Biol Blood Marrow Transplant 25 (2019) 1116–1121

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

Allogeneic – Adult

Fludarabine/Melphalan 100 mg/m^2 Conditioning Therapy Followed by Allogeneic Hematopoietic Cell Transplantation for Adult Patients with Secondary Hemophagocytic Lymphohistiocytosis

Han-Seung Park, Jung-Hee Lee*, Je-Hwan Lee, Eun-Ji Choi, Sun-Hye Ko, Miee Seol, Young-Shin Lee, Young-Ah Kang, Mijin Jeon, Kyoo-Hyung Lee

Department of Hematology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

Article history:
Received 3 September 2018
Accepted 27 November 2018

Key Words:
Hemophagocytic lymphohistiocytosis
Allogeneic hematopoietic cell transplantation
Reduced-intensity conditioning regimen
Epstein-Barr virus

A B S T R A C T

Our previous research indicated that a reduced-intensity conditioning regimen (fludarabine and melphalan at 100 mg/m^2) was useful in allogeneic hematopoietic cell transplantation (HCT) for patients with lymphoma. This retrospective study evaluated the reduced-intensity conditioning regimen in allogeneic HCT for adult patients with hemophagocytic lymphohistiocytosis (HLH). Sixteen patients with HLH were evaluated, including 6 patients who were enrolled in a prospective clinical trial (NCT00772811) and 10 patients who received the same conditioning regimen (fludarabine at 30 mg/m^2/day on days −6 to −2 and melphalan at 100 mg/m^2 on day −2). The median age was 42 years (range, 18 to 64), and 12 patients had Epstein-Barr virus (EBV)-associated HLH. Donors were an HLA matched sibling for 10 patients, an unrelated matched volunteer for 4 patients, and a mismatched family member for 2 patients. After excluding 3 patients who died soon after HCT, 12 patients achieved an engraftment (neutrophil median, day 12; platelet median, day 16). Five patients experienced acute graft-versus-host disease (GVHD), including 1 case of grade II and 4 cases of grades III to IV. Chronic GVHD occurred in 3 patients (moderate, 1 case; severe, 2 cases). After a median follow-up of 33.8 months 1 patient progressed, 3 patients relapsed, and 9 patients died. Five deaths were unrelated to relapse or progression and were caused by infection (n = 3), bleeding (n = 1), and GVHD (n = 1). No deaths or relapses were observed at >124 days post-transplant. The overall survival rate was 48.6%, and significant differences were observed according to pretransplant ferritin level (P = .007) and cytopenia lineage (P = .021). Before allogeneic HCT 10 of 12 patients still tested positive for EBV DNA: 6 patients tested negative for EBV DNA after HCT, 2 patients had persistent EBV DNA, and 2 patients were unassessable because of early death. Conditioning therapy using a lower dose of melphalan combined with fludarabine appears to be promising in allogeneic HCT for adults with HLH. However, strategies are needed to reduce the risk of early death.

INTRODUCTION

Adult hemophagocytic lymphohistiocytosis (HLH) is a rare but fatal disease [1,2]. Furthermore, the diagnosis of HLH remains challenging, despite recent advances in diagnostic methods, including molecular testing [3,4]. The occurrence of HLH is associated with dysfunction of natural killer cells and cytotoxic T lymphocytes, which is caused in primary HLH by genetic mutations affecting the cytotoxic granule activity pathway or is caused in secondary HLH by malignancies, infections, autoimmune diseases, and medications [5,6].

The treatment of HLH consists of suppressing the uncontrolled immune response and cytokine-stimulated cells through immunochemotherapy (HLH-directed treatment), regulating the disease trigger, and controlling any opportunistic infection [2,7]. However, immunochemotherapy alone is insufficient in many cases, and allogeneic hematopoietic cell transplantation (HCT) is needed to eliminate and replace the defective immune system [1]. In addition, HLH can cause a severe uncontrolled immune reaction or cytokine storm [8]. Moreover, the global immunosuppression during HLH treatment makes it critical to control opportunistic infections [7,9]. Adult cases of HLH, which require allogeneic HCT, are often complicated and involve significant pre-HCT comorbidities.

Reduced-intensity conditioning (RIC) regimens with various combinations of fludarabine and melphalan have been used in allogeneic HCT for HLH, and previous studies have indicated that allogeneic HCT with a RIC regimen may improve the

Financial disclosure: See Acknowledgments on page 1120.

* Correspondence and reprint requests: Jung-Hee Lee, MD, PhD, Department of Hematology, Asan Medical Center, University of Ulsan College of Medicine, 88, Olymphic-ro 43-gil, Songpa-gu, Seoul, 05505 Republic of Korea.
E-mail address: jhleecr@amc.seoul.kr (J.-H. Lee).

https://doi.org/10.1016/j.bbmt.2018.11.032
1083-8791/

survival of pediatric patients with HLH [10-15]. However, little is known regarding the feasibility and outcomes of allogeneic HCT for adults with HLH [16,17]. Our prior study used a less-intensive conditioning regimen involving fludarabine and melphalan at 100 mg/m^2 (Flu/Mel 100) and revealed promising results in allogeneic HCT for lymphoma [18]. Therefore, this retrospective study evaluated the Flu/Mel 100 conditioning regimen in allogeneic HCT for adult patients with HLH.

