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ORIGINAL ARTICLE

Outcomes in children with hemophagocytic lymphohistiocytosis treated using HLH-2004 protocol in Japan

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Abstract

Recent advances in intensive chemo- and immunotherapy have contributed to the outcome of hemophagocytic lymphohistiocytosis (HLH); however, the prognosis of HLH in children differs by HLH subtype. In Japan, secondary HLH, particularly Epstein–Barr virus-associated HLH (EBV-HLH), is the most common HLH subtype. The prognosis of HLH has improved in recent years. We here conducted a prospective study of 73 patients who were treated with HLH-2004 protocol in Japan. EBV-HLH, familial HLH (FHL), and HLH of unknown etiology were seen in 41, 9, and 23 patients, respectively. Patients with resistant or relapsed disease after HLH-2004 treatment and those with FHL received hematopoietic stem cell transplantation (HSCT). The induction rate after initial therapy was 58.9%, and the 3-year overall survival (OS) rate of all patients was 73.9% and differed significantly among those with EBV-HLH, FHL, and HLH of unknown etiology. Of the 17 patients who received HSCT, the 3-year OS rates of those with and without complete resolution before HSCT were 83.3% and 54.5%, respectively. Outcomes in children with HLH who were treated with the same protocol differed among HLH subtypes. Appropriate strategy for each subtype should be established in future studies.

Keywords HSCT · HLH-2004 · Epstein–Barr virus · FHL · EBV-HLH

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Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a rare childhood disorder that is characterized by clinical findings, such as persistent fever and hepatosplenomegaly, and abnormal laboratory findings, including pancytopenia, hypertriglyceridemia, hypofibrinogenemia, hyperferritinemia, and high soluble interleukin-2 receptor [1–3]. The infiltration of lymphocytes and histiocytes with hemophagocytic activity is evident in the reticuloendothelial system, including the bone marrow and central nervous system [1, 2]. HLH can be divided into two subtypes: primary and secondary. Primary HLH includes familial HLH (FHL) and X-linked lymphoproliferative syndrome, whereas secondary HLH is induced by various triggers, including the Epstein–Barr virus (EBV) or other infections, lymphoma or other malignancies, and autoimmune diseases [1–3].

Although all subtypes of HLH exhibit common clinical features, the distribution of each subtype varies among countries [2, 4]. Although several genetic defects that cause FHL have been identified, perforin (*PRF1*), a causative gene of FHL type 2, and Munc13-4 (*UNC13D*), responsible for FHL type 3, are among the most common causes in Japan [1]. In secondary HLH, infection-associated HLH, particularly EBV-HLH, accounts for a relatively high incidence in Japan and is most commonly seen in children [2].

Prompt introduction of an appropriate treatment is essential for the survival of patients with primary or refractory HLH. The HLH-94 protocol presented in 1994 by the Histiocyte Society comprised an initial immunochemotherapy with etoposide (VP-16) and dexamethasone (DEX) for 8 weeks, with intrathecal methotrexate administration for patients with central nervous system disease, followed by a maintenance therapy with VP-16 and DEX pulses, in combination with cyclosporine A for maintaining suitable conditions for hematopoietic stem cell transplantation (HSCT) [5].

The HLH-94 protocol confirmed the efficacy of chemoimmunotherapy by demonstrating substantial improvements in the 5-year overall survival (OS) rates to approximately 60% [5, 6]. However, the mortality remained high during the acute phase of HLH, and more deaths were observed in patients before receiving HSCT. To reduce the mortality before HSCT, the HLH-2004 protocol was used in a subsequent study on HLH [6, 7]. In this study, cyclosporine A was incorporated in the induction therapy; however, the outcome of patients with HLH was not different between two protocols [6, 7].

Both HLH-94 and HLH-2004 protocols mainly focused on primary HLH, which accounts for most of the HLH cases in Europe and North America. On the other hand, the common HLH subtype in Japan is secondary HLH, mainly EBV-HLH, raising the question of whether the same treatment protocol could be used for all HLH subtypes. In this study, we aimed to evaluate the outcome of patients with different HLH subtypes treated using the HLH-2004 protocol in Japan to establish an optimal treatment regimen for each subtype.

