Fludarabine-based reduced-intensity conditioning regimen for hematopoietic stem cell transplantation in primary hemophagocytic lymphohistiocytosis

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Abstract

Objective: Primary hemophagocytic lymphohistiocytosis (HLH) is a life-threatening condition that clinically characterized by fever, hepatosplenomegaly, and cytopenia. Hematopoietic stem cell transplantation (HSCT) is the only curative treatment option for patients diagnosed with primary HLH. Methods: In this prospective study, we analyzed the outcome of 10 pediatric patients with primary HLH who had received HSCT, using reduced-intensity conditioning (RIC) regimen from 2007 to 2012. The median age at transplantation was 22.6 months (range: 6–60). All of the patients received the same RIC regimen based on the use of fludarabine in combination with melphalan and horse antithymocyte globulin (ATG). Cyclosporine and methylprednisolone were used as graft-vs.-host disease (GvHD) prophylaxis. Results: Hematopoietic engraftment occurred in all patients. At the present time, 8 patients with a median follow-up of 39 months are still alive and all of them are disease free. Acute and chronic GvHD developed in 6 and 2 patients, respectively. Two patients died of sepsis and chronic GvHD during the study. Conclusion: Because of pretransplant infections caused by underlying immunodeficiency in patients with primary HLH, the use of less toxic regimen with RIC seems to be highly effective in this regard. Recipients of RIC transplant, with either full or mixed chimerism, had a long-term survival rate with no manifestation of primary HLH symptoms.

Key words primary hemophagocytic lymphohistiocytosis; hematopoietic stem cell transplantation; reduced-intensity conditioning

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Primary hemophagocytic lymphohistiocytosis (HLH) is a rare hereditary disorder characterized by fever, pancytopenia, hypofibrinogenemia, elevated ferritin, hypertriglyceridemia, and hemophagocytosis in bone marrow in childhood period (1–9). Primary HLH described in two subgroups: Familial hemophagocytic lymphohistiocytosis (FHLH) and separate primary immune deficiencies included Chediak–Higashi syndrome (CHS), Griscelli syndrome type II (GS-II), and X-linked lymphoproliferative (XLP) syndrome (4, 10, 11). In HLH syndrome, mutations in different genes bring continuous activation of macrophage and antigen-driven T cell that cause massive sustained release of the inflammatory mediators, leading to infiltration in the liver, spleen, lymph nodes, bone marrow, and central nervous system (1–6, 9, 10).

HLH syndrome is initial manifestation of FHLH and one of delayed presentation of GS-II, CHS, and XLP syndrome (2). Patients with primary HLH are not able to be cured with induction chemotherapy alone because the disease ultimately relapses in all patients and leads to death (1–3, 5, 9, 12).

Hematopoietic stem cell transplantation (HSCT) remains the only curative treatment of choice for patients with primary HLH (2, 4, 9, 12, 13). Similar to other primary immunodeficiency disorders, reduced-intensity conditioning (RIC) and myeloablative conditioning (MAC) regimens have been employed in treatment of HLH. Although using RIC
regimen often exhibits mixed chimerism, it decreases the risk of mortality among patients with comorbidities such as infections (6, 14, 15).

In this prospective study, we reported the results of HSCT in pediatric patients with primary HLH who underwent transplantation, using the same fludarabine-based RIC regimen between 2007 and 2012.

Patients and methods

Patient characteristics

Ten pediatric patients (8 boys, 2 girls) with primary HLH were enrolled in our study. Their diagnoses were confirmed by genetic testing, clinical and laboratory finding in Immunology, Asthma and Allergy Research Institute (IAARI). Genetic testing confirmed GS-II, and RAB27 was identified as the gene mutated in patients with GS-II. CHS was confirmed based on bone marrow biopsy, clinical examination, and family history. Genetic testing was also used to confirm FHLH in two patients. The history of child’s death in the past, parental consanguinity, and having characteristics of the disorder were documented to confirm the disease in other patient with FHLH. CSF evaluation was performed at the time of diagnosis and no evidence of CNS involvement was observed. Patients referred to the Hematology-Oncology Research Center and Stem Cell Transplantation (HORCSCT) between 2007 and 2011. All participants had experienced at least one HLH accelerated phase and were treated according to the HLH-2004 chemotherapy protocol (10). Due to congenital immunodeficiency, patients had a history of multiple hospitalizations that stemmed from different and recurrent infections. One of our patients developed disseminated BCGosis at the time of transplantation and was started on antimycobacterial therapy. In this study, one patient underwent transplantation in the second accelerated phase of HLH.