METHODS

Patients

Between October 2008 and July 2016, 16 adult patients with HLH underwent allogeneic HCT with the Flu/Mel 100 regimen and were included in this study. All patients were >18 years old and fulfilled ≥5 of the 8 HLH criteria at the diagnosis [6].

Epstein-Barr virus (EBV) DNA load was quantified using the commercially available Artus EBV QS-RGQ assay (Qiagen, Germantown, MD) according to the manufacturer's protocol. Mutations of the *UNC13D* and *PRF1* genes were evaluated for patients who were aged <30 years, based on a Korean nationwide survey that revealed *UNC13D* and *PRF1* gene mutations were common among Korean pediatric patients with HLH [19]. Patient comorbidity scores were obtained using the HCT-specific comorbidity index [20].

One patient had received dexamethasone and cyclosporine, whereas the other 15 patients were treated using the etoposide, dexamethasone, and cyclosporine-based HLH-2004 protocol [6]. All patients received allogeneic HCT because they still had active disease despite proper treatment or had experienced HLH reactivation during continuation therapy. The first 6 patients were enrolled in a prospective clinical trial that evaluated the Flu/Mel 100 regimen in allogeneic HCT for lymphoid malignancies (NCT00772811), and the other 10 patients underwent the same conditioning regimen but outside of the clinical trial. This retrospective study complied with the tenets of the Declaration of Helsinki and was approved by the Asan Medical Center Institutional Review Board.

Conditioning Regimen and Transplantation Procedure

Patients were typed for the HLA-A, -B, -C, and -DRB1 alleles. Donor searches were performed in the order of HLA-matched sibling donors, HLA-matched unrelated donors, and HLA-mismatched familial donors. The conditioning regimen consisted of fludarabine at 30 mg/m^2/day administered i.v. for 5 consecutive days (days −6 to −2) and melphalan at 100 mg/m^2 i.v. on day −2. Melphalan was administered after completion of the fludarabine infusion. In cases involving unrelated or mismatched familial donors, the patients received rabbit antithymocyte globulin (ATG; Thymoglobulin; Sang-Stat Medical Corp., Lyon, France) at 3 mg/kg/day i.v. on days −4, −3, and −2. On day 0 all patients received a peripheral blood progenitor cell graft without T cell depletion, which had been mobilized using granulocyte colony-stimulating factor.

Ciprofloxacin, acyclovir, and micafungin were administered for infection prophylaxis. Immunoglobulin (0.5 g/kg i.v.) was administered on days 7, 30, 60, and 90. Treatment using granulocyte colony-stimulating factor (450 μg/day) began on day 5 and continued until the absolute neutrophil count reached >3000/μL. Graft-versus-host disease (GVHD) prophylaxis was provided using cyclosporine and methotrexate. Cyclosporine (1.5 mg/kg i.v. every 12 hours) was started on day −1 and subsequently switched to an oral dose after oral intake became possible. Patients also received methotrexate at a dose of 15 mg/m^2 on day 1 and then at a dose of 10 mg/m^2 on days 3, 6, and 11. Methotrexate on day 11 was omitted in patients undergoing matched sibling donor HCT. For pneumocystis prophylaxis trimethoprim-sulfamethoxazole was started after the absolute neutrophil count reached >3000/μL and continued 6 months after transplantation.

Patient Monitoring

The date of bone marrow engraftment was defined as the first of 2 consecutive days with an absolute neutrophil count ≥ 500/μL. The date of platelet engraftment was defined as the first of 7 consecutive days with unsupported platelet counts ≥ 20,000/μL. Bone marrow examinations were performed at 4 to 6 weeks after HCT. Acute GVHD [21] and chronic GVHD [22] were assessed as described. At approximately 1 month after HCT, hematopoietic chimerism was assayed using a PCR-based method and the assay repeated every 3 months for 1 year. Presence of EBV and cytomegalovirus DNA in the blood was evaluated using quantitative PCR every 2 weeks until day 100.

Statistical Analysis

Overall survival (OS) was calculated from the date of HCT to the date of death from any cause. Intergroup comparisons of OS were performed using the Kaplan-Meier method and the log-rank test. Statistics were performed using IBM SPSS software (version 21.0; IBM Corp., Armonk, NY). Treatment-related mortality (TRM) and relapse or progression were considered competing risks and were compared using the Gray method with R version 3.3.2 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Patient Characteristics

The median age was 42 years (range, 18 to 64), and 12 patients had EBV-associated HLH. Allogeneic HCT was performed because of HLH reactivation in 9 patients and failure to achieve a complete response in 7 patients (partial response, 6 patients; refractory disease, 1 patient). Donors were an HLA-matched sibling for 10 patients, an unrelated volunteer for 4 patients, and a mismatched family member for 2 patients. The median time from diagnosis to allogeneic HCT was 2.7 months (range, 1.5 to 7.2) (Table 1).