Materials and methods

Evaluation of HLH

In our experience, among all the subtypes, mild secondary HLH is most frequently observed in Japan. Therefore, all children < 18 years that fulfilled three of the following four criteria were initially registered with possible HLH in the Japan Pediatric Leukemia/Lymphoma Study Group (JPLSG): fever with unknown etiology, bicytopenia (any two of the following: hemoglobin < 90 g/L, platelets < 100,000/μL, and absolute neutrophil count < 1000/μL), and ferritin ≥ 500 ng/mL and lactate dehydrogenase (LDH) that was either ≥ 3 times of the upper limit of the facility reference range or ≥ 3 SD of the upper limit of the normal range by age group, and hemophagocytosis without malignant findings.

All the samples from the registered patients were further screened using quantitative PCR for EBV and natural killer (NK) cell activity. Finally, only the patients who fulfilled the HLH-2004 criteria [7] were registered in this study. For those who showed low NK activity, age < 2 years, positive central nervous system disease, or low EBV load, FHL genetic screening was performed using flow cytometry and/or Western blotting, followed by genetic confirmation [8, 9]. Patients who had high EBV load (i.e., $\geq 10^{2.5}$ copies/μg DNA in the buffy coat or plasma) were diagnosed as having EBV-HLH [10].

Study population and setting

This study was conducted as a prospective study. Between March 2007 and November 2011, 153 patients diagnosed with possible HLH were registered in the JPLSG. Among them, 90 were registered for the HLH-2004 protocol, and 8 were later excluded for the presence of disqualifying medical conditions. Of the remaining 82 patients who were eligible for this study, 9 were further excluded because of protocol violations ($n = 5$) and no treatment given ($n = 4$), resulting in the final study group comprising 73 patients (per protocol set): 9 with FHL (7 with FHL2 and 2 with FHL3), 41 with EBV-HLH, and 23 with other HLH. HSCT was used for treatment in 17 patients; 6 with EBV-HLH, 7 with FHL, and 4 with other HLH. Two patients with FHL died before HSCT due to disease progression.

Treatment protocol

All 73 patients were treated in accordance with the HLH-2004 protocol [6, 7]. For patients with confirmed FHL or possible FHL having family history of HLH, the initial induction therapy was administered for 8 weeks, followed by continuation therapy until an acceptable donor for HSCT was available. HSCT was also used in those with resistance for the initial therapy or with relapse after treatment. Although a conditioning regimen for HSCT with busulfan, cyclophosphamide, and VP-16 was proposed in the HLH-2004 protocol, the donor source, conditioning regimen, and GVHD prophylaxis varied among the patients according to the treatment institution doctors' decision.

Assessment of complete resolution after initial treatment

After 8 weeks of initial induction therapy, patient condition was judged and defined as complete resolution if all of the following criteria were fulfilled: (1) fever < 37.5 °C; (2) no splenomegaly; (3) no cytopenia (hemoglobin ≥ 90 g/L, platelets ≥ 100,000/μL, and absolute neutrophil count ≥ 500/μL); (4) fasting triglyceride < 265 mg/dL; (5) fibrinogen > 150 mg/dL; (6) ferritin < 500 ng/mL; and (7) no abnormality of cerebrospinal fluid.

Evaluation of adverse events

The signs and symptoms of toxicity were evaluated according to the Common Terminology Criteria for Adverse Events v3.0. In all the patients included in this trial, the following 12 examination items were evaluated at week 8: allergic reaction, hypertension, hypotension, rash desquamation, glucose intolerance, bilirubin, serum transaminases, infection, avascular osteonecrosis, creatinine, glomerular filtration rate, and dyspnea.

Statistical analysis

Cumulative survival curves and 3-year OS rates were calculated according to the Kaplan–Meier method. Baseline values by HLH subtype, age, and proteins in the cerebrospinal fluid at diagnosis were compared among the patients using the Mann–Whitney *U* test. The other clinical manifestations and adverse events were evaluated by the Chi square test. All statistical analyses were performed using EZR, a software program (Saitama Medical Center, Jichi Medical University, Japan) [11]. A p value of < 0.05 was considered statistically significant.

