

Treatment outcomes and prognostic factors in adult patients with secondary hemophagocytic lymphohistiocytosis not associated with malignancy



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ABSTRACT

Hemophagocytic lymphohistiocytosis is an overwhelming systemic inflammatory process that is life-threatening if not treated appropriately. We analyzed prognostic factors in patients with secondary hemophagocytic lymphohistiocytosis excluding malignancy. In this retrospective study, we analyzed 126 adult cases between 2001 and 2017. Treatment was based on dexamethasone with or without etoposide and cyclosporine. Patients who achieved a complete response by 4 weeks were defined as early stable responders, those who failed to achieve a complete response but showed continuous improvement until 8 weeks were defined as late responders, and those whose conditions waxed and waned until 8 weeks were defined as unstable responders. Patients with hemophagocytic lymphohistiocytosis caused by Epstein-Barr virus had a worse 5-year overall survival compared to those whose disease was secondary to autoimmune disease, other infections, or unknown causes (25.1% versus 82.4%, 78.7% and 55.5%, respectively; $P < 0.001$). We observed that the overall response rate at 4 weeks was similar, but decreased at 8 weeks in the Epstein-Barr virus subgroup from 75.5% to 51.0%, and finally decreased to 30.6%. Multivariate analysis revealed that 8-week treatment response was the most relevant factor for overall survival. Excluding 8-week response, the presence of Epstein-Barr virus, old age, hyperferritinemia, and thrombocytopenia were associated with poor survival. We established a prognostic model with the parameters: low-risk (score 0-1), intermediate-risk (score 2), and high-risk (score ≥ 3). These groups had 5-year overall survival rates of 92.1%, 36.8%, and 18.0%, respectively ($P < 0.001$). We found that 8-week treatment response was a good predictor for overall survival, and that Epstein-Barr virus, old age, thrombocytopenia, and hyperferritinemia were associated with poor survival outcomes. Physicians should take care to identify high-risk patients for appropriate treatment strategies.

Introduction

Secondary hemophagocytic lymphohistiocytosis (HLH) without a family history or known genetic predisposition is considered a rare clinical condition and its underlying pathophysiological mechanisms have not yet been well elucidated. The distinction between primary HLH and secondary HLH is not clear because of the emergence of new gene defects, and the diagnosis is difficult because the symptoms and signs of HLH overlap with those of other severe conditions such as sepsis, multi-organ failure, and progression of malignancies or rheumatologic disorders.^{1,2} The incidence and prevalence of HLH may, therefore, be under- or overestimated if clinicians lack suspicion of and specific knowledge about HLH, especially in adult patients.³

HLH is a life-threatening inflammatory disease, and therefore early diagnosis and urgent treatment, including dexamethasone, cyclosporine, and etoposide, are

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important for survival.^{4,6} However, even when specific therapies are administered promptly, treatment response and overall survival rates remain poor, especially when the condition is associated with malignancies.^{7,8} Poor survival and frequent relapse are common not only in patients with malignancy-associated HLH, but also in those with active Epstein-Barr virus (EBV) infection and in some high-risk patients with HLH of unknown cause.⁹⁻¹¹ For relapsed or refractory HLH patients, several salvage treatments¹²⁻¹⁸ followed by allogeneic hematopoietic cell transplantation (HCT) are now considered,^{19,21} but many of these patients die of rapid deterioration due to severe sepsis and multi-organ failure. It is, therefore, important for physicians to identify high-risk patients earlier in the course of treatment, and to apply second-line therapy and prepare the patient immediately for allogeneic HCT. However, there are few data identifying relevant prognostic factors and enabling risk-stratification for adult patients with secondary HLH.^{8,11}

In this study, we initially analyzed treatment response and survival outcomes among 126 adult patients with non-malignancy-associated secondary HLH according to the underlying causes of the disease. We then assessed factors predictive of treatment response and survival outcome. Finally, we constructed a prognostic model using risk-stratification according to predictive factors in order to identify high-risk patients who should be considered for early second-line therapies, including salvage regimens and allogeneic HCT.

Methods

Patients

We retrospectively reviewed records for 165 adult patients who were diagnosed with HLH from 2001 to 2017. All patients pre-

sented with fever, some criteria of HLH2004,^{5,22} and increased histiocytosis with active hemophagocytosis in bone marrow. Of these 165 patients, we excluded 12 who did not satisfy the full diagnostic criteria and 27 with malignancy-associated HLH (Figure 1 and *Online Supplementary Methods* for Identification of the causes of HLH). Ultimately, we analyzed 126 patients (median age 45 years, range 15-85 years) with non-malignancy-associated secondary HLH due to several causes. This research was conducted in accordance with Institutional Review Board and Ethics Committee guidelines of the Catholic Medical Center (KC15RISI0863) and the principles of the Declaration of Helsinki.

