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Original Article

Haploidentical Hematopoietic Stem Cell Transplantation for Paediatric Patients with X-linked Lymphoproliferative Syndrome

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Abstract. The aim of this study was to investigate the prognostic factors of haploid hematopoietic stem cell transplantation in the treatment of X-linked lymphoproliferative syndrome. Seven children with X-linked lymphoproliferative syndrome diagnosed by XIAP gene analysis were enrolled. The conditioning regimens were tolerated in all seven patients, and the median time of neutrophil engraftment was 10 days (8-13 days), and that of platelet engraftment was 21 days (14-24 days). STR-PCR analysis on the peripheral blood cells showed complete donor origins. Four cases developed Grade I acute graft versus host disease (aGVHD), one developed Grade III aGVHD (intestinal tract), and two cases had limited chronic GVHD. Four cases had cytomegalovirus (CMV) reactivation, and two cases had Epstein–Barr virus (EBV) reactivation. One case was diagnosed as pneumocystosis, and thrombotic microangiopathy (TMA) occurred in three cases. During the follow-up period (median time of 42 months), one patient died of TMA and six patients survived. Statistical analysis showed that the status of disease remission and the positive result of virus in blood before transplantation were independent prognostic factors. Haplo-HSCT might be a curative option for children with refractory X-linked lymphoproliferative syndrome. Low-intensity conditioning regimens may reduce transplant-related mortality and improve overall survival.

Keywords: Haploidentical Hematopoietic Stem Cell Transplantation; X-linked Lymphoproliferative Syndrome; Hemophagocytic lymphohistiocytosis; children; malignancies.

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Introduction. Hemophagocytic lymphohistiocytosis (HLH), also nominated as hemophagocytic syndrome, includes two categories according to the pathogenesis, namely primary HLH and secondary HLH. According to different genetic backgrounds or acquired pathogenic factors, it is further divided into different subtypes.[1] The optimal treatment options for HLH depend upon the causes and progression of the disease. For the different precipitating factors, there is one type of primary HLH that is driven by EBV, X-linked Lymphoproliferative Syndrome (XLP). This disorder is the most common classic HLH driven by EBV[1-4] and includes two subtypes, XLP-1 and XLP-2 (XIAP), that correspond to the BIRC4 gene mutation. In addition to hemophagocytic symptoms, these patients are often associated with chronic colitis, and the minority of them

Table 1. Clinical characteristics of HLH patients with XIAP gene positive before HSCT.

NO.	Age (Years)	Course of Disease (months)	EBV-DNA copy/ml at diagnosis	Before HSCT EBV-DNA copy/ml	Therapy before HSCT	XIAP Protein	Status Of Remission	Complications before HSCT
1	5.6	28	2×10^4	——	Simple hormone HLH-1994	Lower	PR	Lung infection Mesenteritis
2	1.4	5	$6X10^5$	——	HLH-1994	Lower	PR	Lung infection
3	1.2	8	$5X10^6$	——	HLH-1994	Lower	PR	Lung infection
4	3.5	13	$3X10^3$	——	HLH-2004 E-CHOP	Lower	PR	Mesenteritis
5	4.6	10	——	——	HLH-1994	Lower	PR	——
6	2.3	9	$3X10^4$	$1.5X10^3$	HLH-2004	Lower	Disease progression	Lung infection
7	3.1	9	$2X10^3$	——	HLH-2004	undetermined	PR	——

has the presentation of hypogammaglobulinemia. Lymphoma has not been reported so far in this setting, although it is an immunodeficiency disease.[5-7] Clinical observations from the HLH-1994/2004 Study have shown that CD20 monoclonal antibody and Alemtuzumab have temporary mitigation on active HLH.[8-12] Previous reports have shown that HSCT for XLP-2 had poor efficacy,[13] though it is a curative treatment for other subtypes.

Here, we summarize the therapeutic effect of Haplo-HSCT in seven children with hemophagocytic syndrome with XIAP gene mutation. The factors affecting the curative effect are statistically analyzed. The results are generally acceptable as reduced-intensity conditioning regimens were utilized.

Methods.

Patients. Seven patients with XIAP gene-positive HLH were enrolled in our hospital from June 2015 to September 2020. The diagnosis of the disease met the criteria of Hemophagocytic lymphohistiocytosis, which was revised by the Histiocyte Society in 2004.[1]All children were male, and the genetic test results were positive for the XIAP gene on the X chromosome (**Table 1**). The median age was 3.1 years (1.2-5.6 years), and the median time from the onset to transplantation was 9 months (5-28 months). XIAP protein decreased in 5 cases, while in 1 case, XIAP function was normal. XIAP gene mutation was found in all the patients' mothers. The function of this protein was not measured in 1 case. Assessing was made before the start of HSCT, and 6 patients were in partial remission (PR) and one in disease progression. Among the seven patients, in six cases at the initial stage, the EBV-DNA copies were 10^3-10^6 copies/ml, and in 1 case, it was still positive before transplantation. The main symptoms before transplantation were intestinal and pulmonary infections. The modes of transplantation. Five patients received paternal grafts and 2 cases received hematopoietic grafts from mothers. Graft failure occurred in these two cases, and they received secondary transplantation with their mothers as the donors.