Ethical considerations

This study was approved by the Clinical Research Review Board of the Japanese Society of Pediatric Hematology and

Table 1 Clinical findings of 73 patients with 3 HLH subtypes at diagnosis

	EBV-HLH ($n=41$)	FHL ($n=9$)	Others ($n=23$)	p value
Age (years)				
Median (range)	2.6 (0.8–14.5)	0.2 (0-1.4)	1.5 (0-14.3)	0.011
Sex				
Male:female	20:21	4:5	10:13	0.944
Patient activity				
Normal	8 (19.5%)	2 (22.2%)	1 (4.3%)	0.284
Mild to moderate	19 (46.3%)	3 (33.3%)	9 (39.1%)	
Moderate to severe	14 (34.1%)	4 (44.4%)	13 (56.5%)	
Fever				
> 38.5 °C	40 (97.6%)	9 (100.0%)	23 (100.0%)	1.000
< 38.5 °C	1 (2.4%)	0 (0.0%)	0 (0.0%)	
Hemophagocytosis	40 (97.6%)	8 (88.9%)	18 (78.3%)	0.024
Hepatomegaly	38 (92.7%)	9 (100.0%)	18 (78.3%)	0.167
Splenomegaly	28 (68.3%)	7 (77.8%)	13 (56.5%)	0.463
Neurologic alterations	10 (24.4%)	2 (22.2%)	7 (30.4%)	0.741
Coma	1 (2.4%)	0 (0.0%)	4 (17.4%)	0.048
Protein in cerebrospinal fluid				
Evaluated number of patients	24	8	17	
Median (range)	18 (6–42)	37.5 (14–101)	21 (9–156)	0.018

EBV-HLH Epstein–Barr virus-associated HLH, *FHL* familial HLH, *HLH* hemophagocytic lymphohistiocytosis

Oncology. All researches performed were approved by the independent ethics committee or institutional review board of each participating institution and were in accordance with the principles expressed in the Declaration at Helsinki. Written informed consent was obtained from all participants who were between 16 and 18 years or from the parents or legal guardians of the participants under 16 years.

Results

Clinical and laboratory findings

Table 1 shows clinical characteristics of 73 HLH patients with three different subtypes. Children with FHL were significantly younger than those with EBV-HLH or other subtypes. The frequency of hemophagocytosis in bone marrow was higher in children with EBV-HLH than those with other two subtypes. The frequency of elevated protein in cerebrospinal fluid was higher in those with FHL than in those with others.

Table 2 shows laboratory findings of 73 HLH patients with three different subtypes. Among the 15 parameters, creatinine and LDH were significantly higher and absolute neutrophil count and sodium were significantly lower in patients with EBV-HLH than in those with others. Notably, soluble IL-2 receptor was higher in those with FHL than in those with others.

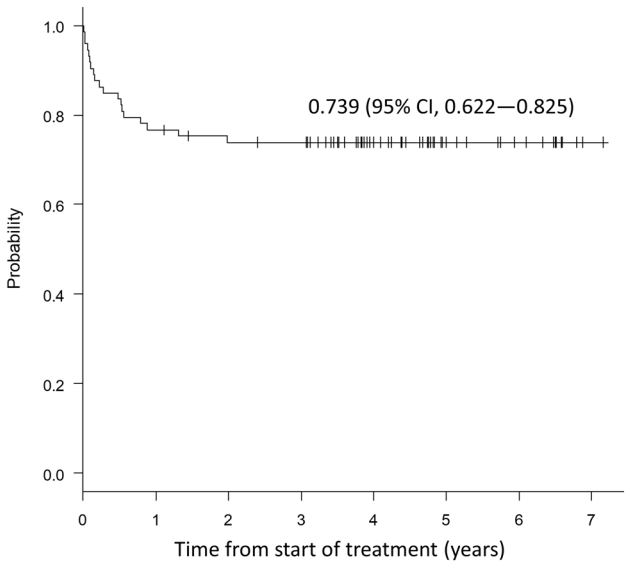

Fig. 1 Overall survival rate of all patients. Overall survival rate of 73 patients treated using the HLH-2004 protocol in Japan