Parameters associated with hemophagocytic lymphohistiocytosis

All parameters included in the diagnostic guidelines for HLH were reviewed: fever, splenomegaly, cytopenia affecting at least two lineages, triglycerides, fibrinogen, ferritin, and natural killer (NK)-cell cytotoxic activity. Laboratory findings were serially accessed and the lowest levels were captured for complete blood cell counts, fibrinogen, albumin, and prothrombin time before treatment. The highest levels within 4 weeks after initial treatment were used for ferritin, aspartate aminotransferase, alanine aminotransferase, total bilirubin, lactate dehydrogenase, and triglycerides in order to assess clinical outcomes. We have analyzed NK-cell cytotoxicity by a flow cytometry-based assay since 2012.²³ We did not check levels of soluble CD25. Thus, the majority of patients enrolled in the current study satisfied at least five out of six criteria for the diagnosis of HLH, excluding the soluble CD25 and NK cytotoxicity criteria. An association with EBV was evaluated by both serology and EBV DNA real-time quantitative polymerase chain reaction (RQ-PCR) analysis (See the *Online Supplementary Methods* for Identification of the causes of HLH).

Treatments

Treatment strategies were based on HLH-94 protocols, which consisted mainly of dexamethasone, etoposide, and additional

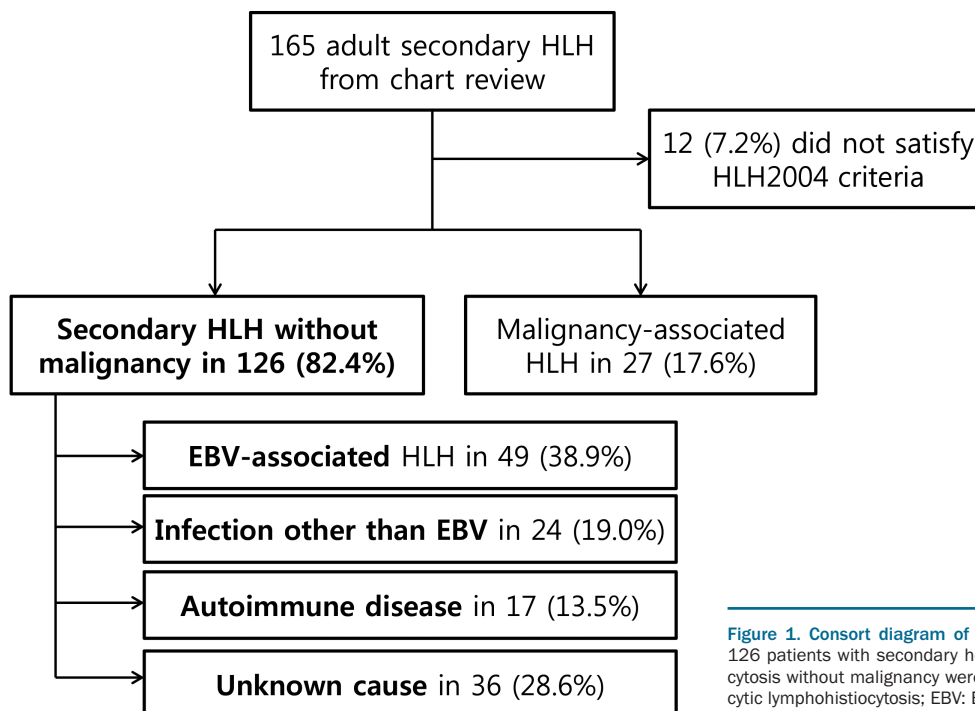


Figure 1. Consort diagram of enrolled patients. A total of 126 patients with secondary hemophagocytic lymphohistiocytosis without malignancy were enrolled. HLH: hemophagocytic lymphohistiocytosis; EBV: Epstein-Barr virus.

cyclosporine.^{5,6,24} Final treatment response was evaluated after 8 weeks of induction according to the HLH-94 protocol. Patients exhibiting a complete response at 8 weeks were given maintenance therapy with low-dose steroids and cyclosporine for 2 to 4 more weeks, while patients who did not achieve a complete response or who were positive for EBV according to RQ-PCR (> 3-log) were considered for continuation therapy. If the EBV RQ-PCR level increased by at least 1-log with features of relapsing HLH, rituximab or alemtuzumab was given. Five patients who relapsed or had refractory disease were treated with allogeneic HCT using fludarabine (30 mg/m²/day for 5 days), busulfan (3.2 mg/kg/day for 2 days), and antithymocyte globulin (ATG, Thymoglobulin®, 2.5 mg/kg for 2 days).