Transplant preparation

HLA-matched siblings are generally the first choices for donor selection. For those patients who did not have an HLA-matched sibling donor due to consanguineous marriage, we searched in extended family for finding a suitable donor. When a fully matched related donor is not available, unrelated donor should be investigated as a feasible alternative hematopoietic stem cell (HSC) source. HLA typing of all recipient-sibling donor pairs was performed by low-resolution molecular typing. In other related and unrelated donors, high-resolution HLA typing was conducted.

Transplant procedure

Signed informed consent forms were obtained from parents who agreed to participate. HSCs can be obtained from three different sources: bone marrow, peripheral blood, and umbilical cord blood. Peripheral blood stem cells (PBSCs) were obtained with continuous-flow leukapheresis after release of stem cells into the peripheral blood by granulocyte colony-stimulating factor (G-CSF). On the day of transplant, bone marrow stem cells (BMSCs) were extracted from marrow cavities of the iliac crests with heparinized syringes under general anesthesia. The defined protocol for HSCT conditioning regimen was approved by the institutional review board and ethics committee.

All of our patients received the same RIC regimen included fludarabine 30 mg/m² administrated intravenously (i.v.) for five consecutive days (days −8 to −4), melphalan 70 mg/m² i.v. for two consecutive days (days −3 and −2), and horse ATG (Atgam, Pfizer Inc, New York, NY, USA) 10 mg/kg for four consecutive days (days −4 to −1).

Cyclosporine A (CsA) (1.5 mg/kg daily i.v. on day −1; then 3 mg/kg from day +1 to day +7 in PBSC and day +11 in BMSC) and methylprednisolone [1 mg/kg i.v. on day −5 (day −5 to 7+), then 0.5 mg/kg/d by day 14+] were used as graft-vs.-host disease (GVHD) prophylaxis. Level of CsA was checked twice weekly (normal range: 100–250 ng/mL).

Supportive care

All patients received the same supportive care and were admitted to an isolated room equipped with high-efficiency particulate arresting (HEPA) filter. Antimicrobial prophylaxis that used against common germs was made up of acyclovir 750–1500 mg/m²/d administrated i.v. for 10 d (from−10 to −1) and then was changed to 1200–2400 mg/m²/d. It was given as prophylaxis for herpes simplex and varicella–zoster virus. Trimethoprim–sulfamethoxazole against the Pneumocystis jiroveci was prescribed from days −10 to −1, then restarted on day +36, and continued until 2 yr after HSCT. To prevent fungal infections, itraconazole was given from the start of conditioning regimens until 3 months after transplant.

All of patients were screened for cytomegalovirus (CMV) infection by quantitative polymerase chain reaction (PCR) or CMV pp65Ag twice weekly and positive cases were treated by ganciclovir for at least 21 d or until antigen tests became negative. Empiric broad-spectrum antibiotics were given for febrile neutropenia. All blood products were irradiated before using. IVIG was prescribed 0.5 g/kg once every 3 wk. G-CSF 5 μg/kg was started from day +8 until the number of absolute neutrophil count (ANC) exceeded more than 1 × 10⁹/L.

Follow-up

After discharge, we visited patients weekly during the first month and every 2 wk until day +100 and individually, thereafter. The assessment of chimerism by PCR assay of genomic DNA for short tandem repeats (STR) was performed on bone marrow on +15 and +30 d after transplantation. Chimerism
assessments were also carried out on peripheral blood on days +60, +90, +180, +365, and thereafter, it was repeated every 6 months. For evaluation of disease status, we followed up immune reconstitution after HSCT with measurement of CD3, CD4, CD8, CD19, and CD20 of immune cells on +30, +90, +180, and +365 d after transplantation.

**Definition**

Full chimerism is defined as >95% donor’s cells in peripheral blood or bone marrow of recipients. Mixed chimerism is defined as the detection of 5–95% of cells of donor origin in peripheral blood or bone marrow. Grafts were defined as rejected when chimerism decreased to <5%.