Engraftment and Post-Transplant Adverse Events

Excluding 3 patients who died early after HCT, 13 patients achieved an absolute neutrophil count of ≥500/μL at a median interval of 12 days after HCT (range, 10 to 18). Twelve patients achieved transfusion-independent platelet counts ≥ 20,000/μL at a median interval of 16 days after HCT (range, 10 to 230). One patient (patient 5) did not achieve transfusion-independent platelet recovery until he died (with 98% donor chimerism at 264). After follow-up 3 fully engrafted patients experienced relapse/progression. The overall rate of graft failure was 30.8%.

Except 4 patients (including 3 patients who died early after HCT), hematopoietic chimerism studies were performed in 12 patients. Ten of 12 patients showed complete donor chimerism at 1 month after HCT. Two patients demonstrated 99% donor chimerism at 1 month after HCT. These patients converted to complete donor chimerism at 3 months after HCT (without any intervention), but patient 5 showed 98% donor chimerism at last follow-up. Another 2 patients showed complete donor chimerism at 1 month after HCT but had a re-emergence of mixed chimerism at relapse (patients 11 [91%] and 13 [43%]). All other patients (75.0%) showed sustained complete chimerism at the time of last follow-up.

Table 1
Patient Characteristics at the Time of HCT

Characteristic	Value
Median age at HCT, yr (range)	42 (18-64)
Male sex	9 (56.3)
Cause of HLH	
EBV	12 (75.0)
Idiopathic	4 (25.0)
Clinical status at HCT	
Reactivation of disease	9 (56.2)
Failure to achieve CR	7 (43.8)
Partial response	6
Refractory disease	1
Comorbidity index*	
0	2 (12.5)
1–2	3 (18.8)
≥3	11 (68.7)
Donor type	
MSD	9 (56.2)
URD	5 (31.3)
HFD	2 (12.5)
Median time from diagnosis to HCT, mo (range)	2.7 (1.5-7.2)

Values are n (%) unless otherwise defined. CR indicates complete response; MSD, matched sibling donor; URD, unrelated donor; HFD, haploidentical family donor.

* Patient comorbidity scores were obtained using the HCT-specific comorbidity index [20].

Infectious complications occurred in 6 of 16 patients (37.5%). Patient 1 had grade 4 neutropenia/thrombocytopenia and hepatic fungal infection at the time of conditioning therapy and subsequently died of sepsis (*Stenotrophomonas maltophilia*) on post-transplant day 21 without recovery from the pancytopenia. Patient 8 experienced central nervous system involvement of HLH and perianal abscess (*Escherichia coli* and *Klebsiella pneumoniae*) during the HLH continuation therapy and subsequently died of rapidly progressed fungal pneumonia on post-transplant day 8. Patient 16 had fungal pneumonia (*Fusarium* spp.) and eventually died of pneumonia despite a slow recovery from the pancytopenia after HCT. Nine patients had cytomegalovirus infection and 1 patient progressed to cytomegalovirus gastritis (patient 12).

Gastrointestinal adverse events related to the conditioning regimen were frequent, including 4 episodes of grade 3/4 toxicity (oral mucositis, 3 cases; vomiting, 1 case). One patient experienced grade 2 hematuria. Five of 16 patients developed acute GVHD at a median interval of 20 days after HCT (range, 12 to 32), including grade II in 1 case and grades III to IV in 4 cases. Patient 15 had reactivated disease at the time of HCT and subsequently died of grade IV acute gastrointestinal GVHD. Chronic GVHD occurred in 3 patients at a median interval of 88 days after HCT (range, 70 to 102) (Table 2).

Survival after Transplantation

Among surviving patients and after a median follow-up of 6.5 months after allogeneic HCT (range, .2 to 97.8), 9 patients (56.3%) subsequently died. Four deaths were related to relapse (n = 3) or progression (n = 1) after HCT, and 5 deaths were not related to relapse or progression (infection, 3 cases; bronchopulmonary hemorrhage, 1 case; GVHD, 1 cases). Patient 5 died of diffuse alveolar hemorrhage because of incomplete platelet recovery on post-transplant day 264. Five of 9 deaths occurred within 100 days after HCT, and no deaths or relapses were observed after post-transplant day 264. In total, the 1-year cumulative incidence of TRM was 31.2% (95% confidence interval, 10.7% to 54.6%).

Eight patients (50.0%) received ATG during conditioning. Four patients in the ATG group (infection, 1 case; relapse, 2 cases; progression, 1 case) and 5 patients in the non-ATG group (infection, 2 cases; bronchopulmonary hemorrhage, 1 case; relapse, 1 case; GVHD, 1 cases) died. The 5-year OS rate was not significantly different between groups (50.0% versus 37.5%, $P = .990$).