Response assessment

Complete response was defined as resolution of all clinical signs and symptoms, as well as recovery of the complete blood count and normalization of abnormal laboratory findings associated with HLH. Progressive disease was identified when both cytopenia and abnormal laboratory findings remained and partial response was defined when patients achieved either complete blood count recovery alone without normalization of laboratory findings or improvement of HLH-related laboratory findings alone without complete blood count recovery. We evaluated treatment response at 4 and 8 weeks after treatment, and also evaluated dynamic responses according to the response time and disease progression within 8 weeks. Patients with stable complete response at both 4 weeks and 8 weeks were classified as early stable responders, patients who failed to achieve a complete response by 4 weeks but showed continuous response until 8 weeks were classified as late stable responders, patients who showed only transient response and eventually progressed until 8 weeks were defined as unstable responders, and patients exhibiting no response at all were classified as non-responders. Details on the statistical analysis are provided in the *Online Supplementary Methods*.

Results

Baseline characteristics of the patients

The causes of HLH in the 126 patients enrolled in this study were categorized as EBV-associated (n=49), infection other than EBV (n=24), and autoimmune disease (n=17). Among the 49 cases of EBV-associated HLH, five were concomitantly related with an autoimmune disease. We were unable to identify related causes in 36 (28.6%) patients (Figure 1). The baseline characteristics of the patients and their responses to treatment are presented in Table 1. Fever was observed in all patients and 118 (93.6%) patients presented with splenomegaly. Infections other than EBV included hepatitis A virus (n=5), cytomegalovirus (n=2), *Staphylococcus aureus* (n=3), mycoplasma (n=3), parvovirus (n=3), human immunodeficiency virus (n=2), mycobacterium (n=2), malaria (n=2), amebiasis (n=1), anaplasmosis (n=1), Hantaan virus (n=1), herpes simplex virus (n=1), influenza virus (n=1), and enterococcus (n=1). Patients with Kaposi sarcoma caused by human immunodeficiency virus were considered to have malignancy-associated HLH. Autoimmune diseases and similar features included systemic lupus erythematosus (n=8), Kikuchi disease (n=6), Behçet syndrome (n=2), Sjögren syndrome (n=2), Graves disease (n=1), rheumatoid arthritis (n=1), autoimmune hemolytic anemia (n=1), and ulcerative colitis (n=1). Among the 35 EBV-associated

HLH patients with available serology results, seven showed a recent primary infection, five reactivation, and 23 a past infection. In addition, initial bone marrow findings revealed that 36 out of 49 patients (73.5%) with EBV-associated HLH had active and frequent hemophagocytosis, while 17 out of the remaining 77 patients (22.1%) showed active hemophagocytosis.

Treatment outcomes according to causes of hemophagocytic lymphohistiocytosis

After excluding four patients (2 with hepatitis A and an unknown cause of HLH who showed a self-limiting disease course, and 2 cases with progressive multi-organ failure before treatment), 122 patients were treated. The HLH-94 protocol was applied in 81 (64.3%) patients: 23 (63.8%) with HLH of unknown cause, 40 (81.6%) with EBV-HLH, 13 (54.2%) with an infection other than EBV, and five (29.4%) with an autoimmune disease). Dexamethasone plus cyclosporine treatment was admin-

Table 1. Baseline characteristics and treatment outcome of patients with non-malignancy-associated secondary HLH (n=126).

Gender, male; n(%)	64 (50.8%)
Age, years; median (range)	45.0 (15-85)
Time from symptom to diagnosis, days; median (range)	13 (0-82)
Fever; n(%)	126 (100%)
Splenomegaly; n(%)	118 (93.6%)
Lowest level prior to therapy; median (range)	
Leukocytes (x10 ⁹ /L)	2.39 (0.14-31.9)
Neutrophils (x10 ⁹ /L)	1.08 (0.01-24.1)
Hemoglobin (g/dL)	9.3 (3.7-16.3)
Platelets (x10 ⁹ /L)	44.0 (5-307)
Fibrinogen (mg/dL)	215.0 (4-513)
Albumin (g/dL)	2.8 (1.7-4.1)
Prothrombin time (%)	69.0 (10-145)
Highest level within 4 weeks after treatment; median (range)	
Ferritin (ng/mL)	4702 (278-214000)
Aspartate aminotransferase (U/L)	196 (12-4990)
Alanine aminotransferase (U/L)	134 (5-5117)
Total bilirubin (mg/dL)	1.17 (0.07-19.9)
Lactate dehydrogenase (U/L)	1670 (285-30000)
Triglyceride (mg/dL)	173.5 (7-813)
C-reactive protein (mg/dL)	4.9 (0.02-34.0)
Causes of HLH; n(%)	
EBV-associated	49 (38.9%)
Median highest level (log)	4.5 (2.7-7.5)
≥ 5.0-log	20 (41.0%)
< 5.0-log	29 (59.0%)
Infection other than EBV	24 (19.0%)
Autoimmune disease	17 (13.5%)
Unknown cause	36 (28.6%)
Treated patients; n(%)	122 (96.8%)
HLH94-based therapy	81 (64.3%)
Steroid + cyclosporine	18 (14.3%)
Steroid alone	23 (18.2%)
Complete remission achieved after treatments; n(%)	81 (64.3%)
Treatment response at 8 weeks; n(%)	
Early stable responder	42 (34.4%)
Late stable responder	36 (29.5%)
Unstable responder	19 (15.6%)
Non-responder	25 (20.5%)

