



ORIGINAL ARTICLE

Clinical features, genetics, and outcome of pediatric patients with hemophagocytic lymphohistiocytosis in Korea: report of a nationwide survey from Korea Histiocytosis Working Party

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Abstract

Background: We analyzed a nationwide registry of pediatric patients with hemophagocytic lymphohistiocytosis (HLH) in Korea to assess the clinical and genetic features and treatment outcomes in pediatric HLH. **Methods:** The Korea Histiocytosis Working Party retrospectively analyzed data on 251 pediatric patients diagnosed with HLH between 1996 and 2011. **Results:** In the study cohort, 25 cases were categorized with familial HLH, 64 with presumed secondary HLH, and 162 with unspecified HLH. Of 217 evaluable patients, 91 (42%) had concomitant Epstein–Barr virus infection. Of 238 evaluable patients, central nervous system (CNS) involvement, which was more frequent in the familial group, was evident in 81 cases (34%). Genetic tests revealed a predominant *UNC13D* mutation with a high incidence of two recurrent splicing mutations (c.118-308C>T and c.754-1G>C). The 5-yr overall survival rate was 68% (38% in the familial group and 81% in the presumed secondary group). The 5-yr overall survival rate among 32 patients who underwent allogeneic hematopoietic stem cell transplantation was 64%. In multivariate analysis, a younger age at diagnosis, severe transaminasemia, and a coagulation abnormality were independent prognostic factors for survival. Responses during initial treatments were also significant indicators of outcome. **Conclusion:** Our study showed the unique predominance of a *UNC13D* mutation and vulnerability to Epstein–Barr virus infection in Korean children with HLH and emphasizes the prognostic significance of age, liver dysfunction, and treatment responses in this disease. A multicenter prospective trial that builds on the present results is warranted to identify subgroups of patients with a poor prognosis and identify optimal treatments.

Key words hemophagocytic lymphohistiocytosis; genetic mutation; prognostic factors; survival; allogeneic hematopoietic stem cell transplantation

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Hemophagocytic lymphohistiocytosis (HLH) is a potentially fatal disease caused by dysregulated immune responses and overwhelming inflammation (1). HLH can be categorized into two distinct forms: primary or familial HLH (FHL) and secondary HLH. FHL can be further subcategorized into five subtypes, FHL1 to FHL5, according to causative genes. Mutations in *PRF1*, *UNC13D*, *STX11*, and *STXBP2* have been linked to FHL2, FHL3, FHL4, and FHL5, respectively (1–3). Secondary HLH is triggered by infections, malignancy, or rheumatic disease, without a known genetic predisposition. Interestingly, ethnic differences in genetic susceptibility to FHL and vulnerability to Epstein–Barr virus-associated HLH have been reported (1, 4).

The establishment of diagnostic criteria and the use of immunochemotherapy with or without appropriate allogeneic hematopoietic stem cell transplantation (HSCT) have significantly improved survival outcomes in patients with HLH (5–8). Early recognition and the prompt initiation of treatment are mandatory for an improved prognosis. However, reliable outcome predictors for HLH and risk-defined treatment strategies remain to be adequately established.

Although our understanding of HLH has increased significantly, a large-scale, nationwide study of this heterogeneous disease is still lacking. Our present study was conducted to analyze a Korean national registry of pediatric HLH and thereby investigate the epidemiologic features and ethnic characteristics of Korean pediatric patients with HLH, as well as the general clinical features and prognostic factors of HLH.

Patients and methods

Study population and data acquisition

The Korea Histiocytosis Working Party retrospectively collected data on children and adolescents with HLH from member hospitals. A total of 251 patients diagnosed with HLH between 1996 and 2011 were registered from 22 Korean institutions. The case report form included information on demographic characteristics, clinical, laboratory, and radiological findings at presentation, genetic mutation analysis, type of treatment and responses to treatment, allogeneic hematopoietic stem cell transplantation, and survival outcomes. Patients with malignancy-associated HLH were not registered in this study.

Classification

HLH was diagnosed based on the diagnostic criteria proposed by the Histiocyte Society in 1991 and updated in 2004 (6, 9). Since 2006, the Korea Histiocytosis Working Party has recommended that patients who present with suspicious clinical and laboratory features for HLH should undergo genetic testing for this disorder at a central laboratory, irrespective of

their age. In our present study, patients who were found to have a genetic abnormality or early-onset disease (≤ 2 yr) with family history without genetic evaluation were designated as having familial HLH. Patients whose genetic testing for *UNC13D*, *PRF1*, *STX11*, and *STXBP2* revealed no genetic abnormality and who had no family history of HLH were designated as having presumed secondary HLH. Patients who could not be defined as having familial or secondary HLH due to unavailability of genetic test and data were designated as having unspecified HLH.

