Original Article

JHSMR Journal of Health Science and Medical Research

Prognostic Factors of Early Death in Childhood Hemophagocytic Lymphohistiocytosis: Experience From A Single Tertiary Center in Thailand

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Received 18 November 2021 ● Revised 26 April 2022 ● Accepted 29 April 2022 ● Published online 30 May 2022

Abstract:

Objective: To establish the clinical profile, outcomes, and risk factors of mortality in pediatric hemophagocytic lymphohistiocytosis (HLH) patients admitted to a tertiary care hospital in the south of Thailand.

Material and Methods: The medical records of HLH patients aged under 15 years were retrospectively reviewed. Survival times were estimated using the Kaplan–Meier estimator. Univariate associations between covariates and survival were visualized with graphs and compared using the log–rank test. Factors with statistical significance in the univariate analysis were included in a multivariate cox regression analysis.

Results: A total of 24 childhood HLH cases were identified over the 20–year study period. Central nervous system (CNS) involvement at diagnosis (hazard ratio [HR]: 7.7, 95% CI: 1.2–51.2), absolute neutrophil count (ANC) <1,000.0 cells/μL (HR: 33.3, 95% CI: 2.7–39.8) and renal insufficiency (HR: 28.2, 95% CI: 2.1–373.8) were adverse risk factors. Forty–six percent of the children survived at least 30 days after diagnosis with a median survival time of 21 days.

Conclusion: CNS involvement, low ANC and renal insufficiency at diagnosis were adverse risk factors for early death in HLH children.

Key words: hemophagocytic lymphohistiocytosis, prognostic factors, survival outcome

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J Health Sci Med Res .....
doi: 10.31584/jhsmr.2022880
www.jhsmr.org

Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a rare condition, with reported incidences ranging from 1.2 to 10 per 1,000,000 children per year. It is a frequently fatal disorder of immune regulation.[1-3] HLH comprises two types[1-4]: primary HLH or familial hemophagocytic lymphohistiocytosis (FHL), and secondary HLH (sHLH). FHL is a potentially lethal disease with a median survival of fewer than two months after diagnosis, with typical onset during infancy and early childhood.[4] Secondary HLH (sHLH) develops due to strong immunological activation of the immune system caused by a severe infection, malignancy or autoimmune disorder.[1,2,4] The currently used diagnostic criteria and treatment of HLH are based on the HLH-2004 treatment protocol.[4]

Previous studies have identified many different factors correlated with the survival rates of HLH patients. In a large cohort study of the treatment outcomes of HLH patients during 1994-1998 from 21 countries[4], there were no significant factors associated with survival outcomes. More recent retrospective studies[1,5-8] have identified different prognostic factors for estimating survival outcomes, notably early age of diagnosis, central nervous system (CNS) involvement, low albumin, high bilirubin, hypertriglyceridemia, hypoferritinemia, and coagulopathy.

According to data from the HLH-94 trial[9], the majority of HLH deaths occur during initial therapy, especially during the first month, indicating different phases of the disease may have different risks of death. Thus, we hypothesized that identifying risk factors in the early stage of HLH would be important in tailoring the appropriate treatment and reducing mortality.

There have been to date few studies examining survival outcomes and prognostic factors of early mortality in HLH children. The objective of this study was to establish the clinical profile, outcomes, and risk factors of mortality in pediatric HLH patients treated in a tertiary care hospital in the south of Thailand.

Material and Methods

We retrospectively reviewed the hematological records of all children aged under 15 years diagnosed with HLH between January 2000 and December 2019 at the Hematologic Clinic, Department of Pediatrics, Faculty of Medicine, Prince of Songkla University, the major tertiary care institution and referral center in southern Thailand. Patients are referred here for investigation, treatment, and follow-up for HLH. The information recorded for each child included sex, age, onset of symptoms, clinical manifestations at diagnosis (including fever, hepatosplenomegaly, lymphadenopathy and CNS involvement), laboratory investigations at diagnosis (including complete blood count, renal function, liver function, ferritin, triglycerides, fibrinogen), pathology from bone marrow aspiration, evidence of infection and cause of HLH. CNS involvement was defined as having neurological symptoms or an elevated white blood cell count (WBC) in the cerebrospinal fluid.