HLH: hemophagocytic lymphohistiocytosis; EBV: Epstein-Barr virus.

istered to 18 (14.3%) patients, while 23 (18.2%) patients were given dexamethasone alone. Patients who had mild symptoms and improved rapidly were treated with dexamethasone alone and further therapy was not needed. In contrast, we were unable to administer cyclosporine and etoposide to patients with significant deterioration of organ function or a poor general condition. Of the 81 (64.3%) patients who achieved a complete response after treatments, 43 achieved this response by 4 weeks after treatment and the other 38 achieved it by 8 weeks or later. Of the 27 patients who relapsed, one relapsed between 4 and 8 weeks and 26 relapsed after 8 weeks during the course of treatment. After relapsing, most of the patients were treated with the HLH-94 protocol again. For relapsed EBV-HLH, we considered rituximab (n=6) or alemtuzumab (n=2) concomitantly, and two patients were treated with allogeneic HCT.

With regards to dynamic responses, 42 (34.4%) patients were early stable responders, 36 (29.5%) were late stable responders, 19 (15.6%) were unstable responders, and 25 (20.5%) were primary refractory non-responders (Table 1). Detailed responses according to causes of HLH are as follows. In patients with EBV-HLH, the overall response rate (complete plus partial responses) at 4 weeks was 75.5% (complete response in 16, partial response in 21) but decreased to 51.0% (complete response in 17, partial response in 8) at 8 weeks, and finally decreased to 30.6% (complete response in 15). The overall response rates at 4 weeks and 8 weeks for patients with HLH associated with infections other than EBV were 62.5% (complete response in 10, partial response in 5) and 75% (complete response in 14, partial response in 4), respectively, for those with autoimmune diseases, 82.3% (complete response in 9, partial response in 5) and 82.3% (complete response in 12, partial response in 2), respectively, and for those with HLH of unknown cause, 66.7% (complete response in 8, partial response in 16) and 66.7% (complete response in 19, partial response in 5), respectively. Patients with HLH associated with an autoimmune disease or infection other than EBV had superior 5-year overall survival rates (82.4% and 78.7%, respectively) and lower cumulative incidences

of progression (29.4% and 22.7%, respectively) compared to those of patients with EBV-associated HLH. The estimated 5-year overall survival rate and cumulative incidence of progression of patients with EBV-associated HLH were 25.1% and 77.5%, respectively (Figure 2).

Survival outcomes according to treatment response

Estimated 5-year overall survival rates of patients who achieved a complete response by 4 weeks (n=43) and 8 weeks (n=62) were 82.8% and 82.7%, respectively, while the overall survival rate of patients who achieved a partial response until 8 weeks (n=19) was 68.4% (Figure 3A,B). As we recognized four subgroups defined according to dynamic treatment response, there were 42 patients in the early stable responder group, 36 in the late stable responder group, 19 in the unstable responder group, and 25 in the non-responder group. Figure 3C,D presents the overall survival and cumulative incidence of progression curves of patients divided according to dynamic treatment response. Early stable responders and late stable responders had 5-year overall survival rates of 80.7% and 73.7%, respectively, and 5-year cumulative incidences of progression of 20.2% and 43.1%, respectively, while almost all patients in the unstable and non-responder groups died of disease progression (P<0.001).