CNS involvement was defined as either of the presence of neurological symptoms or pleocytosis and/or proteinosis in cerebrospinal fluid (CSF), or the demonstration of abnormalities on magnetic resonance imaging (MRI) such as high signal intensity lesions, hemorrhage, atrophy, and leptomeningeal enhancement.

Statistical methods

A *t*-test was used to compare differences between parametric variables. A chi-square test or Fisher's exact test was used to assess differences between groups. The Kaplan–Meier method was used to estimate survival probabilities, and a log-rank test was used to test the prognostic significance of various risk factors. Multivariate Cox analysis was performed to assess associations between clinical variables and prognosis. $P < 0.05$ were considered statistically significant. All statistical analyses were performed using *SPSS* version 21.0 (Statistical Package for the Social Sciences; IBM, Armonk, NY, USA).

Results

Patient characteristics

Demographic data and clinical features of 251 patients with HLH at their initial presentation are shown in Table 1. Twenty-five patients (10%) were categorized with familial HLH based on family history and/or genetic mutation, 64 (25%) with presumed secondary HLH, and 162 (65%) with unspecified HLH. Patients with clinical symptoms or signs suggesting Chédiak–Higashi syndrome, Griscelli syndrome, or Hermansky–Pudlak syndrome were not reported.

In our total HLH cohort, 123 patients were male and 128 were female (male : female ratio = 0.96) with a median age at diagnosis of 3.2 yr (range, 0–18.7 yr) and 43 patients younger than 12 months (17%). The proportion of infants younger than 12 months was significantly higher in the familial HLH group than in the presumed secondary HLH group ($P < 0.001$). The median age at diagnosis of the familial group was significantly younger than that of the presumed secondary HLH group (0.4 yr vs. 3.5 yr; $P < 0.001$). The proportion of patients with familial HLH tended to

Table 1 Demographics, clinical manifestations, laboratory and histopathological findings, and CNS involvement at presentation of patients with HLH

	Total (n = 251)	Familial (n = 25)	Presumed secondary (n = 64)	Unspecified (n = 162)	P-value*
Age at diagnosis					
≤12 months	43 (17%)	16 (64%)	10 (16%)	17 (10%)	<0.001
>12 months	208 (83%)	9 (36%)	54 (84%)	145 (90%)	
Median age (year, range)	3.2 (0.1–18.7)	0.4 (0.1–13.2)	3.5 (0.1–18.7)	3.6 (0–18.2)	<0.001
Sex (M/F)	123/128	9/16	36/28	78/84	0.086
Manifestations at presentation					
Fever	248/251 (99%)	25/25 (100%)	64/64 (100%)	159/162 (98%)	NA
Hepatomegaly	208/241 (86%)	22/24 (92%)	54/58 (93%)	129/156 (83%)	0.820
Splenomegaly	201/243 (83%)	24/24 (100%)	48/60 (80%)	129/159 (81%)	0.018
Neurological symptoms	41/233 (18%)	8/24 (33%)	14/61 (23%)	19/148 (13%)	0.325
Diagnostic criteria					
Bicytopenia	226/248 (91%)	25/25 (100%)	53/62 (86%)	148/161 (92%)	0.044
Hb <9.0 g/dL	142/251 (57%)	20/25 (80%)	22/64 (50%)	90/162 (56%)	0.016
Platelets <100 000/μL	202/251 (81%)	24/25 (96%)	45/64 (70%)	133/162 (82%)	0.01
ANC <1000/μL	136/235 (58%)	13/22 (59%)	32/56 (57%)	91/157 (58%)	1
Triglycerides >265 mg/dL	90/225 (40%)	7/21 (33%)	22/56 (39%)	61/148 (41%)	0.793
Fibrinogen <150 mg/dL	144/233 (62%)	13/22 (59%)	34/59 (58%)	97/152 (64%)	1
Ferritin >500 μg/dL	204/226 (90%)	16/19 (84%)	56/59 (95%)	132/148 (89%)	0.151
NK-cell activity <10%	4/16 (25%)	1/2 (50%)	2/11 (18%)	1/3 (33%)	0.423
sCD25 ≥2400 U/L	7/8 (86%)	2/2 (100%)	4/4 (100%)	1/2 (50%)	NA
Hemophagocytosis	236/243 (97%)	19/22 (86%)	60/62 (97%)	157/159 (99%)	0.110
Others					
AST and/or ALT >200 IU/L	153/242 (63%)	11/24 (46%)	39/59 (66%)	103/159 (65%)	0.087
Bilirubin >3 mg/dL	83/239 (35%)	7/22 (32%)	15/58 (26%)	61/159 (38%)	0.594
Albumin <2.5 g/dL	38/222 (17%)	2/16 (13%)	10/49 (20%)	26/157 (17%)	0.479
aPTT >60 s	62/226 (27%)	8/21 (38%)	14/58 (24%)	40/147 (27%)	0.261
LDH >500 IU/L	212/224 (95%)	16/19 (84%)	58/59 (98%)	138/145 (95%)	0.043
Na <130 mEq/L	36/171 (21%)	2/10 (20%)	5/30 (17%)	29/131 (22%)	1
CNS involvement	81/238 (34%)	15/25 (60%)	21/61 (34%)	45/152 (30%)	0.034
Neurological symptoms	41/233 (18%)	8/24 (33%)	14/61 (23%)	19/148 (13%)	0.410
CSF pleocytosis	38/106 (36%)	8/13 (62%)	10/29 (35%)	20/64 (31%)	0.101
CSF proteinosis	29/94 (31%)	8/12 (67%)	4/23 (17%)	17/59 (29%)	0.007
MRI abnormalities	26/77 (34%)	13/10 (77%)	8/27 (30%)	8/37 (22%)	0.007