The 5-year OS rate was 48.6% (Figure 1A), with significantly better OS associated with a lower pretransplant ferritin level (<20,000 ng/mL versus ≥20,000 ng/mL, 58.3% versus 0%, $P = .007$; Figure 1B) and unilineage cytopenia based on the HLH

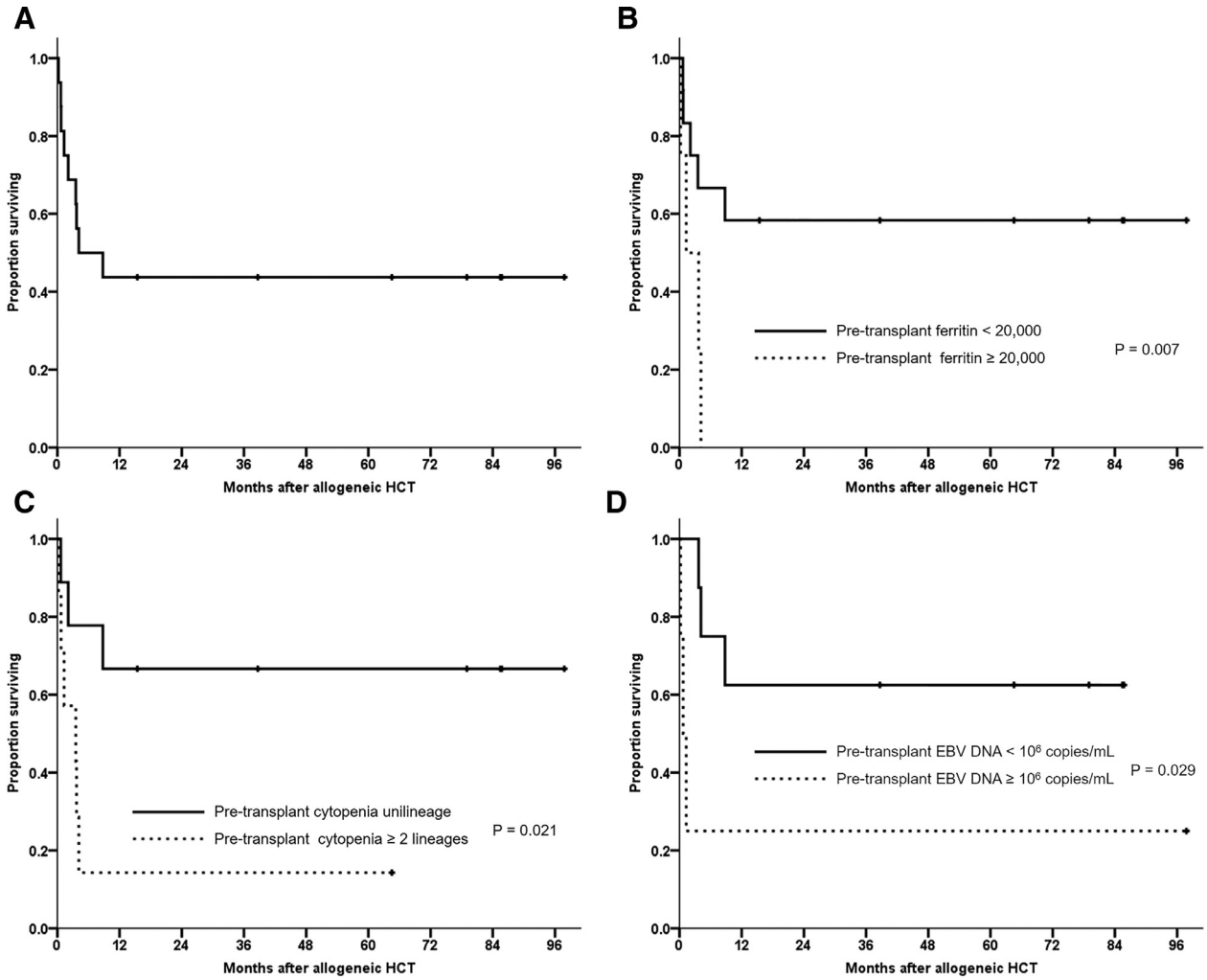

Figure 1. Overall survival. (A) All 16 patients. (B) Survival according to pretransplant ferritin level (<20,000 ng/mL [n = 12] versus ≥20,000 ng/mL [n = 4]). (C) Survival according to pretransplant cytopenia lineage number (unilineage [n = 9] versus ≥2 lineages [n = 7]). (D) Survival according to pretransplant EBV DNA load (<10^6 copies/mL [n = 8] versus ≥10^6 copies/mL [n = 4]).

diagnostic guidelines [6] (unilineage versus ≥2 lineages, 66.7% versus 14.3%, $P = .021$; Figure 1C).