Outcome

The 3-year OS rate was 73.9% (95% CI 62.2–82.5%) for all 73 patients treated with the HLH-2004 protocol. The outcome was significantly different among the patients with EBV-HLH, FHL, and other HLH and the 3-year OS was 85.3% (95% CI 70.2–93.1%), 66.7% (95% CI 28.2–87.8%), and 56.2% (95% CI 33.9–73.6%), respectively (Figs. 1, 2) ($p = 0.0284$). Seventeen patients underwent HSCT. The

Table 2 Laboratory findings of 73 patients with 3 HLH subtypes at diagnosis

Examination	EBV-HLH	FHL	Others	*p* value
ANC ($\times 10^9$/L)	0.51 ± 0.82	0.85 ± 1.69	0.95 ± 2.58	0.021
Hemoglobin (g/L)	84 ± 20	80 ± 13	85 ± 14	0.434
Platelet ($\times 10^9$/L)	31 ± 29	27 ± 23	41 ± 64	0.181
Albumin (g/dL)	2.90 ± 0.52	2.90 ± 0.44	2.75 ± 0.65	0.476
Creatinine (mg/dL)	0.32 ± 0.21	0.24 ± 0.10	0.27 ± 0.20	0.011
AST (U/L)	294 ± 592	143 ± 436	328 ± 1300	0.283
ALT (U/L)	147 ± 592	114 ± 436	167 ± 570	0.686
Total Bilirubin (mg/dL)	1.11 ± 1.70	1.00 ± 5.78	0.70 ± 2.06	0.563
LDH (U/L)	2549 ± 3375	605 ± 1731	2307 ± 3338	0.002
Sodium (mmol/L)	129 ± 5	136 ± 4	133 ± 5	0.004
Triglyceride (mmol/L)	3.37 ± 1.53	1.94 ± 1.33	3.36 ± 2.62	0.069
Ferritin (μg/L)	12568 ± 38840	2266 ± 5021	14200 ± 369852	0.078
Soluble IL2R (U/mL)	14334 ± 16698	25290 ± 10359	8570 ± 10379	0.025
Fibrinogen (g/L)	0.86 ± 0.52	0.97 ± 0.42	1.00 ± 0.84	0.365
APTT (seconds)	44.6 ± 50.3	56.0 ± 45.4	47.3 ± 51.9	0.896

ALT alanine aminotransferase, *ANC* absolute neutrophil count, *APTT* activated partial thromboplastin time, *AST* aspartate aminotransferase, *EBV-HLH* Epstein–Barr virus-associated HLH, *FHL* familial HLH, *HLH*, hemophagocytic lymphohistiocytosis, *IL2R* interleukin-2 receptor, *LDH* lactate dehydrogenase

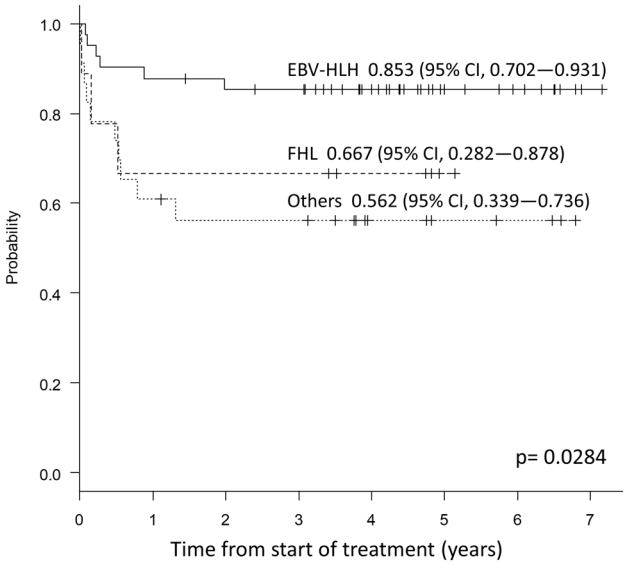

Fig. 2 Overall survival rates by HLH subtype. Difference in overall survival rates according to the HLH subtypes. The numbers of patients based on the HLH subtype are 41 with EBV-HLH, 9 with FHL, and 23 with other HLH. *HLH* hemophagocytic lymphohistiocytosis