Prognostic factors and risk stratification

We found that most parameters associated with HLH disease activity were predictive of survival outcomes, and many were correlated with each other. Age was correlated with albumin and alanine aminotransferase levels, EBV-association with triglyceride and fibrinogen levels, anemia with albumin levels, thrombocytopenia with prothrombin time, total bilirubin and erythrocyte sedimentation rate, and ferritin with prothrombin time, fibrinogen, erythrocyte sedimentation rate, and lactate dehydrogenase levels. Multivariate analyses were performed using age, EBV-association, thrombocytopenia, ferritin, and dynamic treatment response after excluding correlated parameters (Table 2). Remaining an unstable responder or non-responder at 8 weeks was the most powerful predictive

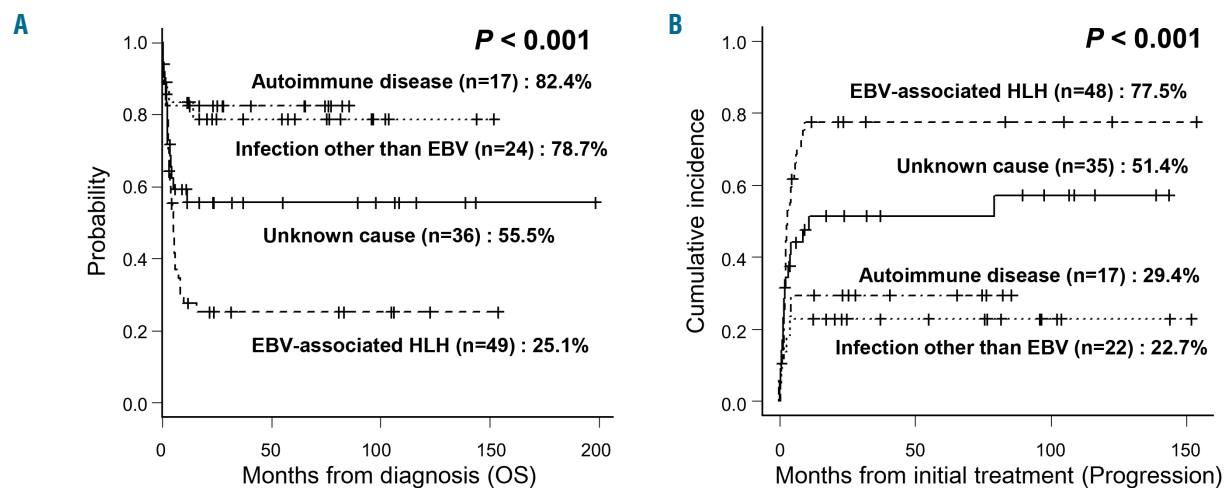


Figure 2. Survival outcomes according to the cause of secondary hemophagocytic lymphohistiocytosis. (A) Comparison of 5-year overall survival rates according to the cause of secondary hemophagocytic lymphohistiocytosis. (B) Comparison of cumulative incidences of progression after treatment according to the cause of secondary hemophagocytic lymphohistiocytosis. OS: overall survival; HLH: hemophagocytic lymphohistiocytosis; EBV: Epstein-Barr virus.

factor for poor overall survival; age >45 years and low platelet counts ($<35 \times 10^9/L$) were also significant predictive factors for poor overall survival. When we excluded treatment response as a parameter, age >45 years, EBV-association, thrombocytopenia (platelets $<35 \times 10^9/L$), and hyperferritinemia ($>20,000$ ng/mL) were associated with poor overall survival. All of these factors were also associated with higher cumulative incidences of progression and poor 8-week response (*data not shown*). We ultimately selected four factors and weighted them based on the hazard ratio: age >45 years (2 points), EBV-association (1 point), low platelet count ($<35 \times 10^9/L$) (1 point), and hyperferritinemia ($>20,000$ ng/mL) (1 point). Using these variables, we performed risk scoring and stratification. The 5-year overall survival rates of low-risk (score 0-1), intermediate-risk (score 2), and poor-risk (score ≥ 3) patients were 92.1%, 36.8%, and 18.0%, respectively, while the corresponding 5-year cumulative incidences of progression were 18.5%, 59.4% and 87.0% (both $P < 0.001$) (Figure 4A,B). In the EBV-HLH subgroup, the 5-year overall survival rate was 59.2% in early stable responders, 44.4% in the late responders, and 0.0% in non-responders.

Multivariate analysis in the EBV-HLH subgroup revealed that maximal EBV RQ-PCR level greater than 5-log, age >45 years, and hyperbilirubinemia were associated with poor overall survival

Discussion

Causes of HLH and the proportions of each identified in the current study differed from those of previous studies, in which the most common cause of secondary HLH was a hematologic malignancy.^{3,7,8,25} Some patients with malignancy-associated HLH die early from rapid deterioration without a suspicion of HLH and their prognosis depends on the outcome of the malignant disease. In the current study we, therefore, analyzed treatment response and prognostic factors for adult secondary HLH after excluding those with malignancy-associated HLH. To the best of our knowledge, this is the largest data set used to analyze prognostic factors in adult patients with secondary HLH without malignancies.