HLH, hemophagocytic lymphohistiocytosis; NA, not applicable; ANC, absolute neutrophil count; sCD25, soluble CD25; AST, aspartate transaminase; ALT, alanine transaminase; LDH, lactate dehydrogenase; CNS, central nervous system; CSF, cerebrospinal fluid; MRI, magnetic resonance imaging.

*Comparison between familial and presumed secondary HLH groups.

decrease as the age of the patients increased with 10% of the patients over 5 yr of age (3/29) having genetic mutations consistent with FHL. Notably, 59% of the infant patients (16/27) were shown to have a genetic background associated with HLH. In particular, young infants younger than 6 months of age were highly likely to have a genetic predisposition to HLH (14/20, 70%) (Fig. 1).

At initial presentation, nearly all of our patient subjects had a fever, and most had hepatosplenomegaly. Splenomegaly was more prevalent in the familial HLH group than in the presumed secondary HLH group ($P = 0.018$). Of 233 evaluable patients, 41 (17.6%) had neurological symptoms. The proportion of patients with neurological symptoms was not different between the familial and presumed secondary HLH groups.

Laboratory and histopathological findings

The characteristics of laboratory and histopathological findings are shown in Table 1. Most patients had bicytopenia with predominant anemia and thrombocytopenia, with significantly higher incidence of anemia ($P = 0.016$), thrombocytopenia ($P = 0.01$), and bicytopenia ($P = 0.044$) in the familial HLH group than in the presumed secondary HLH group. Elevated lactate dehydrogenase (LDH) levels (>500 IU/L) were more common ($P = 0.043$) and transaminasemia marginally more common ($P = 0.087$) in the presumed secondary HLH group. The differences of other laboratory findings were statistically not significant between the two groups.

Notably, many patients had liver dysfunction, with elevated levels of aspartate transaminase (AST) and/or alanine

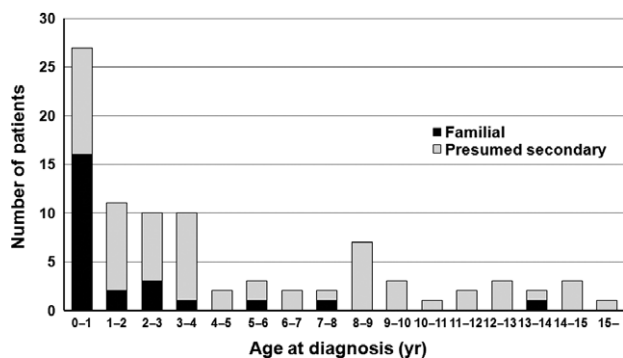


Figure 1 Distribution of familial and presumed secondary HLH by age for 89 classifiable patients.

transaminase (ALT) (>200 IU/L), hyperbilirubinemia (>3.0 mg/dL), and hypoalbuminemia (<2.5 g/dL), and more than a quarter of the patients had significant coagulation abnormalities (aPTT >60 s). Also, most patients had elevated levels of serum LDH.

