patients with AL who underwent haplo-HSCT at our center between January 2020 and June 2022. Of these, 76 patients underwent haplo-HSCT combined with UCB (haplo-HSCT + UCB), 31 underwent haplo-HSCT combined with MSC (haplo-HSCT + MSC), and 45 underwent haplo-HSCT alone. There were no significant differences among the three groups for sex, age, diagnosis, pretransplant BM morphology, pretransplant minimal residual disease (MRD) positivity (detected by either flow cytometry or quantitative reverse transcription polymerase chain reaction (qRT-PCR) or both methods), conditioning intensity, graft source, GVHD prophylaxis regimen, ABO blood type compatibility between donor and recipient, donor–recipient sex match, HLA typing, mononuclear cell count, and CD34+ cell count ($p > 0.05$; Table 1).

ES within 30 days

The incidence of ES within 30 days posttransplantation was 69.7% (53/76), 51.6% (16/31), and 73.3% (33/45) in the haplo-HSCT + UCB, haplo-HSCT + MSC, and haplo-HSCT groups, respectively, with no significant differences observed ($\chi^2 = 4.400$, $p = 0.1$).

Univariate analysis showed that HLA matching $\geqslant$6/10 was a protective factor against ES. Multivariate analysis revealed that haplo-HSCT combined with MSC transplantation and HLA matching $\geqslant$6/10 were independent factors associated with a reduced incidence of ES (Table 2 and Figure 1).

Among 61 patients with ALL, the ES incidence within 30 days after HSCT was 57.7% (15/26) in the haplo-HSCT + UCB group, 53.8% (7/13) in the haplo-HSCT + MSC group, and 77.3% (17/22) in the haplo-HSCT group, with no significant differences observed ($\chi^2 = 2.711$, $p = 0.258$).

Among 87 patients with acute myeloid leukemia (AML) patients, the incidence of ES within 30 days after HSCT was 75.0% (36/48) in the haplo-HSCT + UCB group, 47.1% (8/17) in the haplo-HSCT + MSC group, and 68.2% (15/22) in the haplo-HSCT group, with no significant differences observed ($\chi^2 = 4.492$, $p = 0.106$).

Hematopoietic reconstruction

The median time to neutrophil engraftment was 11 (range 10–21 days) in the haplo-HSCT + UCB group, 12 (range 10–16 days) in the haplo-HSCT + MSC group, and 11 (range 10–17 days) in the haplo-HSCT group, with no significant differences observed ($H = 0.818$, $p = 0.664$). The median time to platelet engraftment was 11 days (range 5–110 days) in the haplo-HSCT + UCB group, 11 days (range 7–19 days) in the haplo-HSCT + MSC group, and 11 days (range 8–68 days) in the haplo-HSCT group, with no significant differences observed ($H = 0.451$, $p = 0.798$) The rate of delayed platelet engraftment was 8.0% (6/75) in the haplo-HSCT + UCB group (one patient received intermittent platelet transfusions until death at 1 month without achieving platelet engraftment), 0% (0/31) in the haplo-HSCT + MSC group, and 4.4% (2/45) in the haplo-HSCT group, with no significant differences observed ($p = 0.313$). All the patients received complete engraftment of haploid donor cells.

aGVHD within 30 days

In the cohort of 152 patients, the incidence of aGVHD within 30 days of HSCT was 66.4% (101/152 patients). The rates of grades II–IV aGVHD and grades III–IV aGVHD were 24.3% (37/152) and 14.5% (22/152), respectively. In patients with ALL, haplo-HSCT combined with UCB significantly reduced the incidence of gut aGVHD and grades II–IV gut aGVHD within 30 days after HSCT compared to Haplo-HSCT + MSC and haplo-HSCT (0 vs 15.4% and 18.2%, respectively, $p = 0.046$). In patients with AML, haplo-HSCT combined with MSC significantly reduced the incidence of grades III–

Table 1. Clinical characteristics of 152 pediatric patients with acute leukemia.