Excluding malignancies, EBV was the most common

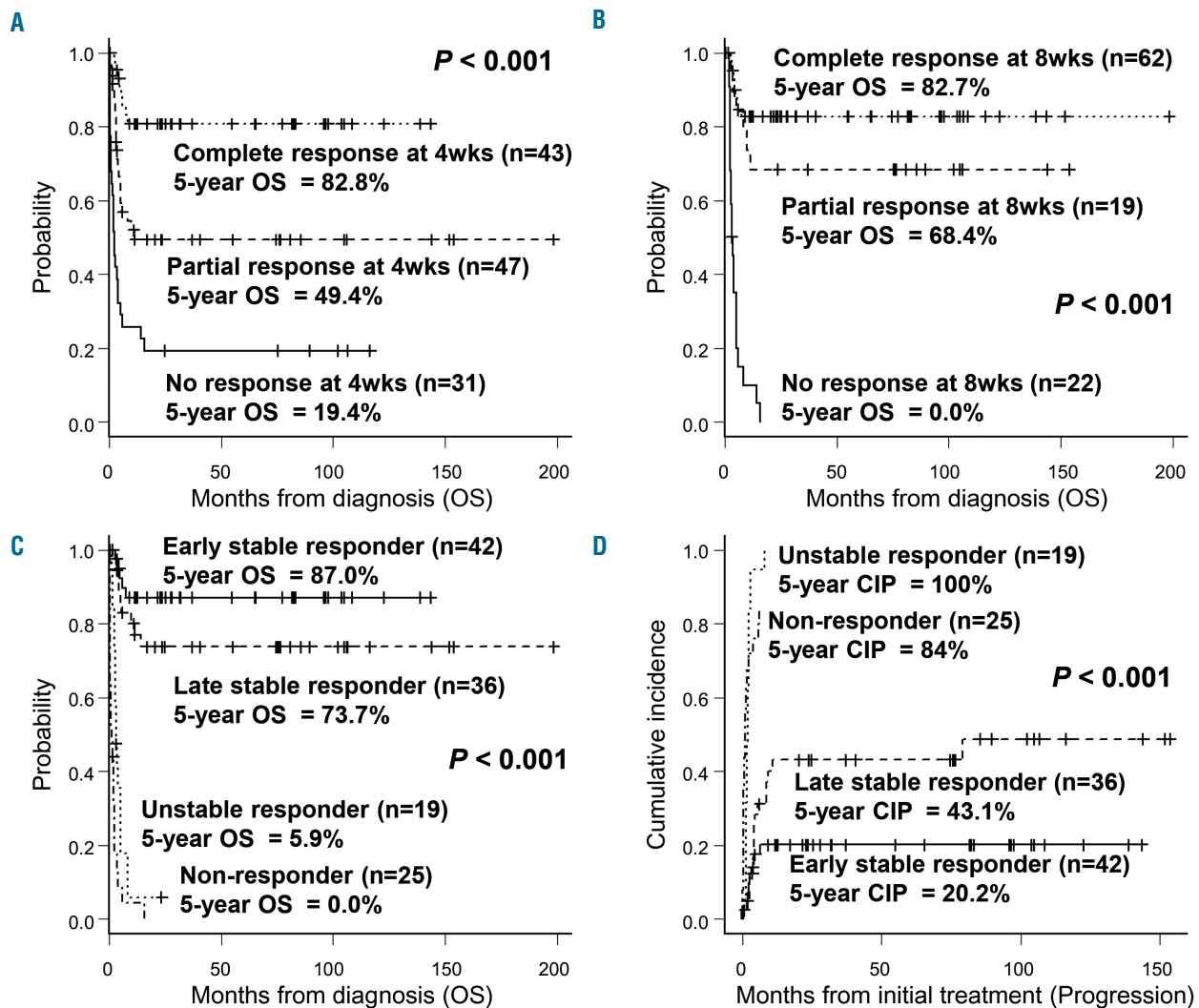


Figure 3. Survival outcomes according to treatment response. (A, B) Five-year overall survival according to treatment response at (A) 4 weeks and (B) 8 weeks. (C, D). Five-year (C) overall survival and (D) cumulative incidence progression according to dynamic treatment response at 8 weeks. OS: overall survival; CIP: cumulative incidence of progression.