For analysis, each laboratory investigation at diagnosis was divided at the normal cut-off point. Low absolute neutrophil count (ANC), anemia and thrombocytopenia at diagnosis were defined as <1,000.0 cells per microliter (cells/μL), <9.0 grams per deciliter (g/dL) and <100,000.0 cells/μL, respectively, following the HLH-2004 criteria for cytopenia. Hyper direct bilirubinemia was defined as serum direct bilirubinemia >1.0 milligrams per deciliter (mg/dL), high alanine transaminase (ALT) was defined as ALT >40.0 units per liter (U/L), high alkaline phosphatase (ALP) was defined as ALP >150.0 units per deciliter (U/dL), hypoalbuminemia was defined as albumin <4.0 g/dL, coagulopathy was defined as international normalized ratio (INR) >1.5, hyperferritinemia was defined as serum ferritin >500.0 ng/mL, hypertriglyceridemia was defined as serum triglycerides (TG) >265.0 mg/dL, hypofibrinogenemia was defined as serum fibrinogen <150.0 mg/dL, and renal insufficiency was defined as creatinine clearance less than 90.0 milliliters per minute per 1.7 meters

squared (mL/min/1.73 m^2), using Schwartz's formula.[10] Suspected Epstein-Barr virus (EBV) infections were confirmed serologically from initial or previous exposure to EBV (elevated viral capsid antigen IgM or IgG) and/or a positive human herpes virus serology test. The overall survival (OS) was defined as time from diagnosis to death or last follow-up. The prognostic factors of death are presented as hazard ratios (HRs) with 95% confidence intervals (CI).

Descriptive data are presented as means and standard errors (S.D.) or medians and interquartile ranges (IQR) for continuous outcomes and numbers and percentages (%) for categorical outcomes. Survival times were estimated using the Kaplan-Meier estimator. Univariate associations between covariates and survival are visualized with graphs and compared using the log-rank test. Factors with statistical significance in the univariate analysis were included in the multivariate cox regression analysis. A p-value less than 0.050 was considered significant. The data were analyzed using R program version 4.1.0.

Results

Over the 20-year period, a total of 29 children diagnosed with HLH were identified. We excluded five children who had incomplete data, so a total of 24 children remained in the analysis. Of these, 12 were infection-associated, 1 was malignancy-associated (MAHS), 1 had rheumatologic-associated disease and 10 were idiopathic HLH. No patients had a family history of HLH and no consanguinity was reported. Of the 24, 11 (45.8%) were alive and 13 (54.2%) had died by 30 days after diagnosis, all deaths from infection. The median age at diagnosis was 2.1 years (IQR 0.3-7.3 years). The percentages of males and females were 54.1 and 45.9%, respectively. The presentations were fever 100%, splenomegaly 79.2%, hepatomegaly 95.8%, lymphadenopathy 41.7%, and CNS involvement 12.5%. All three children with CNS involvement had clinically significant changes in consciousness. Laboratory investigations at diagnosis found low ANC in 33.3% of the patients, anemia in 95.8%, thrombocytopenia in 95.8%, hyper direct bilirubinemia in 58.3%, high ALT in 91.7%, high ALP in 79.2%, hypoalbuminemia in 95.8%, coagulopathy in 20.0%, hyperferritinemia in 95.2%, hypertriglyceridemia in 86.4%, hypofibrinogenemia in 72.7%, and renal insufficiency in 17.4%. Evidence of infection, including EBV infection, was found in 40.9%, with dengue infection in 4.2% and mycoplasma infection in 8.3%. The demographic characteristics, clinical presentations and initial laboratory investigations of the study group at the time of diagnosis are presented in Table 1.