Characteristics	Total (*N* = 152)	Haplo-HSCT + UCB (*N* = 76)	Haplo-HSCT + MSC (*N* = 31)	Haplo-HSCT (*N* = 45)	*p* value
Age (m, M)	108.5 (9–192)	104 (11–192)	99 (17–188)	113 (9–180)	0.518
Sex (*n*, %)					0.8
Male	94 (61.8)	45 (59.2)	20 (64.5)	29 (64.4)	
Female	58 (38.2)	31 (40.8)	11 (35.5)	16 (35.6)	
Diagnose (*n*, %)					0.53
ALL	61 (40.1)	26 (34.2)	13 (41.9)	22 (48.9)	
AML	87 (57.2)	48 (63.2)	17 (54.8)	22 (48.9)	
MPAL	4 (2.6)	2 (2.6)	1 (3.2)	1 (2.2)	
BM morphology before HSCT (*n*, %)					0.843
CR	141 (92.8)	71 (93.4)	28 (90.3)	42 (93.3)	
Non-CR	11 (7.2)	5 (6.6)	3 (9.7)	3 (6.7)	
MRD before HSCT (*n*, %)					0.267
Negative	119 (79.3)	55 (74.3)	25 (80.6)	39 (86.7)	
Positive	31 (20.7)	19 (25.7)	6 (19.4)	6 (13.3)	
Conditioning chemotherapy intensity (*n*, %)					0.059
MAC	21 (13.8)	7 (9.2)	3 (9.7)	11 (24.4)	
RIC	131 (86.2)	69 (90.8)	28 (90.3)	34 (75.6)	
GVHD prevention (*n*, %)					0.301
CSA + MMF + MTX	136 (89.5)	66 (86.8)	27 (87.1)	43 (95.6)	
FK506 + MMF + MTX	16 (10.5)	10 (13.2)	4 (12.9)	2 (4.4)	
ABO blood type of the donor and recipient (*n*, %)					0.932
Same	83 (54.6)	42 (55.3)	16 (51.6)	25 (55.6)	
Different	69 (45.4)	34 (44.7)	15 (48.4)	20 (44.4)	
Sex of donor and recipient (*n*, %)					0.287
Male donors to male recipients	75 (49.3)	37 (48.7)	13 (41.9)	25 (55.6)	
Male donors to female recipients	42 (27.6)	22 (28.9)	6 (19.4)	14 (31.1)	
Female donors to male recipients	19 (12.5)	8 (10.5)	7 (22.6)	4 (8.9)	
Female donors to female recipients	16 (10.5)	9 (11.8)	5 (16.1)	2 (4.4)	

(Continued)

Table 1. (Continued)

Characteristics	Total (*N* = 152)	Haplo-HSCT + UCB (*N* = 76)	Haplo-HSCT + MSC (*N* = 31)	Haplo-HSCT (*N* = 45)	*p* value
Grafts (*n*, %)					0.079
BM + PB	118 (77.6)	64 (84.2)	20 (64.5)	34 (75.6)	
PB alone	34 (22.4)	12 (15.8)	11 (35.5)	11 (24.4)	
HLA matching (*n*, %)					0.811
5/10	101 (66.4)	49 (64.5)	22 (71.0)	30 (66.7)	
⩾6/10	51 (33.6)	27 (35.5)	9 (29.0)	15 (33.3)	
MNCs (*n*, %)					0.613
$<7\times10^6$/kg	77 (50.7)	38 (50.0)	18 (58.1)	21 (46.7)	
$\geqslant7\times10^6$/kg	75 (49.3)	38 (50.0)	13 (41.9)	24 (53.3)	
CD34+ (*n*, %)					0.483
$<7.1\times10^8$/kg	77 (50.7)	35 (46.1)	18 (58.1)	24 (53.3)	
$\geqslant7.1\times10^8$/kg	75 (49.3)]	41 (53.9)	13 (41.9)	21 (46.7)	

AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; BM, bone marrow; CLS, capillary leak syndrome; CR, complete remission; CSA, cyclosporine A; FK506, tacrolimus; GVHD, graft-versus-host disease; HSCT, hematopoietic stem cell transplantation; MAC, myeloablative conditioning; MMF, mycophenolate mofetil; MNC, mononuclear cell; MPAL, mixed phenotype acute leukemia; MRD, minimal residual disease; MSC, mesenchymal stem cell; MTX, methotrexate; PB, peripheral blood; RIC, reduced intensity conditioning; UCB, umbilical cord blood.

IV gut aGVHD compared to haplo-HSCT combined with UCB (0 vs 22.9%, $p=0.024$).

Virus infection within 30 days

The incidence rates of CMV viremia were 69.7% (53/76) in the haplo-HSCT + UCB group, 64.5% (20/31) in the haplo-HSCT + MSC group, and 51.1% (23/45) in the haplo-HSCT group, within 30 days after HSCT. Similarly, the incidence rates of EBV viremia were 64.5% (49/76), 61.3% (19/31), and 57.8% (26/45), respectively. The incidence rates of VB19 infection were 1.3% (1/76), 0.0% (0/31), and 2.2% (1/45), respectively. Statistical analysis revealed no significant differences in the incidence rates of CMV viremia ($\chi^2=4.245$, $p=0.12$), EBV viremia ($\chi^2=0.542$, $p=0.763$), or VB19 viral infection ($p=1.000$) among the three groups.

Other complications within 30 days

After haplo-HSCT + UCB transplantation, the incidence rates of HC, CLS, VOD, and TMA within 30 days of transplantation were 32.9% (25/76), 19.7% (15/76), 2.6% (2/76), and 2.6% (2/76), respectively. In the haplo-HSCT + MSC group, the incidence rates were 35.5% (11/31), 12.9% (4/31), 0% (0/31), and 0% (0/31), respectively. In the haplo-HSCT group, the incidence rates were 24.4% (11/45), 17.8% (8/45), 0% (0/45), and 0% (0/45), respectively. There were no significant differences in the incidence rates of HC ($\chi^2=1.324$, $p=0.516$), CLS ($\chi^2=0.704$, $p=0.703$), VOD ($p=0.702$), and TMA ($p=0.702$) among the three groups within 30 days.

3-Year OS and RFS

At the last follow-up, the 3-year OS for the 152 pediatric patients was 84.3% ± 3.3%. The median OS time was 23.5 months (range 1–41 months). The 3-year RFS was 75.7% ± 3.6%. In the haplo-HSCT + UCB group, the 3-year OS was 81.8% ± 4.6%, with 13 deaths reported (relapse, 5 cases; infection, 5; GVHD, 1). In the haplo-HSCT + MSC group, the 3-year OS was

Table 2. Risk factor analysis for ES.

ES within 30 days	Univariate analysis	Multivariate analysis		
	p value	HR	HR 95% CI	*p* value
HC within 30 days	0.096	2.028	0.875–4.7	0.099
Neutrophils implanted after 11 days	0.085	0.509	0.243–1.063	0.072
HLA matching ⩾6/10	0.023	0.391	0.181–0.843	0.017
Age ⩾9 years	0.1	0.491	0.234–1.031	0.06
Haplo-HSCT + MSC	0.1	0.301	0.106–0.852	0.024

ES, engraftment syndrome; HC, hemorrhagic cystitis; HSCT, hematopoietic stem cell transplantation; MSC, mesenchymal stem cell.