Plasma EBV-DNA Status and Post-Transplant Outcomes

Two of 12 patients with EBV-associated HLH had no detectable EBV DNA at HCT, and both patients remained alive without relapse. Among 5 patients with no detectable EBV DNA after HCT, 4 patients remained alive without relapse, although 1 patient died of hemorrhage. Among 5 patients with detectable EBV DNA at HCT, 2 patients died of sepsis and disease progression during the early post-transplant period, whereas the other 3 patients died later because of disease relapse, GVHD, and pneumonia. The 5-year OS was significantly better for patients with an EBV DNA load $< 10^6$ copies/mL (62.5% versus 25%, $P = .029$; Figure 1D).

DISCUSSION

HLH involves severe and uncontrolled hyperinflammation [2], which can be caused by a genetic defect or various nongenetic factors [2,6]. The introduction of immunochemotherapy has resulted in approximately 80% of familial HLH patients responding to the initial treatment, and HCT after immunochemotherapy is a curative option for primary HLH [1]. However, there are no established therapeutic strategies for adult patients with HLH, especially in relapsed or refractory cases, because of their variable clinical courses and disease triggers. Furthermore, the outcomes of relapsed or refractory adult HLH are generally poor. Allogeneic HCT has a poorly defined role in this setting [23], although it appears to be effective for relapsed or refractory adult HLH [9,24]. Li et al. [25] have reported that 19 of 30 patients survived after transplantation for refractory EBV-associated HLH, whereas all patients who did not undergo stem cell transplantation died.

Myeloablative conditioning–based allogeneic HCT for pediatric HLH is associated with a TRM rate of approximately 30% to 35% [26,27]. To reduce the post-transplant TRM rate, previous studies have introduced RIC-based transplantation for pediatric HLH, which was associated with a reduced TRM rate and improved survival [10,13]. The present study evaluated adult patients with HLH who received RIC (a reduced dose of melphalan plus fludarabine) and revealed a 5-year OS rate of 48.6%, which is slightly lower than the OS rates in the pediatric population (54.5% to 89%), as well as a higher incidence of grades II to IV acute GVHD (31.3%) relative to the pediatric population [10,11,15]. Machowicz at al. [17] reported similar outcomes in patients with adult HLH, resulting in a 3-year OS of 41%, and engraftment of 77% (RIC, 37%). Nevertheless, previous studies that used RIC-based transplantation for pediatric HLH revealed a relatively high incidence of mixed chimerism (31% to 79%) [10,11,13], whereas the present study revealed sustained complete donor chimerism in 75.0% of patients after HCT.

The previous study of pediatric primary HLH has reported that a sustained remission was achieved in patients with a donor chimerism of more than 20% of leukocytes [28]. Therefore, although a result of RIC-based transplantation for pediatric HLH trials showed high incidence of mixed chimerism, OS rate was improved to myeloablative conditioning–based transplantation. In our study 2 patients relapsed 108 and 64 days after transplantation (donor chimerism at relapse, 91% and 43%, respectively). Cause of HLH of these patients was idiopathic. It may suggest that the mechanism of relapse of adult HLH is different from pediatric primary HLH.

A recent phase II trial reported an outcome of RIC-based allogeneic HCT (fludarabine, melphalan, and alemtuzumab) for a largely pediatric cohort. The 1-year OS rate was 80.4%, with a

Table 2
Post-Transplant Outcomes

Patient no.	Age (yr)	Sex	Cause of HLH	Disease Status at the Time of HCT	Comorbid diseases	Comorbidity Index*	EBV Status (copies/mL)			Time to HCT (month)	Donor Type	Donor GVHD	Chronic GVHD	Donor Chimerism (%)	Outcome
							At Diagnosis	At HCT	Post-Transplant						
1	60	M	EBV	Reactivation	Hepatic fungal infection	4	12,000	1,000,000	–	3.1	MSD	–	–	–	Died at day 21 (sepsis)
2	27	F	EBV	Reactivation	EBV hepatitis	7	5,325,000	1,697,500	Negative	1.5	MSD	None	None	100	Alive
3	42	M	EBV	PR		4	26,250	Negative	Negative	2.5	URD	Grade II	Severe	100	Alive
4	46	F	EBV	PR		1	4,050,000	Negative	Negative	2.3	MSD	Grade III	Severe	100	Alive
5	37	M	EBV	Reactivation	Pulmonary nocardiosis	7	2,417,500	592,500	Negative	7.2	MSD	None	None	99	Died at day 264 (hemorrhage)
6	22	F	EBV	PR	Nontraumatic brain hematoma	5	1,360,000	245,500	Negative	1.8	MSD	None	None	100	Alive
7	22	M	EBV	Reactivation	Fungal pneumonia	3	103,000	85,750	Negative	4.3	URD	None	None	100	Alive
8	33	F	Idiopathic	Reactivation	CNS HLH, perianal abscess	5				4.1	MSD	–	–	–	Died at day 8 (pneumonia)
9	43	F	EBV	Refractory		10	19,054,600	4,530	–	3.6	URD	–	–	–	Died at day 7 (refractory)
10	47	F	EBV	Reactivation	Cytomegalovirus ileitis	5	741,310	873	Negative	2.4	MSD	None	None	100	Alive
11	44	F	Idiopathic	PR	CNS HLH	1				2.9	MSD	None	None	100	Died at day 108 (relapse)
12	29	M	EBV	Reactivation		0	3,311,300	4,304	3,967,000	2.1	HFD	Grade III	–	–	Died at day 39 (relapse)
13	64	M	Idiopathic	Reactivation		1				2.9	HFD	None	None	100	Died at day 64 (relapse)
14	18	M	Idiopathic	PR		0				3.5	URD	Grade III	Moderate	99	Alive
15	41	M	EBV	Reactivation		3	9,550	6,920	24,000	2.3	MSD	Grade IV	–	100	Died at day 111 (GVHD)
16	44	M	EBV	PR	Fungal pneumonia	4	6,025	12,590	1,170	2.3	MSD	None	None	100	Died at day 124 (Pneumonia)