CNS manifestations

The CNS involvement in our HLH cohort is summarized in Table 1. Of 238 evaluable patients, 81 (34%) had CNS involvement. CSF and MRI abnormalities were observed in more than 30% of the evaluable patients. Patients with familial HLH had more frequent CNS involvement with more frequent MRI abnormalities ($P = 0.007$) and CSF proteinosis ($P = 0.007$) than those with presumed secondary HLH, whereas the proportion of patients with clinical neurological symptoms was not different between the two groups. The rate of concomitant EBV infection was significantly lower in patients with CNS involvement than those without (30% vs. 47%; $P = 0.021$).

Conditions associated with HLH

Possible conditions associated with HLH included viral infection in 125 (Epstein–Barr virus (EBV) in 91, cytomegalovirus in 8, adenovirus in 5, parainfluenza virus in 5, herpes simplex virus in 3, influenza virus in 3, hepatitis A virus in 2, varicella zoster virus in 2, metapneumovirus in 2, parvovirus B19 in 1, coronavirus in 1, respiratory syncytial virus in 1, rhinovirus in 1), bacterial infection in 9 (*Coxiella burnetii* in 1, *Escherichia coli* in 1, *Mycoplasma* in 1, *Pseudomonas aeruginosa* in 1, *Salmonella typhi* in 1, *Staphylococcus aureus* in 1, *Streptococcus mitis* in 1, *Streptococcus pneumoniae* in 1, *Orientia tsutsugamushi* in 1), and rheumatological disorders in 10 (Kawasaki disease in 5, systemic juvenile rheumatoid arthritis in 3, systemic lupus erythematosus in 2), and an unknown etiology or insufficient data in 107.

Of 217 evaluable patients for EBV, 91 (42%) had concomitant EBV infection. EBV infection tended to be more common in the presumed secondary HLH group than in the

familial HLH group, but without statistical significance (42% vs. 20%; $P = 0.116$), and was significantly associated with concomitant hyperbilirubinemia (46% vs. 29%; $P = 0.014$).

Molecular genetics

Genetic testing for *UNC13D*, *PRF1*, and *STX11* was carried out for 85 patients and testing for *STXPB2* for 23 patients, with 19 (22%) showing genetic mutations. The most common genetic mutation was in *UNC13D* (*UNC13D* mutations in 15 and *PRF1* mutations in 4). No patient had mutation in *STX11* and *STXPB2*. Among 15 patients with *UNC13D* mutations, 13 had biallelic mutations and 2 had monoallelic mutations. Of 28 mutations in *UNC13D*, 24 were splicing mutations, 3 frameshift mutations, and 1 nonsense mutation. Notably, 2 recurrent splicing mutations—c.118-308C>T (IVS1-308C>T) (7/15) and c.754-1G>C (IVS9-1G>C) (8/15)—predominated, with either mutation found in 80% of the patients with *UNC13D* mutations (12/15). Detailed data on mutations were separately reported (10). All two patients, whose detailed data on *PRF1* mutations were available, had same frameshift mutation of c.1090_1091delCT (p.Leu364Glufs*93) (1 with biallelic and 1 with monoallelic mutation).

Treatment and outcomes

The majority of our study patients (167 of 222 evaluable patients) were treated with HLH-94- or HLA-2004-based immunochemotherapy (5, 6), whereas 9% of these cases (21/222) were treated with other treatments such as methylprednisolone, intravenous immunoglobulin, or antithymocyte globulin; 15% of the patients (34/224) had no treatment due to spontaneous resolution or rapid progression of the disease (Table 2).

Data for analysis of treatment response were available in 175 among 188 patients with HLH who received treatment. Eighty patients (46%) achieved complete resolution, and 43 (25%) showed improvement. In contrast, 27 (15%) of these patients had reactivated or persistent disease with minimal or no improvement after 8 wk of initial treatment, and 25 (14%) patients died of refractory disease (Table 2). Of the total cohort of 251 enrolled HLH patients, 45 (18%) died within 8 wk of diagnosis, and 32 (13%) beyond 8 wk, leading to a 5-yr overall survival (OS) rate of 68% (Fig. 2A). The 5-yr OS rate was significantly poorer in the familial HLH group than in the presumed secondary HLH group and unspecified HLH group (38% vs. 81% vs. 68%; $P = 0.001$; Fig. 2B).