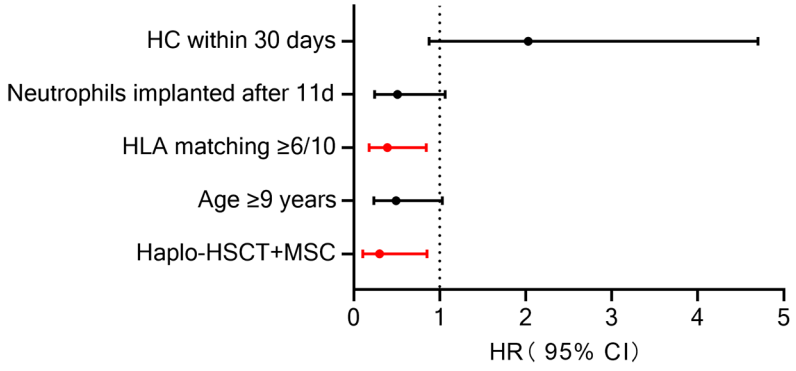

Figure 1. Risk factor analysis for engraftment syndrome.

88% ± 6.8%, with three deaths reported (relapse, 1; infection 1; GVHD 1). In the haplo-HSCT group, the 3-year OS was 85% ± 6.4%, with five deaths reported, two due to relapse, two due to infection, and one due to GVHD. There was no significant difference in 3-year OS among the groups ($\chi^2 = 1.183$, $p = 0.553$). The 3-year RFS were 75.8% ± 5.0% in the haplo-HSCT + UCB group, 69.2% ± 8.8% in the haplo-HSCT + MSC group, and 79.2% ± 6.8% in the haplo-HSCT group, with no significant difference among the groups ($\chi^2 = 1.384$, $p = 0.501$).

In 61 patients with ALL and 87 patients with AML, there was no statistically significant difference in 3-year OS and RFS among the three groups (Please refer to Figure 2 in the Supplemental File for details).

3-Year CIR and TRM

Up to the follow-up endpoint, the 3-year CIR in the 152 pediatric patients was 22.7% ± 4.2%, with a 3-year cumulative hematological relapse rate of 11.2% ± 3.7% and a 3-year TRM of 10.4% ± 2.9%. The haplo-HSCT + UCB group had a 3-year CIR of 21.3% ± 6.2%, the haplo-HSCT + MSC group had a 3-year CIR of 26.7% ± 8.1%, and the haplo-HSCT group had a 3-year CIR of 20.8% ± 6.8%, with no significant difference among the three groups ($\chi^2 = 1.402$, $p = 0.496$). The 3-year cumulative hematological relapse rates were 12.1% ± 5.6% in the haplo-HSCT + UCB group, 3.3% ± 3.3% in the haplo-HSCT + MSC group, and 13.2% ± 5.7% in the haplo-HSCT group, with no significant difference among the three groups ($\chi^2 = 1.326$, $p = 0.515$). The 3-year TRM was 11.3% ± 3.8%

in the haplo-HSCT + UCB group, 8.9% ± 6.3% in the haplo-HSCT + MSC group, and 11% ± 6.6% in the haplo-HSCT group, with no significant difference among the three groups ($\chi^2 = 0.658$, $p = 0.72$).

In 61 patients with ALL and 87 patients with AML, there was no statistically significant difference in 3-year CIR, cumulative hematological relapse rates, and TRM among the three groups (Please refer to Figure 3 in the Supplemental File for details).

Furthermore, a subgroup analysis was conducted on 20 patients who achieved CR in BM morphology but remained positive for MRD prior to transplantation. The patients were divided into two groups based on whether they received UCB infusion: the haplo-HSCT + UCB group ($n = 14$) and nonhaplo-HSCT + UCB group ($n = 6$). The 2.5-year CIR was 14.3% ± 9.4% in the haplo-HSCT + UCB group and 50.0% ± 20.4% in the nonhaplo-HSCT + UCB group. Although this difference was not significant ($\chi^2 = 2.319$, $p = 0.128$), the haplo-HSCT + UCB group demonstrated a lower 2.5-year CIR compared to the nonhaplo-HSCT + UCB group (Please refer to Figure 4 in the Supplemental File for details).