The methods of transplantation.

Conditioning regimen. A conditioning regimen consisting of Etoposide (VP-16), Fludarabine (Flu), Busulfan (BU), Anti-thymocyte globulin (ATG), and cyclophosphamide (CTX) was performed before the transplantation as previously reported, according to the conditioning regimen.[20] The doses were as follows: VP16, 600mg/m^2 from days -11 to -9; Bu, 9.6-14.4mg/kg from days -8 to -6; Flu, 30mg/m^2 from days -5 to -3; ATG, 8.5mg/kg within 4 days, from days -5 to -2, and CTX, 10mg/Kg from days -4 to -3. For the two cases that had received maternal grafts and engraftment failure had occurred, Melphalan (MEL) at a total dose of 130mg/m^2 injected from days -6 to -5 was added to the regimen described above.

Mobilization and Collection of Hematopoietic Stem Cells. Hematopoietic mobilization and collection of the grafts were performed as previously described [19]. Briefly, the related donors received recombinant human granulocyte colony-stimulating factor (G-CSF) at a dose of 5-10ug/kg/d for 5 continuous days. On the fourth day, the bone marrow was collected under continuous epidural anesthesia, and on the 5th day, peripheral stem cells were collected by a cell separator. The median count of bone marrow plus peripheral stem cells was 9.07 (8.45-9.98)$\times10^8$/kg, and $CD34^+$ was 6.45 (4.67-8.53) $\times10^6$/kg.

The Criteria of Hematopoietic Reconstruction or Stem Cell Engraftment. DNA fingerprinting was used to determine donor origins, and blood type identification was performed if the donor and the recipient had different blood types. Myeloid reconstruction was identified if the absolute peripheral blood neutrophil count was above 0.5×10^9/L without injection of G-CSF and the platelet above 20×10^9/L without platelet transfusion for more than two weeks.

Table 2. Outcome of the patients post-transplantation.

No.	Chimerism (%)	Granulocyte reconstruction	Platelet Reconstruction	a-GVHD	c-GVHD	Infections	HLA-matching	Survival	Follow-up duration (months)
1	100	8d	16d	Grade I Skin	None	CMV viremia TMA	father HLA 5/10	Survive	42
2	0	——	——	——	——	——	father HLA 5/10	Survive	38
2*	100	9d	14d	Grade I Skin	None	CMV viremia EBV viremia	mother HLA 5/10		
3	0	——	——	——	——	——	father HLA 6/10	Survive	38
3*	100	9d	23d	None	Limited	CMV viremia PCP	mother HLA 6/10		
4	100	13d	15d	None	None	CMV viremia	mother HLA 5/10	Survive	56
5	100	10d	21d	Grade I Liver	None	TMA EBV viremia	father HLA 5/10	Survive	44
6	100	11d	22d	Grade III Intestinal tract	None	TMA	Father HLA 5/10	Death	21
7	100	13d	24d	Grade I Liver	Limited	Cystitis	Mother HLA5/10	Survive	63

* These two patients experienced secondary transplantation after graft failure.

Prevention of Complications.

Graft Versus Host Disease (GVHD). The prophylactic was Cyclosporin or Tacrolimus (FK506), Mycophenolate Mofetil, and Anti-CD25 monoclonal antibody. The Intravenous dosage of Cyclosporin was 2.5mg/kg/d from -10 days, and the dosage was adjusted according to the blood concentration; Tacrolimus was taken orally at -10 days and reduced by half after stem cell transplantation, then stopped till the 28th day; all patients' therapy contains Anti-CD25 monoclonal antibody (from +1day and 10mg once time) and infusing

Disease monitoring. Post-HSCT, Bone marrow morphology, chimerism, XIAP gene mutation, and protein function were monitored regularly.

Results

Stem cell Engraftment and the Toxicity of conditioning Regimen. Five of the seven cases were successfully implanted, and the other two cases failed in the primary engraftment, but all were successfully implanted after secondary transplantation. The chimerism rate of 7 patients was 100%, and the median time of neutrophils above 0.5×10^9/Kg was 10 (8-13) days. The median time of platelets above 20×10^9/L without platelet transfusion was 21 (14-24) days. All the patients tolerated the conditioning regimen well. Among them, five patients had no toxicity, but two cases had Toxicity associated with the conditioning regiments. It should be manifested as fever, diarrhea, reactions of the digestive tract, etc., without complications of major organ bleeding, severe infection, organ failure, and so on.

GVHD. Five cases of GVHD were reported, including four cases of Grade I GVHD, mainly involving the liver and skin, and one case of Grade III aGVHD in the intestinal tract. Two cases developed into limited cGVHD, mainly involving the liver. After adjustment of immunosuppressive agents, the symptoms were alleviated.