PR indicates partial response.
* Patient comorbidity scores were obtained using the HCT-specific comorbidity index [20].

Table 3
Comparison of Fludarabine and Melphalan–Based Conditioning Regimens

Day	Cooper et al. [10]	Park et al. (this study)	Allen et al.* [29]
−14			Alemtuzumab (test dose)
−13			Alemtuzumab (max 21.75 mg)
−12			Alemtuzumab (max 21.75 mg)
−11			Alemtuzumab (max 21.75 mg)
−10			Alemtuzumab (max 21.75 mg)
−9			Rest
−8	± Alemtuzumab .2 mg/kg†		Fludarabine 30 mg/m^2
−7	Fludarabine 30 mg/m^2 ± alemtuzumab .2 mg/kg†		Fludarabine 30 mg/m^2
−6	Fludarabine 30 mg/m^2 ± alemtuzumab .2 mg/kg†	Fludarabine 30 mg/m^2	Fludarabine 30 mg/m^2
−5	Fludarabine 30 mg/m^2 ± alemtuzumab .2 mg/kg† ± ATG 3 mg/kg‡	Fludarabine 30 mg/m^2	Fludarabine 30 mg/m^2
−4	Fludarabine 30 mg/m^2 ± alemtuzumab .2 mg/kg† ± ATG 3 mg/kg‡	Fludarabine 30 mg/m^2 ± ATG 3 mg/kg§	Fludarabine 30 mg/m^2
−3	Fludarabine 30 mg/m^2 ± ATG 3 mg/kg‡	Fludarabine 30 mg/m^2 ± ATG 3 mg/kg§	Melphalan 140 mg/m^2
−2	Melphalan 140 mg/m^2 ± ATG 3 mg/kg‡	Fludarabine 30 mg/m^2 + melphalan 140 mg/m^2 ± ATG 3 mg/kg§	Rest
−1	Melphalan 125 mg/m^2*,† ± ATG 3 mg/kg‡	Rest	Rest
0		Cell infusion	Cell infusion

* For adults and children > 15 kg.
† For matched sibling /unrelated donor transplantation.
‡ For mismatched familial donor transplantation.
§ For unrelated or mismatched familial donor transplantation.

relative high rate of suboptimal engraftment [29]. In our study all patients were conditioned with fludarabine and melphalan, and those undergoing unrelated or mismatched familial HCT received ATG. ATG was used in allogeneic HCT to prevent GVHD, also used in treatment of primary HLH as a part of immunotherapy [30]. We performed subgroup analysis according to use of ATG, but the 5-year OS rate was not significantly different between groups. Our study was retrospective and analyzed a small number of patients, and we did not observe an effect of ATG on the survival and engraftment (Table 3).

The optimal dose of melphalan for RIC remains controversial, although a melphalan dose of 140 mg/m^2 has been most commonly used. We further reduced the melphalan dose to 100 mg/m^2 because adult patients with relapsed or refractory HLH frequently have complications before the HCT. In the present study 7 patients had infectious complications at HCT and another patient had a nontraumatic brain hematoma. Nine patients died after HCT, and 5 of those deaths were not related to HLH. In addition, 5 patients died at <100 days after HCT, although there were no deaths or relapses at >264 days after HCT. It is possible that infectious complications during the early post-transplant period are related to the immunosuppressive nature of HLH-directed treatment before HCT rather than to toxicity of the conditioning therapy [31]. Uncontrolled disease at the time of conditioning is also considered a risk factor for poor post-transplantation outcomes [27]. Moreover, a multicenter study of 63 adults with refractory HLH revealed that complete response to salvage treatment was associated with better survival outcomes after HCT [32]. Thus, effective disease control before allogeneic HCT, using appropriate salvage treatment for relapsed or refractory HLH, seems to be essential to improve post-transplant outcomes.