Adverse reactions to UCB/umbilical cord MSC infusion

The incidence of adverse reactions within 4h of UCB infusion in the haplo-HSCT + UCB group was 97.3% (74/76). Specifically, the incidence of hypertension was 94.7% (72/76), increased heart rate was 7.8% (6/76), decreased heart rate was 31.5% (24/76), allergic reactions were 2.6% (2/76) (manifested as rash, dry cough), gastrointestinal reactions were 7.8% (6/76) (manifested as nausea, abdominal pain), headache was 1.3% (1/76), and gross hematuria was 25.0% (19/76). All 74 patients with elevated blood pressure returned to baseline levels without specific treatment or symptomatic drug treatment. Allergic reactions, gastrointestinal reactions, and adverse reactions in the nervous and urinary systems were graded as 1–2 according to CTCAE (5.0).

Based on administration of UCB infusion, the 152 patients were divided into two groups: haplo-HSCT + UCB (76 cases) and nonhaplo-HSCT + UCB (76 cases). In the haplo-HSCT + UCB group, 48.7% (37/76) of patients required long-term oral antihypertensive medication after transplantation, with 34.2% (26/76) requiring treatment with one antihypertensive drug and 14.5% (11/76) requiring concurrent treatment with two or more antihypertensive drugs. In the nonhaplo-HSCT + UCB group, 51.3% (39/76) of patients required long-term oral antihypertensive medication after transplantation, with 39.5% (30/76) requiring treatment with one antihypertensive drug and 11.8% (9/76) requiring concurrent treatment with two or more antihypertensive drugs. There was no significant difference in the incidence of long-term oral antihypertensive medication between the two groups ($\chi^2 = 0.105$, $p = 0.746$), and no significant difference in the number of antihypertensive drugs required ($\chi^2 = 0.538$, $p = 0.764$).

In the haplo-HSCT + MSC group, no infusion-related adverse reactions were observed after umbilical cord MSC infusion.

Discussion

Allo-HSCT remains a highly effective approach for children with high-risk refractory leukemia. To improve the applicability of haplo-HSCT, some studies have combined it with UCB infusion for the treatment of adult hematologic malignancies, with the advantages of both methods achieving rapid hematopoietic recovery and a low incidence of GVHD while obtaining similar rates of GVHD, OS, RFS, and TRM as fully HLA-matched transplantation.[11–13] Studies have demonstrated the satisfactory efficacy of haplo-HSCT combined with MSC infusion in BM failure syndromes, accelerating hematopoietic reconstitution and improving GVHD-free and failure-free survival rates.[14,15] In this study, we examined the effectiveness and safety of haplo-HSCT combined with UCB/umbilical cord MSC therapy in 152 children with AL who underwent haplo-HSCT.

The reported incidence of ES varies widely, ranging from 5% to 79%, which may be attributed to different underlying diseases, transplant types, medication usage, and varying diagnostic criteria used by different institutions.[16–19] In this study, we used the clinical criteria proposed by Maiolino et al.[5] The results showed that the incidence of ES within 30 days after haplo-HSCT in children with AL was 67.1% (102/152), which is consistent with the findings of Yanagisawa et al. (70.4%, 19/27).[20] Furthermore, multivariate analysis

indicated that the transplantation method for haplo-HSCT + MSC was an independent factor associated with a lower incidence of ES, which has not been reported in similar domestic or international studies. The infusion of MSC-derived exosomes into a mouse model of allogeneic GVHD increased the number of Tregs, reduced GVHD severity, and improved survival.[21] The regulatory effects of MSCs on GVHD are primarily mediated by Tregs. The mechanisms underlying the role of MSCs in the autoimmune process include B cells and T cell inhibition, the reduction of Th1/Th2, Th17/Treg, and M1/M2 ratios, downregulation of pro-inflammatory cytokines such as IL-1, IL-6, IL-17, TNF-α, IFN-γ, and upregulation of anti-inflammatory cytokines such as IL-4, IL-10, and TGF-β.[22] This suggests that MSCs can alleviate systemic inflammation via multiple pathways, thereby preventing and controlling ES.