Prognostic factors

The results of univariate analysis on the putative prognostic factors for OS according to the type of disease are shown in

Table 2 Treatment and outcomes of patients with HLH

	No. of patients
Treatment characteristics	
Treatment (+)	188/222 (85%)
HLH-94/2004	167/222 (75%)
Other treatment	21/222 (9%)
No treatment	34/222 (15%)
No data	29/251
Treatment response (after 8 wk)	
Complete resolution	80/175 (46%)
Improvement without complete resolution	43/175 (25%)
Reactivation or persistence	27/175 (15%)
Death	25/175 (14%)
No data	13/188
Survival outcome	
Death before 8 wk	45/251 (18%)
Death after 8 wk	32/251 (13%)
Alive at last follow-up	174/251 (69%)

HLH, hemophagocytic lymphohistiocytosis.

Table 3. Overall, a younger age, CNS involvement, severe transaminasemia (AST or ALT >800 IU/L), severe cholestasis (total bilirubin >6 mg/dL), and coagulation abnormalities were significant indicators of a poor prognosis. In the familial HLH group, only a coagulation abnormality was a marginally significant prognostic factor, and CNS involvement and age were not found to be significantly associated with survival outcome. In the presumed secondary HLH group, a younger age, severe transaminasemia, a coagulation abnormality, and CNS involvement were found to be predictors of poor survival outcomes. Overall, disease status and treatment response represented by the ferritin level after 8 wk of treatment were found to be significant indicators of outcome. Disease status after 8 wk was an indicator of poor outcome in both the familial and presumed secondary HLH groups, whereas the ferritin levels after 8 wk were significant indicators in the familial group, not in the presumed secondary group.

Overall, multivariate Cox regression analysis revealed that a younger age at diagnosis (hazard ratio [HR] = 2.40; $P = 0.002$; 95% confidence interval [CI], 1.37–4.22), severe

transaminasemia (HR = 1.82; $P = 0.028$; 95% CI, 1.07–3.11), and a coagulation abnormality (HR = 2.52; $P < 0.001$; 95% CI, 1.51–4.20) are independent prognostic factors for survival.

Allogeneic hematopoietic stem cell transplantation

Thirty-two of the patients with HLH in our study cohort underwent allogeneic hematopoietic stem cell transplantation (HSCT). The reasons for using HSCT were familial HLH in 10 cases, active disease after the initial treatment in 11, and reactivated disease in 11. Whereas nine patients received transplants from matched related donors, 22 patients received transplants from unrelated donors. Graft sources included the bone marrow in 15 patients, granulocyte colony-stimulating factor-mobilized peripheral blood in 9, and cord blood in 6. Busulfan- ($n = 26$) or total-body irradiation-based ($n = 1$) myeloablative conditioning (MAC) regimens were used in 27 and fludarabine-based reduced-intensity conditioning (RIC) regimens in 5. Twenty-four patients had non-active disease at the time of HSCT, while 8 had active disease. Three patients developed graft failure. All three patients, who developed graft failure, had received cord blood graft after MAC in 2 and RIC in 1. No patient received donor lymphocyte infusion or a stem cell boost. The 5-yr OS rate after allogeneic HSCT was 64%. The type of donor, conditioning regimen, and the status prior to HSCT did not influence the outcome, while cord blood graft tended to be associated with a poorer outcome, but without statistical significance ($P = 0.115$) (Fig. 3). Seven patients died of transplant-related causes (graft failure in three, infection in two, heart failure in one, hemorrhage in one, and graft-versus-host disease in one), and two patients died of refractory or reactivated disease.

Discussion

Our current study represents the first nationwide assessment of HLH in Korea and describes the epidemiological and clinical characteristics of Korean patients with this disorder.

Figure 2 The 5-yr overall survival rate of 251 patients with HLH in the overall group (A) and according to the type of disease (B).