The present study revealed that OS was significantly predicted by the ferritin level and the number of cytopenia lineages at the time of allogeneic HCT. These factors might reflect the activity of HLH, because ferritin is a well-recognized marker of disease activity and prognosis in cases of HLH [8,33]. In the pediatric population a highly elevated ferritin level (≥10,000 mg/L) is considered a suitably specific factor for diagnosing HLH [34]. Nevertheless, ferritin levels in patients with HLH can vary according to other factors, such as sepsis, renal failure, and hepatocellular injury [35]. Thus, pretransplant ferritin levels seem to be associated with disease activity and other comorbidities at the time of HCT.

EBV is a major cause of infection-related HLH [7,36], and the present study revealed that 12 of 16 patients had EBV-associated HLH. However, 2 of 12 patients had undetectable EBV DNA at the time of HCT, and 5 other patients developed undetectable EBV DNA levels after HCT. Six of 7 patients with controlled EBV infection remained alive without relapse, and only 1 patient died of bleeding complications that were caused by thrombocytopenia. In contrast, all 5 patients with persistently detectable EBV DNA levels died. These results suggest that eradication of EBV infection is crucial for post-transplant survival in cases of EBV-associated HLH. Furthermore, allogeneic HCT can control EBV infection by removing the infected immune cells and reconstituting a normal immune system, even in patients who had persistent EBV infection after chemoimmunotherapy.

The present study revealed that the Flu/Mel 100 conditioning regimen, which uses a lower dose of melphalan plus fludarabine, can ensure adequate engraftment and complete donor chimerism and appears to be a promising regimen in allogeneic HCT for adult HLH. However, strategies are needed to prevent early death and further improve these outcomes.

ACKNOWLEDGMENTS

Financial disclosure: The authors have nothing to disclose.

Conflict of interest statement: There are no conflicts of interest to report.

Authorship statement: J.-H.L. contributed to the study design. H.-S.P. contributed to the literature search, data collection, data interpretation, and writing of the manuscript. All authors contributed to the data collection, data analysis, and final approval of the manuscript.

REFERENCES

1. Henter JI, Samuelsson-Horne A, Arico M, et al. Treatment of hemophagocytic lymphohistiocytosis with HLH-94 immunochemotherapy and bone marrow transplantation. *Blood*. 2002;100:2367–2373.
2. Janka GE, Lehmberg K. Hemophagocytic syndromes—an update. *Blood Rev*. 2014;28:135–142.