Previous studies have shown that haplo-HSCT combined with MSCs can achieve lower incidence and severity of aGVHD.[3,23,24] Haplo-HSCT combined with UCB has also demonstrated similar efficacy,[2,25] possibly because of the presence of cells such as CD4+CD25+ Tregs and MSCs in UCB, which play important roles in preventing and treating GVHD.[26] In this study, we observed a reduced incidence of severe gut aGVHD in both ALL and AML patients who underwent haplo-HSCT combined with UCB or MSCs, which is consistent with previous research. However, in a cohort of 152 patients with AL, there was no significant difference in the incidence and severity of aGVHD within 30 days after HSCT among the haplo-HSCT + UCB, haplo-HSCT + MSC, and haplo-HSCT groups, regardless of the affected organs, including the skin, gut, and liver. Studies found no significant difference in the incidence of aGVHD between the haplo-HSCT combined with UCB/MSC group and the noncombined group in patients with hematological disorders, including leukemia and AA,[1,15,27] which is consistent with our findings. But then again, the limited sample size in our study may have contributed to biased results. To address this, it is imperative to increase the sample size in future studies, conduct multicenter clinical trials for confirmation of these findings, and undertake fundamental medical research to elucidate the immunomodulatory mechanism of UCB/MSC.

Zhou et al.[1] showed that haplo-HSCT combined with UCB transplantation was an independent prognostic factor for reducing the 3-year CIR and improving the 3-year OS and RFS in adults with B-cell ALL, particularly in patients with adverse molecular and cytogenetic markers and persistent MRD before transplantation. However, our study found no significant differences in 3-year OS, DFS, CIR, cumulative incidence of hematological relapse, or TRM among the haplo-HSCT + UCB, haplo-HSCT + MSC, and haplo-HSCT groups. This may be attributed to the lower proportion of patients with MRD persistence before transplantation (20.4%, 31/152) compared to that in the aforementioned study (65.9%, 116/176), indicating a lower tumor burden and better disease remission status before transplantation.

Furthermore, our study analyzed the prognosis of 20 patients who achieved CR based on BM morphology but had persistent MRD. The haplo-HSCT + UCB group showed a lower 2.5-year CIR compared to the nonhaplo-HSCT + UCB group, with rates of $14.3\% \pm 9.4\%$ and $50.0\% \pm 20.4\%$, respectively, although the difference was not significant ($p = 0.128$). This suggests that haplo-HSCT combined with UCB transplantation in pediatric AL may induce a stronger GVL effect without increasing the incidence of aGVHD within 30 days of transplantation. This study found that the haplo-HSCT + MSC group had a slightly lower 3-year RFS compared to the other two groups. This is consistent with the findings of Ning et al.,[28] who proposed that MSC combined with HLA-matched sibling HSCT can prevent GVHD, but significantly increase the relapse rate of malignant hematological diseases, resulting in an earlier median time to relapse and decreased 3-year RFS after HSCT. Similarly, in murine experiments, Suzuki et al.[29] demonstrated that mice receiving simultaneous subcutaneous injections of B16-LacZ cells (a murine melanoma cell line) and MSCs exhibited significantly increased tumor volumes compared to mice receiving only B16-LacZ cells. A similar conclusion was reached when B16-LacZ cells were replaced with LLC cells (a lung cancer cell line).