Neutrophil engraftment was defined as an ANC >0.5 \( \times 10^9 \)/L for 3 consecutive days after HSCT without growth factor support. Platelet engraftment was defined as a platelet count >20 \( \times 10^9 \)/L for 3 consecutive days after HSCT without transfusion support for 7 d (16).

**Results**

**Patient and donor characteristics**

Three patients were diagnosed with FHLH and 2 patients had Chediak–Higashi syndrome. Five other patients had GS-II whose data with shorter follow-up period were published in another article (17).

The median time interval between diagnosis of primary HLH and HSCT was 6.7 months (range: 2–14). The median age of recipients and donors was 22.6 months (range: 6–60) and 13.3 yr (range: 4–29), respectively. The characteristics of patients and donors are illustrated in Table 1. Nine patients received transplants from HLA-matched related donors, and one of them received transplant from two locus-mismatched unrelated cord blood. The median number of infused MNC and CD34 cells from BMSC was 5.8 \( \times 10^6 \)/kg (range: 3.6–8.1) and 6.1 \( \times 10^6 \)/kg (range: 2.3–11.7), respectively. The median number of infused MNC and CD34 cells from PBSC was 8.1 \( \times 10^6 \)/kg (range: 7.6–8.4) and 5.7 \( \times 10^9 \) (range: 2.7–7.8), respectively. Total numbers of infused nucleated cell and CD34 from umbilical cord blood were 6 \( \times 10^6 \)/kg and 3.4 \( \times 10^7 \)/kg, respectively.

**Engraftment and chimerism**

All of the 10 patients engrafted. The median time to neutrophil and platelet engraftment was 12 (range: 8–31) and 19 (range: 10–67) days after transplantation.

**GvHD and other complications**

Acute GvHD (aGvHD) occurred in six patients. Except two patients who developed extensive chronic GvHD (cGvHD), other patients with aGvHD had good responses to treatment. One of the 2 patients who developed cGvHD died during the study. Complications and other details on GvHD are described in Table 2.

After transplantation, one of our patients was complicated with veno-occlusive disease (VOD) who responded favorably to fluid restriction. Three patients who infected with CMV treated with ganciclovir showed appropriate responses to the treatment.

**Outcome**

At the time of this report with a median follow-up of 39 months (range: 18–67), among 8 survived patients, 7 demonstrated full chimerism and 1 showed mixed chimerism. No evidence of HLH crisis or recurrent infection caused by underlying immunodeficiency was seen among all patients after transplantation. During a follow-up period, all

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**Table 1** Patient and donor’s characteristics

<table>
<thead>
<tr>
<th>Patients No.</th>
<th>Age at HSCT (months)</th>
<th>Sex</th>
<th>Type of disease</th>
<th>Time from diagnosis to HSCT (month)</th>
<th>Source of HSCT</th>
<th>Ferritin level at the time of HSCT</th>
<th>HLH status at the time of HSCT</th>
<th>Donor characteristics</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>66</td>
<td>Female</td>
<td>CHS</td>
<td>5</td>
<td>PB</td>
<td>178</td>
<td>CR</td>
<td>11</td>
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<tr>
<td>2</td>
<td>30</td>
<td>Male</td>
<td>GS-II</td>
<td>18</td>
<td>PB</td>
<td>1375</td>
<td>AP</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>Male</td>
<td>FHL</td>
<td>12</td>
<td>BM</td>
<td>206</td>
<td>CR</td>
<td>22</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>Male</td>
<td>FHL</td>
<td>22</td>
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<td>186</td>
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</tr>
<tr>
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<td>PB</td>
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<tr>
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<td>Male</td>
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<td>12</td>
<td>PB</td>
<td>194</td>
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<tr>
<td>9</td>
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<td>Female</td>
<td>CHS</td>
<td>3</td>
<td>PB</td>
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<td>CR</td>
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<tr>
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<td>14</td>
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<td>GS-II</td>
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<td>BM</td>
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<td>CR</td>
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</tbody>
</table>

GS-II, Griscelli syndrome type II; FHLH, familial hemophagocytic lymphohistiocytosis; CHS, Chediak–Higashi syndrome; PB, peripheral blood; BM, bone marrow; CB, cord blood; CR, complete remission; and AP, accelerated phase.
of these patients demonstrated normal growth (age appropriate) after transplantation.