cause of secondary HLH in this study. The prognosis of patients with EBV-associated HLH is expected to be better than that of those with malignancies or primary HLH, but if patients are not treated appropriately, their survival outcomes are dismal.^{26,27} Our data revealed that patients with EBV-associated HLH had similar responses to treatment in the early period but many relapsed afterward, which finally resulted in these patients having significantly inferior overall survivals and higher cumulative incidences of progression compared to patients with HLH related to other causes. It is, therefore, important to decide on early salvage treatment, including allogeneic HCT, for poor responders with EBV-associated HLH. Previous reports suggested a role of acyclovir in the management of EBV-associated disease,^{28,29} but no relevant data are available on the additional effects of antiviral therapy.⁹ Salvage treatments include rituximab^{12,17,30} and alemtuzumab³¹ followed by allogeneic HCT.^{32,33} Although we used rituximab in six patients and alemtuzumab in two in the setting of relapsed disease, none of the patients showed a response to additional therapy suggesting that incorporation of rituximab into the HLH protocol might be an alternative approach as initial treatment in EBV-associated HLH. Of the three patients with relapsed/refractory EBV-HLH treated with allogeneic HCT, one died of relapse, one died of septic pneumonia, and one is alive without relapse.

We calculated survival outcomes of responders and non-responders at 4 weeks and 8 weeks, respectively, and

found that patients who did not achieve at least a stable partial response at 8 weeks had very high mortality. A recent prospective study investigating the effects of a salvage regimen using liposomal doxorubicin applied salvage chemotherapy for refractory patients who were defined as non-responsive to initial therapy at 2 weeks.¹⁸ However, our data show that patients who failed to achieve a complete response by 4 weeks after treatment could still achieve long-term survival if they exhibited a continuous and stable response until 8 weeks. The HLH-94 protocol recommends continuation therapy when disease is active at 8 weeks after initial therapy, and our study population included patients who proceeded to continuation therapy. Among our 21 patients who were given continuation therapy, 16 additional patients ultimately achieved a complete response and two of these patients were treated with allogeneic HCT. There are no data comparing survival between patients receiving continuation therapy and other salvage regimens, or on the proper timing for allogeneic HCT, especially in patients with EBV-HLH. However, as our data indicate that the course of EBV-HLH after relapse is very progressive, urgent preparation for allogeneic HCT is recommended. We also identified that the risk of relapse among patients with EBV-HLH was higher in those with an age >45 years, maximal EBV RQ-PCR higher than 5-log, and hyperbilirubinemia.

Apart from non-responders at 8 weeks, who have a particularly poor outcome, we further identified high-risk

Table 2. Univariate and multivariate analyses for parameters affecting overall survival in non-malignancy-associated HLH.

	Univariate analysis		Multivariate analysis			
	5-year OS	P	Treatment response included HR	P	Treatment response excluded HR	P
Age > 45 years old** (n=61, 49.4%)	26.8% (vs. 74.9%)	< 0.001*	2.54 (1.4-4.7)	0.003*	3.38 (1.9-6.1)	< 0.001*
EBV-associated** (n=49, 45.9%)	25.1% (vs. 69.3%)	< 0.001*			2.16 (1.2-3.8)	0.006*
Hemoglobin <9.0 g/dL (n=47, 42.5%)	38.7% (vs. 75.6%)	< 0.001*				
Platelet count <35 x10 ⁹ /L ** (n=47, 36.8%)	32.8% (vs. 64.7%)	< 0.001*	1.99 (1.1-3.5)	0.016*	2.20 (1.3-3.8)	0.004*
Alanine aminotransferase >165 U/L (n=55, 32.2%)	67.7% (vs. 40.6%)	0.0055*				
Bilirubin >1.6 mg/dL (n=51, 43.7%)	30.1% (vs. 68.5%)	< 0.001*				
Albumin <3.0 g/dL (n=76, 49.4%)	34.2% (vs. 80.8%)	< 0.001*				
Triglycerides >200 mg/dL (n=50, 43.7%)	38.1% (vs. 61.4%)	0.0256*				
Ferritin >20,000 ng/mL ** (n=26, 50.6%)	23.1% (vs. 60.1%)	< 0.001*			1.99 (1.1-3.6)	0.021*
Fibrinogen <150 mg/dL (n=45, 35.6%)	33.4% (vs. 63.3%)	0.002*				
Dynamic treatment response**						
Early stable responder (n=42)	87.0%	< 0.001*	1.0			
Late responder (n=36)	73.7%		1.73 (0.6-5.2)	0.326		
Unstable or non-responder (n=44)	0.0%		17.3 (6.5-45.9)	< 0.001*		

OS: overall survival; HLH: hemophagocytic lymphohistiocytosis; HR: hazard ratio; EBV: Epstein-Barr virus; *P < 0.05. **Variables used in multivariate analyses were selected after excluding significant correlations: age, EBV-association, thrombocytopenia, hyperferritinemia, dynamic treatment response