3. Lehmberg K, Ehl S. Diagnostic evaluation of patients with suspected haemophagocytic lymphohistiocytosis. *Br J Haematol*. 2013;160:275–287.
4. Machowicz R, Janka G, Wiktor-Jedrzejczak W. Similar but not the same: differential diagnosis of HLH and sepsis. *Crit Rev Oncol Hematol*. 2017;114:1–12.
5. Emmenegger U, Reimers A, Frey U, et al. Reactive macrophage activation syndrome: a simple screening strategy and its potential in early treatment initiation. *Swiss Med Wkly*. 2002;132:230–236.
6. Henter JI, Horne A, Arico M, et al. HLH-2004: diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer*. 2007;48:124–131.
7. Imashuku S, Tabata Y, Teramura T, Hibi S. Treatment strategies for Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis (EBV-HLH). *Leuk Lymph*. 2000;39:37–49.
8. Janka GE. Familial and acquired hemophagocytic lymphohistiocytosis. *Annu Rev Med*. 2012;63:233–246.
9. Schram AM, Berliner N. How I treat hemophagocytic lymphohistiocytosis in the adult patient. *Blood*. 2015;125:2908–2914.
10. Cooper N, Rao K, Gilmour K, et al. Stem cell transplantation with reduced-intensity conditioning for hemophagocytic lymphohistiocytosis. *Blood*. 2006;107:1233–1236.
11. Marsh RA, Vaughn G, Kim MO, et al. Reduced-intensity conditioning significantly improves survival of patients with hemophagocytic lymphohistiocytosis undergoing allogeneic hematopoietic cell transplantation. *Blood*. 2010;116:5824–5831.
12. Nishi M, Nishimura R, Suzuki N, et al. Reduced-intensity conditioning in unrelated donor cord blood transplantation for familial hemophagocytic lymphohistiocytosis. *Am J Hematol*. 2012;87:637–639.
13. Marsh RA, Bleesing JJ, Chandrakasan S, Jordan MB, Davies SM, Filipovich AH. Reduced-intensity conditioning hematopoietic cell transplantation is an effective treatment for patients with SLAM-associated protein deficiency/X-linked lymphoproliferative disease type 1. *Biol Blood Marrow Transplant*. 2014;20:1641–1645.
14. Patel SA, Allewelt HA, Troy JD, et al. Durable chimerism and long-term survival after unrelated umbilical cord blood transplantation for pediatric hemophagocytic lymphohistiocytosis: a single-center experience. *Biol Blood Marrow Transplant*. 2017;23:1722–1728.
15. Marsh RA, Kim MO, Liu C, et al. An intermediate alemtuzumab schedule reduces the incidence of mixed chimerism following reduced-intensity conditioning hematopoietic cell transplantation for hemophagocytic lymphohistiocytosis. *Biol Blood Marrow Transplant*. 2013;19:1625–1631.
16. Kuriyama T, Kawano N, Yamashita K, Kikuchi I. Cord blood transplantation following reduced-intensity conditioning for epstein-barr virus-associated hemophagocytic lymphohistiocytosis during systemic lupus erythematosus treatment. *J Clin Exp Hematop*. 2016;56:126–129.
17. Machowicz R, Suarez F, Jedrzejczak WW, et al. Allogeneic hematopoietic stem cell transplantation in hemophagocytic lymphohistiocytosis (HLH) in adults: a retrospective study of the Chronic Malignancies and Inborn Errors Working Parties (CMWP and IEWP) of the EBMT [abstract]. *Blood*. 2016;128:3490.
18. Lee JH, Lee JH, Kim DY, et al. A phase II trial of fludarabine/melphalan 100 conditioning therapy followed by allogeneic hematopoietic cell transplantation for patients with lymphoma. *Clin Lymph Myeloma Leuk*. 2015;15:655–663.
19. Koh KN, Im HJ, Chung NG, et al. Clinical features, genetics, and outcome of pediatric patients with hemophagocytic lymphohistiocytosis in Korea: report of a nationwide survey from Korea Histiocytosis Working Party. *Eur J Haematol*. 2015;94:51–59.
20. Sorror ML, Maris MB, Storb R, et al. Hematopoietic cell transplantation (HCT)-specific comorbidity index: a new tool for risk assessment before allogeneic HCT. *Blood*. 2005;106:2912–2919.
21. Harris AC, Young R, Devine S, et al. International, multicenter standardization of acute graft-versus-host disease clinical data collection: a report from the Mount Sinai Acute GVHD International Consortium. *Biol Blood Marrow Transplant*. 2016;22:4–10.
22. Jagasia MH, Greinix HT, Arora M, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease. I. The 2014 Diagnosis and Staging Working Group report. *Biol Blood Marrow Transplant*. 2015;21:389–401.
23. Nikiforow S. The role of hematopoietic stem cell transplantation in treatment of hemophagocytic lymphohistiocytosis. *Hematol Oncol Clin North Am*. 2015;29:943–959.
24. Fu L, Wang J, Wei N, et al. Allogeneic hematopoietic stem-cell transplantation for adult and adolescent hemophagocytic lymphohistiocytosis: a single center analysis. *Int J Hematol*. 2016;104:628–635.
25. Li Z, Wang Y, Wang J, Zhang J, Wang Z. Haploidentical hematopoietic stem cell transplantation for adult patients with Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis. *Leuk Lymph*. 2018;59:77–84.
26. Baker KS, Filipovich AH, Gross TG, et al. Unrelated donor hematopoietic cell transplantation for hemophagocytic lymphohistiocytosis. *Bone Marrow Transplant*. 2008;42:175–180.
27. Horne A, Janka G, Maarten Egeler R, et al. Haematopoietic stem cell transplantation in haemophagocytic lymphohistiocytosis. *Br J Haematol*. 2005;129:622–630.
28. Ouachee-Chardin M, Elie C, de Saint Basile G, et al. Hematopoietic stem cell transplantation in hemophagocytic lymphohistiocytosis: a single-center report of 48 patients. *Pediatrics*. 2006;117:e743–e750.
29. Allen CE, Marsh R, Dawson P, et al. Reduced-intensity conditioning for hematopoietic cell transplant for HLH and primary immune deficiencies. *Blood*. 2018;132:1438–1451.
30. Stephan JL, Donadieu J, Ledeist F, Blanche S, Griscelli C, Fischer A. Treatment of familial hemophagocytic lymphohistiocytosis with antithymocyte globulins, steroids, and cyclosporin A. *Blood*. 1993;82:2319–2323.
31. Janka GE, Lehmberg K. Hemophagocytic lymphohistiocytosis: pathogenesis and treatment. *Hematol Am Soc Hematol Educ Progr*. 2013;2013:605–611.
32. Wang Y, Huang W, Hu L, et al. Multicenter study of combination DEP regimen as a salvage therapy for adult refractory hemophagocytic lymphohistiocytosis. *Blood*. 2015;126:2186–2192.
33. Lin TF, Ferlic-Stark LL, Allen CE, Kozinetz CA, McClain KL. Rate of decline of ferritin in patients with hemophagocytic lymphohistiocytosis as a prognostic variable for mortality. *Pediatr Blood Cancer*. 2011;56:154–155.
34. Allen CE, Yu X, Kozinetz CA, McClain KL. Highly elevated ferritin levels and the diagnosis of hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer*. 2008;50:1227–1235.
35. Schram AM, Campigotto F, Mullally A, et al. Marked hyperferritinemia does not predict for HLH in the adult population. *Blood*. 2015;125:1548–1552.
36. Maakaroun NR, Moanna A, Jacob JT, Albrecht H. Viral infections associated with haemophagocytic syndrome. *Rev Med Virol*. 2010;20:93–105.