Table 1 Demographic and clinical characteristics at initial HLH diagnosis of the 24 study children

Clinical characteristics at diagnosis	Number (%)
Fever	24 (100)
Splenomegaly	19 (79.2)
Hepatomegaly	23 (95.8)
Lymphadenopathy	10 (41.7)
CNS involvement	3 (12.5)
Laboratory investigations at diagnosis	
Low absolute neutrophil count (<1,000 cells/L)	8 (33.3)
Low hemoglobin level (<9 g/dL)	23 (95.8)
Low platelet count (<100,000 cells/L)	23 (95.8)
Hyper direct-bilirubinemia (>1 mg/dL)	14 (58.3)
High alanine transaminase (>40 U/L)	22 (91.7)
High alkaline phosphatase (>150 U/L)	19 (79.2)
Hypoalbuminemia (<4 g/dL)	23 (95.8)
Coagulopathy (INR >1.5)	4 (20.0)
Hyperferritinemia (>500 ng/mL)	20 (95.2)
Hypertriglyceridemia (>265 mg/dL)	19 (86.4)
Hypofibrinogenemia (<150 mg/dL)	16 (72.7)
Renal insufficiency (CrCl <90 mL/min/1.73 m^2)	4 (17.4)
Evidence of EBV infection	9 (40.9)

CNS=central nervous system, CrCl=creatinine clearance, EBV=Epstein-Barr virus, INR=international normalized ratio

Forty-six percent (11/24) of the children survived at least 30 days after diagnosis with an overall median survival time of 21 days. A Kaplan-Meier curve showing the overall survival time after diagnosis of HLH is presented in Figure 1.

Univariate analysis identified 4 clinical parameters that were statistically significant with inferior 30-day OS: CNS involvement at diagnosis (HR: 3.7, 95% CI: 1.01-14.00), ANC at diagnosis less than 1,000.0 cells/μL (HR: 4.8, 95% CI: 1.50-14.50), hyper direct bilirubin more than 1.0 mg/dL (HR: 5.3, 95% CI: 1.20-24.00), and renal insufficiency at diagnosis (HR: 3.3, 95% CI: 1.01-11.00). A summary of the clinical variables based on the univariate analysis is presented in Table 2. Other clinical and laboratory investigation variables including fever, hepatosplenomegaly, lymphadenopathy, hemoglobin, platelet count, ALT, ALP, albumin, INR, ferritin, triglycerides, fibrinogen, and evidence of EBV infection had no associations with survival outcome.

The adverse factors identified in multivariate analysis for predicting survival outcome of HLH are shown in Table 3. They were CNS involvement at diagnosis (HR: 7.7, 95% CI:1.2-51.2), ANC <1,000.0 cells/μL (HR: 33.3, 95% CI:2.7-39.8) and renal insufficiency (HR: 28.2, 95% CI: 2.1-373.8).

The 30-day OS rates were significantly different between patients with and without CNS involvement (52.0% VS 0%; p-value=0.036; Figure 2), ANC or <1,000 cells/μL (62.0% VS 12.0%; p-value=0.001; Figure 3), and patients with and without renal insufficiency (53.0%VS 0.0%; p-value=0.034; Figure 4).