One of the patients with GS-II who were transplanted in the second accelerated phase of HLH expired 15 d after transplantation due to sepsis and intracranial hemorrhage. The other patient with FHLH who received transplant from partially matched unrelated umbilical cord blood died of extensive chronic GvHD on day +165 after HSCT.

### Discussion

Primary HLH is a rare and fatal genetic disorder that usually presents during infancy and early childhood (1–5, 8–10). Despite the significant improvement of chemotherapy protocols for HLH, HSCT remains the only curative option currently available (7, 9). As pretransplant infections in these patients lead to high mortality rates, selecting a conditioning regimen which improves the probability of successful engraftment without increasing the risk of mortality and morbidity is very important. Currently, conditioning regimens used for HSCT in primary HLH (RIC vs. MAC) are still controversial issues among authors.

In this prospective study, we reported the results of patients with primary HLH who underwent transplantation using fludarabine-based reduced-intensity conditioning regimen. In our patients, DFS and OS were 80% at a median follow-up of 39 months (range: 6–55 months).

Ouaché-Charpin et al., (5) reported the results of 48 adult and pediatric patients with familial HLH who had undergone transplantation, using different myeloablative preparative regimens. It was shown that the event-free and overall survival were 58.5% at a median follow-up of 5.8 yr and mortality associated with transplant-related complications occurred within the first 2 yr after transplantation. The results of their study were markedly less than that obtained in our study after a median follow-up of 39 months. Three of 17 (28%) patients died of VOD following bone marrow transplantation, whereas in our study, only 1 (10%) patient developed mild VOD who responded favorably to medication treatment. Patients whose graft failed (n = 12, 25%) underwent retransplantation in their study, while no graft failure was reported in our study.

Marsh et al., (18) conducted a survey to examine 40 adult and pediatric patients with primary and secondary HLH transplanted using different MAC (n = 14) vs. different RIC (n = 26). The 3-yr OS for the MAC and RIC groups was 43% and 90%, respectively. The results indicated that OS was significantly less in MAC recipients compared with those who received RIC regimen. The incidence of grades II–III acute GvHD in the MAC and RIC groups was 14% and 8%, respectively, indicating the decrease compared with our study.

Cooper et al., (7) reported the transplant outcomes in pediatric patients (n = 12) with primary HLH who underwent transplantation using RIC regimen. Similar to our study, engraftment occurred in all patients. The 3-yr OS was 75%. Mixed chimerism was observed in 3 patients who were totally disease free.

It is noticeable that a high incidence of GvHD was found among our patients. It can be resulted from the use of different GvHD prophylaxis regimen and peripheral blood as sources of stem cells in our study.

Due to the rarity of primary HLH, few studies have reported the results of transplantation in these patients (7, 13, 18, 19). There are published reports of patients including adults and pediatric patients diagnosed with HLH (primary and secondary) and treated with MAC and RIC regimens (5, 7, 11, 13, 17). So, it seems difficult to select a suitable conditioning regimen for pediatric patients with primary HLH.

In our study, as opposed to all previous studies on HLH, pediatric patients with primary HLH received the same fludarabine-based conditioning regimen. The study has shown successful transplant outcome in the HLH patients with severe infection such as BCGosis conditioned with RIC regimen. It is important to note that this study largely included patients with GS and CHS instead of FHLH.
Over the last years, MAC regimens were more commonly used for transplantation of adults and children with HLH (7, 8), but in recent years, RIC regimens are more frequently used in patients with HLH. (5, 7, 9, 13, 18). Based on the results of previous studies, patients who received MAC regimens had an increased incidence of transplant-related complications (8, 18–21) and recipients of RIC transplant, with full or mixed chimerism (≥20% donor chimerism) had a long-term survival with no manifestation of HLH symptoms (7, 9, 13, 18–20).

Based on currently available information, pretransplant infections in patients with primary HLH increase the rate of mortality, the use of MAC regimen requires careful consideration, but the use of less toxic RIC as a preparative regimen may seem to be highly effective and safer.

Multicenter prospective studies are needed to present some more definitive suggestions due to the rarity of disease and small sample size in the current and previous studies.

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Conflict of interest

The authors indicated no potential conflict of interests.

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