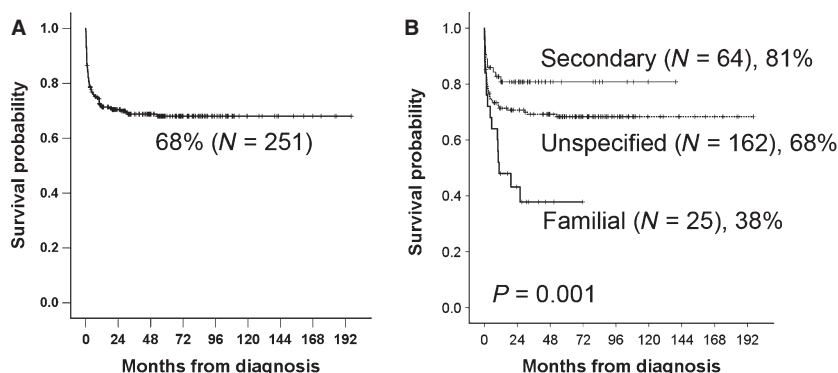


Table 3 Univariate analysis of putative prognostic factors for overall survival

Prognostic factor	Total (<i>n</i> = 251)		Familial (<i>n</i> = 25)		Presumed secondary (<i>n</i> = 64)	
	5-yr OS (%)	<i>P</i> -value	5-yr OS (%)	<i>P</i> -value	5-yr OS (%)	<i>P</i> -value
Age at diagnosis						
>12 months	72 (<i>n</i> = 208)	0.004	22 (<i>n</i> = 9)	0.156	85 (<i>n</i> = 54)	0.047
≤12 months	49 (<i>n</i> = 43)		48 (<i>n</i> = 16)		58 (<i>n</i> = 10)	
EBV						
Positive	71 (<i>n</i> = 91)	0.848	33 (<i>n</i> = 3)	0.452	73 (<i>n</i> = 26)	0.295
Negative	69 (<i>n</i> = 125)		50 (<i>n</i> = 12)		86 (<i>n</i> = 36)	
CNS						
Involvement	56 (<i>n</i> = 81)	0.006	32 (<i>n</i> = 15)	0.2556	60 (<i>n</i> = 21)	0.003
No involvement	76 (<i>n</i> = 157)		60 (<i>n</i> = 10)		92 (<i>n</i> = 40)	
AST/ALT						
≥800 IU/L	53 (<i>n</i> = 58)	0.001	0 (<i>n</i> = 2)	NA	60 (<i>n</i> = 15)	0.009
<800 IU/L	72 (<i>n</i> = 184)		39 (<i>n</i> = 22)		88 (<i>n</i> = 44)	
aPTT						
≥60 s	49 (<i>n</i> = 62)	<0.001	25 (<i>n</i> = 8)	0.065	64 (<i>n</i> = 14)	0.047
<60 s	74 (<i>n</i> = 164)		53 (<i>n</i> = 13)		86 (<i>n</i> = 44)	
Total bilirubin						
≥6.0 mg/dL	58 (<i>n</i> = 41)	0.002	50 (<i>n</i> = 4)	0.972	82 (<i>n</i> = 8)	0.548
<6.0 mg/dL	70 (<i>n</i> = 198)		37 (<i>n</i> = 18)		75 (<i>n</i> = 50)	
Ferritin (at diagnosis)						
≥10 000 ng/mL	71 (<i>n</i> = 58)	0.941	0 (<i>n</i> = 1)	NA	88 (<i>n</i> = 16)	0.418
<10 000 ng/mL	66 (<i>n</i> = 139)		42 (<i>n</i> = 16)		77 (<i>n</i> = 36)	
Ferritin (after 8 wk)						
<500 ng/mL	95 (<i>n</i> = 70)	<0.001	75 (<i>n</i> = 4)	0.048	95 (<i>n</i> = 22)	0.378
≥500 ng/mL	52 (<i>n</i> = 26)		0 (<i>n</i> = 3)		83 (<i>n</i> = 6)	
Response at 8 wk						
Complete resolution	97 (<i>n</i> = 100)	<0.001	86 (<i>n</i> = 7)	0.020	100 (<i>n</i> = 22)	0.029
Without resolution	64 (<i>n</i> = 82)		17 (<i>n</i> = 9)		80 (<i>n</i> = 27)	

OS, overall survival; EBV, Epstein–Barr virus; CNS, central nervous system; AST, aspartate transaminase; ALT, alanine transaminase; NA, not applicable.