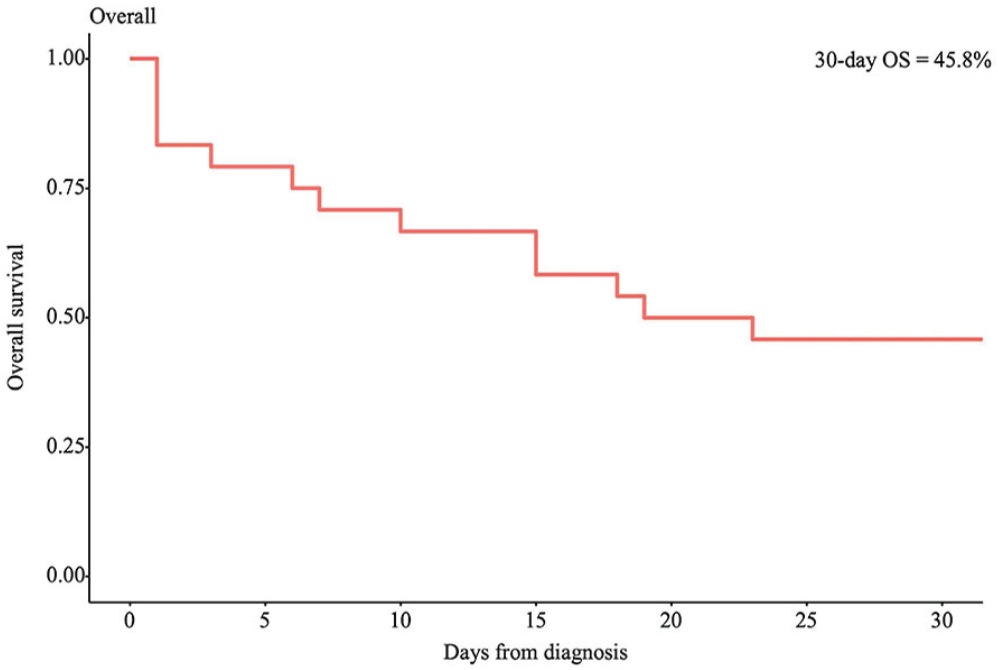


Figure 1 Kaplan-Meier curve of 30-day overall survival of the 24 study children

Table 2 Univariate Cox-regression analysis showing hazard factors of early mortality

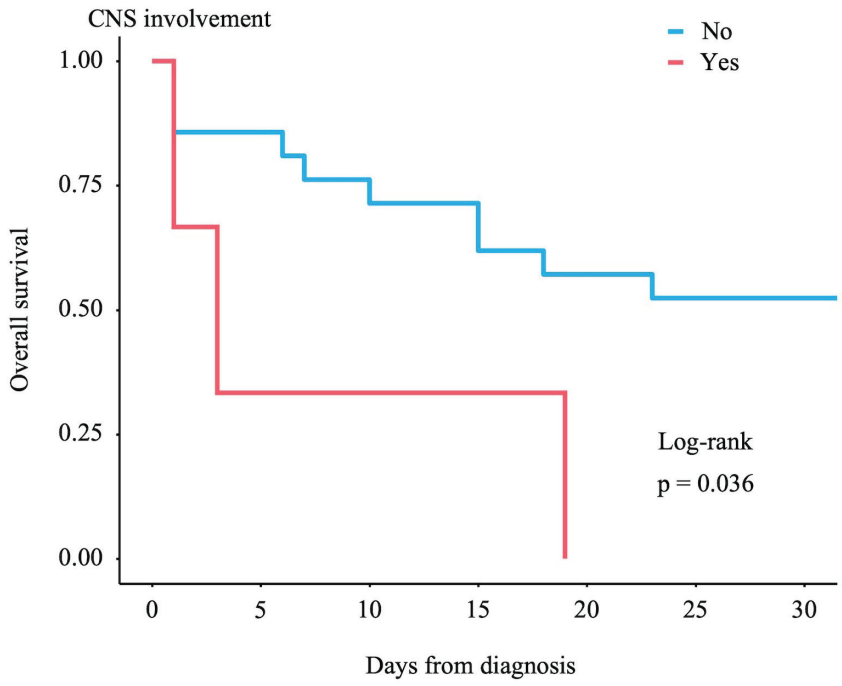
Risk factor	HR (95% CI)	p-value
Splenomegaly		
No	ref	0.35
Yes	2 (0.45-9.30)	
Lymphadenopathy		
No	ref	0.95
Yes	0.97 (0.32-30.00)	
CNS involvement		
No	ref	0.05
Yes	3.7 (1.01-14.00)	
Absolute neutrophil count (cells/μL)		
≥1,000	ref	0.008
<1,000	4.8 (1.50-14.50)	
Direct bilirubin(mg/dL)		
≤1	ref	0.03
>1	5.3 (1.20-24.00)	
Alanine transaminase (U/L)		
≤40	ref	0.88
>40	1.2 (0.15-9.00)	
Alkaline phosphatase (U/L)		
≤150	ref	0.83
>150	0.87 (0.24-3.20)	
Albumin (g/dL)		
<4	ref	0.59
≥4	1.7 (0.22-14.00)	
INR		
≤1.5	ref	0.13
>1.5	2.8 (0.73-11.00)	
Ferritin (ng/mL)		
≤500	ref	0.65
>500	0.29 (0.03-2.50)	
Triglycerides (mg/dL)		
≤265	ref	0.95
>265	0.7 (0.15-3.30)	
Fibrinogen (mg/dL)		
<150	ref	0.12
≥150	0.19 (0.02-1.50)	
Renal insufficiency (CrCl <90 mL/min/1.73 m^2)		
No	ref	0.05
Yes	3.3 (1.01-11.00)	
Evidence of EBV infection		
No	ref	0.65
Yes	0.75 (0.22-2.60)	