In addition to relapse, GVHD and infection are major determinants of prognosis after haplo-HSCT. Clinically, high doses of immunosuppressive agents are often used to control

aGVHD, which further compromises immune function and increases the incidence and severity of infections, thereby affecting long-term patient survival. Forslöw et al.[30] identified the use of MSCs as an independent risk factor for pneumonia-related mortality after HSCT, whereas Qu et al.[31] demonstrated that MSCs improved the prognosis of pediatric patients by increasing the cure rate of HSCT-related pneumonia. In our study, the 10 patients who died from infection-related causes had varying degrees of pulmonary infection. There was no statistically significant difference in infection-related mortality rates among the three groups in our study. This suggests that MSCs and UCB do not significantly affect infection-related mortality after transplantation.

In this study, the incidence of CLS within 30 days of HSCT was 17.8% (27/152), which was slightly lower than that reported by Nürnberger et al.[32] (20.4%, 11/54). The latter study identified unrelated donors as risk factors for CLS after HSCT, whereas all donors in our study were related, which may have contributed to the lower incidence of CLS. Takahashi et al.[33] demonstrated that cord blood monocytes/macrophages exhibit higher sensitivity to pro-inflammatory stimuli than adult PB monocytes/macrophages. Preconditioning-induced tissue damage activates the monocyte-macrophage system in the donor, leading to the production of various pro-inflammatory factors and the development of a recipient inflammatory cytokine storm, which results in endothelial dysfunction. Furthermore, in our study, VOD and TMA occurred exclusively in the haplo-HSCT+UCB group, and 55.5% (15/27) of the CLS cases also occurred in this group, suggesting a potential predisposition for UCB to cause endothelial injury in the early posttransplant period.

In our study, the incidence of adverse reactions within 4 h of UCB infusion in pediatric patients was as high as 97.3% (74/76), with elevated blood pressure being the most common adverse event. Some patients develop secondary hypertension and require long-term oral antihypertensive drug treatment. There was no significant difference in the number of oral antihypertensive drugs administered between the haplo-HSCT + UCB group and nonhaplo-HSCT + UCB group ($p = 0.764$), indicating that blood pressure elevation after UCB infusion was often transient. However, a small number of patients may experience a rapid increase in blood pressure after infusion, even at the risk of hypertensive crisis. Therefore, blood pressure should be dynamically monitored during and on the days following infusion, and prompt antihypertensive treatment should be administered. Other adverse reactions to UCB infusion can be self-resolving or relieved with symptomatic treatment. No long-term sequelae were observed, indicating an overall high safety profile of UCB infusion.

According to the literature, administration of MSCs may lead to adverse reactions and severe complications, including fever, injection site pain, infections (viral or mycoplasma), thromboembolism, tissue fibrosis, and malignant tumor formation.[34] However, in our study, no adverse reactions or MSC-related new-onset tumors were observed in the 31 pediatric patients who underwent umbilical cord MSCs transplantation until the end of the follow-up period, indicating a high level of safety of MSC infusion, which is consistent with previous research findings.[35,36] Of note, the sample size in this study was small, and the follow-up period was relatively short, which may have masked the rare side effects and long-term complications of MSCs.

Limitation

It should be noted that this is a single-center retrospective study with a small sample size and a relatively short follow-up period, which limits its generalizability. Therefore, further in vitro and in vivo experiments, as well as large-scale multicenter randomized controlled trials, are needed to validate these findings.

Conclusion

In summary, we confirmed the effectiveness and safety of haplo-HSCT combined with UCB and umbilical cord MSCs therapy for pediatric AL. UCB and MSCs have demonstrated advantages in preventing ES and severe gut aGVHD. This approach is an efficient and safe alternative in the absence of a fully matched donor.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the Children's Hospital of Soochow University (Ethics No. 2023CS154). The requirement to obtain informed consent was waived due to the retrospective nature of the study and was deemed exempt from review by the Ethics Committee of the Children's Hospital of Soochow University.

Consent for publication

Not applicable.

Author contributions

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Competing interests

The authors declare that there is no conflict of interest.

Availability of data and materials

Data are available from the corresponding author upon reasonable request.

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Supplemental material

Supplemental material for this article is available online.

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