Viruses and Other Complications. CMV reactivation occurred in four cases and EBV reactivation in two cases, while none of them developed viral infections. No patient was associated with viral diseases. One case (No.3) developed PCP months post-transplantation, and the condition was controlled after TMP-SMZ treatment. Three cases developed TMA; remission occurred in two cases after treatment. One case with TMA died of Grade III aGVHD (**Table 2**).

Clinical outcome. Six cases have survived disease-free. The overall median survival time was 42 (21-63) months.

Discussion. Hemophagocytic syndrome (HLH) with XIAP gene mutation, caused by mutations in the BIRC4 gene, is a rare congenital immunodeficiency disease. XLP2 gene, located in the 25th region of the long arm of the X chromosome, encodes the X-linked inhibitor of apoptosis protein (XIAP), which is an apoptotic protein. It is expressed in virtually all normal cells and can inhibit the process of cell apoptosis. In addition to its anti-apoptotic effect, it is also involved in multiple signal pathways.[2-5]

The mechanisms underlying XIAP-linked HLH remain elusive until now. It has been reported that increased sensitivity of lymphocytes to undefined apoptotic signals causes damage to NK and T cells and limits the cytotoxic function of lymphocytes that remain during viral infection. The ineffectiveness of lymphocytes in lysing pathogenic microorganisms leads to the long-term persistence of these pathogenic agents,

which constantly stimulate and activate macrophages and T lymphocytes. The activated immune cells may produce a large number of inflammatory factors, resulting in the occurrence of the life-threatening hyperinflammatory syndrome, HLH.[6-9] Lack of XIAP protein expression detected by flow cytometry and BIRC4 mutation in gene sequencing are utilized as the gold standards for diagnosis of XIAP.[10] Glucocorticoids and etoposide regimens are commonly used in the induction of remission for HLH. In addition, it has been reported that Alemtuzumab and CD20 monoclonal antibodies are also effective for HLH induced by EBV infection.[13-15] HSCT can be performed in refractory cases, and usually acceptable outcomes have been achieved.

In the present study, Haplo-identical HSCT was performed in seven cases with HLH, all of which were in incomplete remission after routine therapy. All the patients had lost the option of accepting HLA-identical HSCT. The overall outcome was generally acceptable, in contrast to the results reported previously. Clinical reports have shown that HSCT early after the induction of remission by traditional therapeutic strategies is recommended for a curative goal.[7] Empirically, for XIAP-positive HLH, the HLH-1994 regimen is commonly used to induce remission.[1]

Meanwhile, transplantation as soon as remission has been achieved might be the key to success. For the conditioning, we recommend a reduced-intensity strategy in order to reduce transplant-related mortalities. The doses used in this report had not elicited fatal toxicities, though primary engraftment failed in two cases, who had experienced successful transplantation when more intense preconditioning was utilized. Analyze the reasons for engraftment, considering that it is highly likely to be associated with hemophagocytic syndrome and lymphocyte activation.[16-17] Most of the patients in this group were found positive for EBV-DNA in plasma in the early stage of the disease, and two cases had EBV viremia post-HSCT. Therefore, virus load before transplantation might not be associated with viremia after transplantation. The conditions of the case who died after transplantation were complex, having experienced a variety of deteriorations that included long-term course of the disease, sustained application of glucocorticoids, severe intestinal symptoms caused by Hemophagocytic syndrome before transplantation, repeated diarrhea and gastrointestinal bleeding, and disorders in the functions of the liver and the kidneys. Despite the successful engraftment, this case had intestinal grade III aGVHD after transplantation.

A fully HLA-matched sibling donor is the primary choice for allogeneic HSCT. However, HLH patients who are prepared for HSCT generally have genetic factors leading to immune deficiency, so HLA-related donors might be excluded from the same genes or immune deficiency due to the fact that some of the primary HLH cannot be diagnosed by existing technical means clearly. Patients with refractory or recurrent HLH cannot exclude the genetic background or immunodeficiency, so it should be considered that the sibling donors may have the same genetic background. Therefore, the advantages and disadvantages should be fully evaluated. For the above reasons, international reports also suggested that the efficacy of non-related all-matched HLA donors was significantly better than related fully matched donors.[7] When the fully matched HLA is not available, HLA-haploidentical transplantation becomes a suitable alternative. Because for primary HLH, the majority of HLA-haploidentical donors are gene carriers, the donor needs to be tested for cellular function. Only the donors without obvious functional abnormalities might be chosen.[18]

In summary, HSCT is an available curative treatment for HLH patients who are fit for the transplant indications. HLH patients within a remission stage provide the best condition for HSCT. The effect of transplantation in the remission stage was significantly better, so it is recommended that HLH patients with XIAP undergo allo-HSCT as early as possible in remission. The status of disease remission before HSCT and the virus presence are independent prognostic factors for the efficacy of transplantation. Virus reactivation after transplantation is a transplant-related complication and should be treated with early intervention. For patients who have already had the disease, timely, effective treatment can alleviate the symptoms as soon as possible, which is helpful in reducing the incidence of complications. It can provide the opportunity for HSCT and improve the overall survival rate.

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