Our results emphasize the prognostic significance of CNS involvement and liver dysfunction (including severe cholestasis, severe transaminasemia, and coagulation abnormalities) and the predictive role of the treatment response, which was represented by the decline in the ferritin level. Moreover, our present findings confirmed the predominance of *UNC13D* mutations in Korean patients with FHL and the high incidence of EBV-associated HLH in Korea.

FHL is usually diagnosed during infancy or early childhood. Our current analyses revealed a median age at diagnosis of FHL of 0.4 yr, with patients younger than 1 yr old highly likely to have a genetic cause associated with HLH. Thus, genetic evaluations should be mandatory for all patients who present with HLH during infancy or early childhood. However, our study findings also showed that older children might have the genetic mutation consistent with FHL. Indeed, 10% of patients in our HLH cohort older than 5 yr of age were shown to have a genetic background. Previous studies have reported that adolescents and even older adults may have a genetic predisposition to HLH (11–14). Thus, all cases of suspicious symptoms, regardless of age, should be evaluated for familial HLH, even when triggering factors are found.

Clinical and laboratory features at presentation in our cohort are comparable to previous reports (15–18). Notably, 97% of patients were found to have hemophagocytosis, which is higher than previous studies (17, 18). This is likely to be due to limited availability of NK-cell activity test and measurements of sCD25, which are elements of diagnostic criteria for HLH. Patients, who were found to have hemophagocytosis, thus meeting the diagnostic criteria for HLH without those two tests, were more likely to be included in the present study. Therefore, high percentage of hemophagocytosis in our study does not emphasize the presence of hemophagocytosis as a necessary requisite for diagnosis of HLH.

CNS involvement has been reported to be a critical factor for predicting the outcomes of patients with HLH (19–21). Our current data indicate that CNS involvement is more common in the familial group, thus suggesting that genetic susceptibility plays an important role in the infiltration of activated lymphocytes and macrophages into the CNS. CNS involvement was found to be a significant prognostic factor in our presumed secondary HLH group but was not associated with a poor outcome in the familial group. Thus, prognostic significance of CNS involvement according to the type of HLH should be evaluated in a larger study.

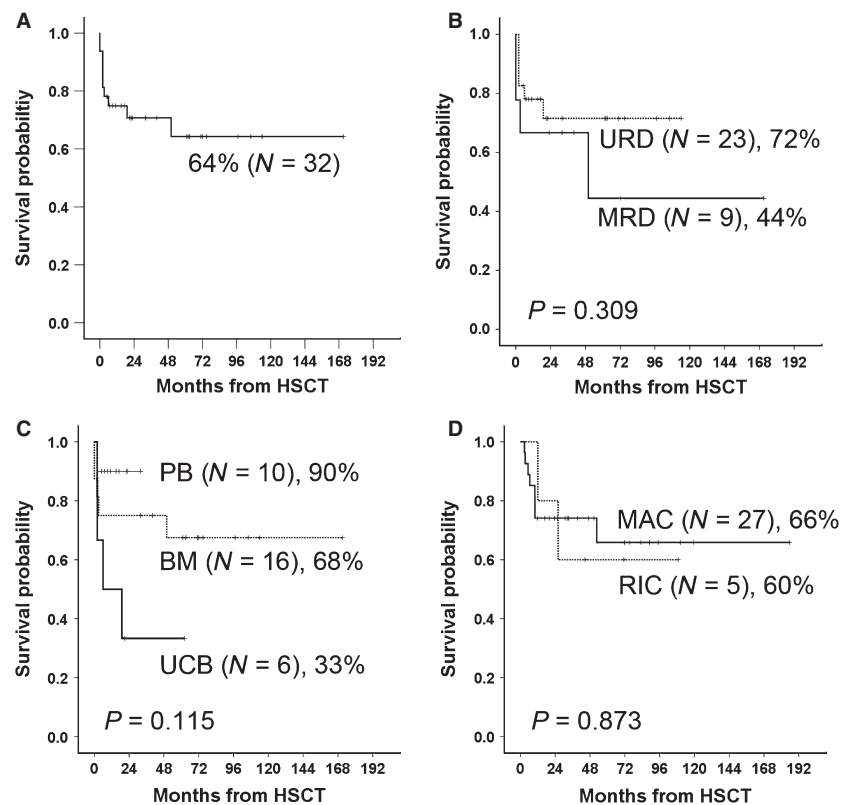


Figure 3 The 5-yr overall survival rate of 32 patients who received allogeneic HSCT in the overall group (A), according to the type of donor (B), graft source (C), and the intensity of conditioning regimen (D). MRD, matched related donor; URD, unrelated volunteer donor; PB, granulocyte colony-stimulating factor-mobilized peripheral blood; BM, bone marrow; UCB, unrelated cord blood; MAC, myeloablative conditioning; RIC, reduced-intensity conditioning.