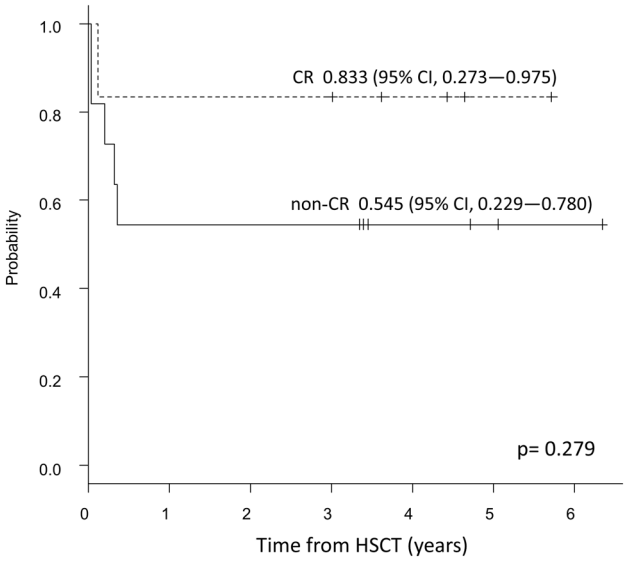

Fig. 3 Overall survival rates by CR before HSCT. Difference in overall survival rates between patients with CR ($n=6$) and with non-CR ($n=11$) before HSCT. *CR* complete resolution, *HSCT* hematopoietic stem cell transplantation

numbers of patients who achieved complete resolution before HSCT were 3 out of 6 for EBV-HLH, 2 out of 7 for FHL, and 1 out of 4 for other causes. Of the 17 patients who underwent HSCT, 14 were evaluable for engraftment, 13 achieved engraftment, and 1 died in the early period after HSCT. The 3-year OS rate of 17 patients who

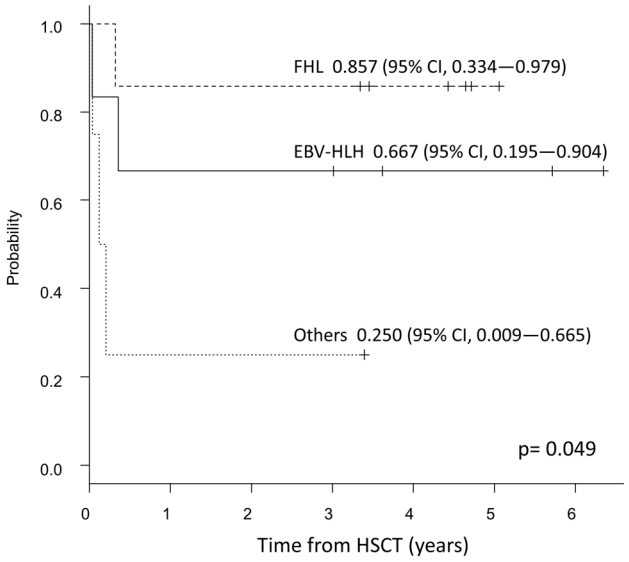

Fig. 4 Overall survival rates of patients who received HSCT according to the HLH subtype. Difference in overall survival rates of patients who received HSCT according to the HLH subtype. The numbers of patients are 6 with EBV-HLH, 7 with FHL, and 4 with other HLH. *HLH* hemophagocytic lymphohistiocytosis, *EBV* Epstein–Barr virus, *FHL* familial HLH, *HSCT* hematopoietic stem-cell transplantation

underwent HSCT was 64.7% (95% CI 37.7–82.3%), and there was no significant difference in the OS rate between patients with and without complete resolution before HSCT (83.3% vs. 54.5%, respectively; $p=0.279$) (Fig. 3). However, the prognosis after HSCT was significantly different among patients with EBV-HLH, FHL, and other HLH (66.7%, 85.7%, and 25.0%, respectively; $p=0.049$) (Fig. 4). There was no statistical difference in terms of patient outcomes according to time from diagnosis to HSCT.

Adverse events

Table 3 shows major adverse events during the 8-week initial treatment. Significance was observed in the elevation of bilirubin and creatinine levels between the three subtypes.