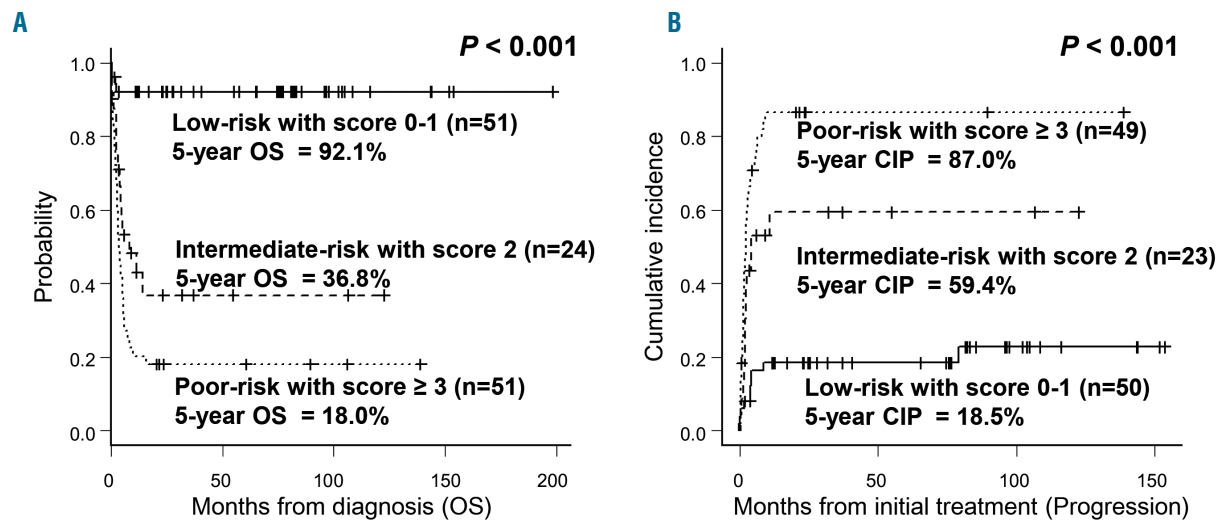


Figure 4. Survival outcomes according to hemophagocytic lymphohistiocytosis risk score. (A) Overall survival according to risk score. (B) Cumulative incidence of progression according to risk score. Low risk (score 0-1), intermediate risk (score 2), high risk (score ≥ 3). OS: overall survival; CIP: cumulative incidence of progression.

patients according to variables such as age, thrombocytopenia, EBV-association, and high ferritin level, which were significant factors for poor overall survival in multivariate analysis. As we observed a high probability of relapse or refractory disease in high-risk patients, early intervention with salvage therapy and allogeneic HCT should be considered in selected patients. In contrast to high-risk or relapsed patients, we also observed patients who showed good responses to initial steroid therapy alone. Response to steroid alone was expected in some patients with an infection other than EBV, an autoimmune disease, or HLH of unknown cause. We may, therefore, consider delaying etoposide in some HLH patients without EBV and those with an autoimmune disease.

Although the distinction between primary and secondary HLH is obvious, several genetic abnormalities found in adult patients support the hypothesis that many cases of secondary HLH have an underlying genetic predisposition.³⁴⁻³⁶ This issue will remain challenging unless we can assess patients for the presence of genetic defects in every suspicious case, and more genes associated with cytotoxic function may be identified in the future.³⁷ We were unable to check for all possible genetic abnormalities, except for some patients with negative results for *UNC13D* and *PRF1* mutations, and we lack reference data regarding gene defects in adult patients. We, therefore, hypothesized that all enrolled patients without gene study data were cases of secondary HLH unless they did not satisfy HLH-2004 criteria.⁵ In addition, all patients enrolled in this study were diagnosed based on increased histiocytosis

with active hemophagocytosis in a bone marrow study, although previous reports showed that 30% of HLH cases may not present with hemophagocytosis in an initial bone marrow study.^{3,38} Conversely, severely ill patients not satisfying the diagnostic criteria for HLH sometimes present with bone marrow hemophagocytosis.³⁹ As we initially identified HLH patients based on bone marrow results, it is possible that we missed some HLH patients without evidence of bone marrow hemophagocytosis or who did not undergo a bone marrow examination.

HLH is a rare disease for which well-organized prospective studies are lacking: analysis of prognostic factors for secondary HLH is, therefore, difficult and few data are available for risk-stratification. Although our data originated from a retrospective study, patients were consistently treated and managed, and the number of patients was sufficient for the final analysis. As we excluded cases of malignancy-associated HLH, treatment response to protocols and survival outcomes were relevant for risk-stratification. Our prognostic risk model should be validated by larger studies. Combinations of several predictive factors at diagnosis and 8-week treatment response can allow clinicians to identify patients at high risk of disease progression, and second-line therapy including allogeneic HCT should be considered earlier in the course of treatment.

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