In our present study cohort, 42% of patients with HLH had an EBV infection. This finding is a further example of the high incidence of secondary HLH associated with EBV infection in an Asian population (4, 22). This unique vulnerability to EBV infection in Asian patients with HLH suggests that different genetic backgrounds can contribute to the development of the disease, even in cases of secondary HLH. In addition, HLH patients with documented EBV infection had a lower rate of CNS involvement and a higher rate of cholestasis, which suggests the tropism of organ involvement in EBV-associated HLH. A future study should include further genetic testing for *SH2D1A*, *XIAP/BIRC4*, and *ITK*, which have been implicated in EBV-associated lymphoproliferative disorders, to differentiate HLH from these disorders presenting as hemophagocytic syndrome (23).

Our present study findings confirmed that *UNC13D* is the gene most commonly mutated in Korean patients with FHL, which was also suggested by a previous pilot study (24). Ethnic differences have been reported in relation to the genetic predisposition to each subtype of FHL (1, 25, 26). Interestingly, the most commonly mutated gene for FHL in the neighboring countries of Korea, Japan and China is *PRF1* (26, 27). Two recurrent splicing mutations—c.118-308C>T and c.754-1G>C—were found in nearly three-quarters of the patients in our current study cohort, which suggest the presence of founder effects of these mutations in

the development of HLH in Korea (10). In contrast, c.1596 + 1G>C, the most common mutation in Japanese FHL3 patients, and c.2346_2349delGGAG and c.753 + 1G>T, the most common mutations in European FHL3 patients, were not found in our series, which suggest a geographic or ethnic specificity of the mutations causing each FHL subtype (28, 29).

Our current analyses showed a 5-yr OS rate of 68%, which is comparable with previous studies (4, 5). Patients with familial HLH in our series showed a significantly poorer outcome. Most deaths occurred within the first 2 months of the disease in our presumed secondary HLH group, whereas many patients with FHL succumbed to the refractory or reactivated disease after the early period of the disease. HSCT was able to rescue a substantial portion of the patients with FHL or refractory disease. Notably, the use of a cord blood graft was associated with a poor outcome. Thus, the optimal conditioning regimen according to a graft source should be evaluated in a future study.

Our present study incorporates the inevitable limitations of being long-term and retrospective in design, such as missing data, inconsistent evaluations, and the use of heterogeneous treatments. Limitations of this retrospective study include the incompleteness of testing for *STXBP2* mutation and the absence of testing for mutations in *SH2D1A*, *XIAP/BIRC4*, and *ITK*, which was why we adopted the term ‘presumed’ secondary HLH for the patients without mutations in

UNC13D, *PRF1*, and *STX11*. This leaves the possibility of the presence of cryptic genetic mutation in the patients designated as having presumed secondary HLH, thus requiring cautious interpretation of our results. Another limitation of the present study is that a larger portion of patients, who were enrolled during early period of the study, has not been tested for genetic mutation and was thus designated as having unspecified HLH. There are no reliable clinical criteria to distinguish familial and secondary HLH. Therefore, we could not further define the patients with HLH without genetic evaluation or family history, even though they were reported to have possible triggering causes. Albeit these limitations, our observations of discrete clinical features between familial and presumed secondary groups and prognostic factors evaluated in our overall cohort could provide clinical implications for a future research agenda.

In conclusion, our current analyses have revealed a unique genetic susceptibility to *FHL3* and vulnerability to EBV infection in Korean children with HLH. Age, liver dysfunction, and treatment response were found to be significant indicators of a poor prognosis. Serial monitoring of changes in the ferritin levels during the initial treatment of HLH may enable the disease status to be followed and allow for the prediction of treatment outcomes, thus helping to identify the subgroup of patients who need more intensive treatment. A multicenter prospective trial is warranted to identify the subgroups of patients with HLH with poor prognoses and find optimal treatment for these cases. In addition, the optimal intensity and duration of treatment for patients with presumed secondary HLH need to be investigated.

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Conflicts of interest

None to declare.

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