Discussion

According to the results of the Histiocyte Society, the OS was not different between HLH-94 and HLH-2004 studies; however, the mortality rate before HSCT was less in patients treated with HLH-2004 than in those treated with HLH-94 [6]. However, the OS rate for patients registered in Japan was 74% and was relatively better than that reported by the results of the Histiocyte Society. The better OS rate could be

Table 3 Comparison of main adverse events during the treatment of HLH-2004 (initial 8ws) by HLH subtype

Adverse event	Grade	EBV-HLH ($n=41$)	FHL ($n=9$)	Others ($n=23$)	p value
Allergy					
Allergic reaction	0	41	9	23	
Cardiac					
Hypertension	0	27 (65.9%)	6 (66.7%)	17 (73.9%)	0.813
	I	3 (7.3%)	0 (0.0%)	1 (4.3%)	
	II	8 (19.5%)	1 (11.1%)	4 (17.4%)	
	III	3 (7.3%)	2 (22.2%)	1 (4.3%)	
Hypotension	0	38 (92.7%)	8 (88.9%)	19 (82.6%)	0.132
	II	1 (2.4%)	0 (0.0%)	0 (0.0%)	
	III	1 (2.4%)	1 (11.1%)	0 (0.0%)	
	IV	1 (2.4%)	0 (0.0%)	4 (17.4%)	
Dermatology/skin					
Rash desquamation	0	41 (100.0%)	8 (88.9%)	23 (100.0%)	0.123
	II	0 (0.0%)	1 (11.1%)	0 (0.0%)	
Endocrine					
Glucose intolerance	0	37 (90.2%)	9 (100.0%)	19 (82.6%)	0.534
	I	3 (7.3%)	0 (0.0%)	4 (17.4%)	
	II	1 (2.4%)	0 (0.0%)	0 (0.0%)	
Hepatobiliary/liver					
Bilirubin	0–II	33 (80.5%)	7 (77.8%)	19 (82.6%)	0.045
	III	7 (17.1%)	1 (11.1%)	0 (0.0%)	
	IV	1 (2.4%)	1 (11.1%)	4 (17.4%)	
Serum transaminases	0–II	27 (65.9%)	6 (66.7%)	17 (73.9%)	0.084
	III	11 (26.8%)	1 (11.1%)	1 (4.3%)	
	IV	3 (7.3%)	2 (22.2%)	5 (21.7%)	
Infection					
Infection	0	23 (56.1%)	5 (55.6%)	11 (47.8%)	0.223
	I	2 (4.9%)	0 (0.0%)	2 (8.7%)	
	II	8 (19.5%)	0 (0.0%)	3 (13.0%)	
	III	7 (17.1%)	4 (44.4%)	3 (13.0%)	
	IV	1 (2.4%)	0 (0.0%)	4 (17.4%)	
Musculoskeletal					
Avascular osteonecrosis	0	41	9	23	
Renal/kidneys					
Creatinine	0	35 (85.4%)	4 (44.4%)	17 (73.9%)	0.025
	I	6 (14.6%)	4 (44.4%)	4 (17.4%)	
	II	0 (0.0%)	0 (0.0%)	1 (4.3%)	
	III	0 (0.0%)	1 (11.1%)	0 (0.0%)	
	IV	0 (0.0%)	0 (0.0%)	1 (4.3%)	
Glomerular filtration rate	0	37 (90.2%)	8 (88.9%)	17 (73.9%)	0.105
	I	2 (4.9%)	1 (11.1%)	2 (8.7%)	
	II	1 (2.4%)	0 (0.0%)	0 (0.0%)	
	III	1 (2.4%)	0 (0.0%)	0 (0.0%)	
	IV	0 (0.0%)	0 (0.0%)	4 (17.4%)	
Pulmonary					
Dyspnea	0	34 (82.9%)	7 (77.8%)	16 (69.6%)	0.720
	I	0 (0.0%)	0 (0.0%)	1 (4.3%)	
	II	1 (2.4%)	0 (0.0%)	0 (0.0%)	
	III	1 (2.4%)	0 (0.0%)	1 (4.3%)	
	IV	5 (12.2%)	2 (22.2%)	5 (21.7%)	

EBV-HLH Epstein–Barr virus-associated HLH, *FHL* familial HLH, *HLH* hemophagocytic lymphohistiocytosis

attributed to the finding that more than half of the patients in Japan had the EBV-HLH subtype, thus having a better prognosis than that of the other HLH subtypes. In fact, when we analyzed only FHL patients in Japan, the 3-year OS rate was 66.7%, which was almost consistent with the results of the Histiocyte Society [6].

Because the modification of protocol from HLH-94 to HLH-2004 did not improve the treatment outcome dramatically, a certain number of patients with HLH, including those with FHL, might have been resistant to the current treatment strategy. In our analysis, approximately 30% of the patients with HLH were not clearly classified into EBV-HLH or FHL. The international HLH-2004 study indicated that there was a group with poor prognosis despite the use of intensive chemo-immunotherapy, including older children [6]. Although several genetic abnormalities causing FHL have been elucidated [1, 3], other unknown genetic backgrounds or unidentified infections may exist. HLH is, therefore, a heterogeneous disease that can manifest similar symptoms due to various causes, and the response to treatment and prognosis may vary according to the cause of HLH. Since further studies on the causes of HLH can be expected, implementation of a more detailed classification for developing treatment strategies is desirable. Although 2 of 9 patients with FHL died before HSCT, prognosis after HSCT was better in patients with FHL than in those with other HLH subtypes, under the planned treatment strategy. In contrast, prognosis after HSCT was worse in patients with other HLH subtypes than in those with FHL or EBV-HLH. Thus, preparation for HSCT as early as possible may be necessary for patients with other HLH subtypes, particularly when refractory. Novel drugs for HLH, such as ATG, alemtuzumab, tocilizumab, ruxolitinib, and a targeted anti-IFN-γ monoclonal antibody, may also be valuable for refractory HLH patients in the future [6].

EBV-HLH is a common disease affecting children in East Asia, including Japan, whereas it is relatively rare in Europe and North America [1, 2, 4, 12]. The clinical course of EBV-HLH can vary widely depending on the disease severity in each patient. Some patients with EBV-HLH can be cured only with steroids or immunoglobulin [2, 4, 12], whereas others may take a fatal course even with intensive treatment [12–16]. In the present study, all patients with EBV-HLH were treated using the HLH-2004 protocol, with favorable outcomes. Because the severity of EBV-HLH can differ among patients, it seems necessary to examine whether disease control can be obtained with less intensive treatment in each patient. We previously showed that reduction of the EBV copy number during the treatment course is a predictor of EBV-HLH relapse [10]. Therefore, the next strategy for each patient with EBV-HLH should be to classify them into several risk groups according to their response to the initial treatment. As the introduction of rituximab for EBV-HLH treatment has been attempted with success [17–20], the next treatment strategy including rituximab to reduce treatment intensity may avoid late complications [21]. In this study, although we did not routinely examine patient condition with proliferation of latently infected cells, such as chronic active EBV infection, examining infection status might be interesting and helpful for improving prognosis of patients with EBV-HLH.

Tables 1 and 2 show clinical and laboratory findings of 73 patients at diagnosis. Although some differences were found among HLH subtypes, it is not reasonable to conclude that clinical findings at diagnosis are useful to predict the prognosis of each case, with the exception of FHL, which is likely to develop at a younger age [1–3]. In addition, the significant differences in the creatinine levels between the three HLH subtypes may also reflect the prevalence of FHL in the younger patients. We thus recommend that all HLH patients should be immediately treated after diagnosis, in addition to conducting screening tests to identify the etiology of HLH and to determine appropriate therapeutic strategy on the basis of patient condition and response to the ongoing treatment.

In the present study, there were no deaths due to adverse events of the HLH-2004 treatment protocol, and there was no apparent difference in the frequency and severity of adverse events between the three HLH subtypes. Therefore, the HLH-2004 protocol can be safely applied for all HLH subtypes. Although the HLH-2004 protocol has provided favorable outcome for most of the EBV-HLH patients in Japan, some patients might have been improved with less intensive treatment. Therefore, in the future, attempts should be made to reduce the risk of late effects by modifying the treatment intensity according to the response to treatment in each patient. On the other hand, there are intractable cases even with the HLH-2004 protocol. For such cases, further strategies with gradual intensification of treatments may also be necessary based on the treatment response in each case.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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