CNS=central nervous system, CrCl=creatinine clearance, EBV=Epstein-Barr virus, INR=international normalized ratio, ref=referent group

Table 3 Multivariate Cox-regression analysis showing hazard factors of early mortality

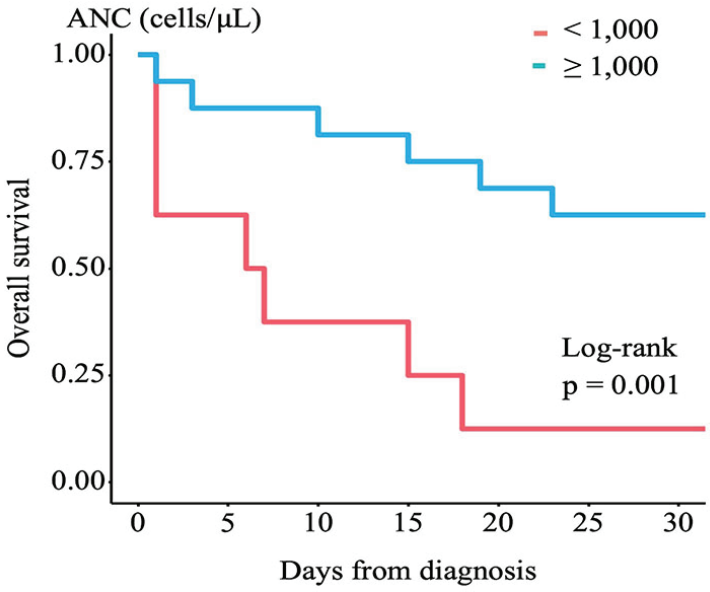
Risk factor	HR (95% CI)	p-value
CNS involvement		
No	ref	0.040
Yes	7.7 (1.2–51.2)	
Absolute neutrophil count (cells/μL)		
≥1,000	ref	0.006
<1,000	33.3 (2.7–39.8)	
Renal insufficiency (CrCl <90 mL/min/1.73 m^2)		
No	ref	0.010
Yes	28.2 (2.1–373.8)	

CNS=central nervous system, CrCl=creatinine clearance, ref=referent group



CNS=central nervous system

Figure 2 Kaplan-Meier curve of patients with and without CNS involvement at 30 days after diagnosis



ANC=absikyte neutrophil count

Figure 3 Kaplan–Meier curve of patients with ANC or <1,000 cells/μL by 30 days after diagnosis

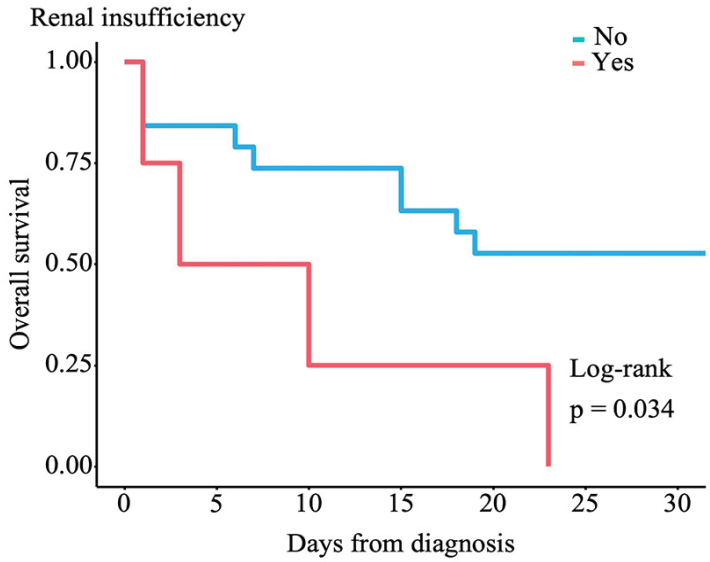


Figure 4 Kaplan–Meier curve of patients with and without renal insufficiency by 30 days after diagnosis

Discussion

Our study covered a 20-year period and included a total of 24 HLH children. We used the survival outcome level 30 days after diagnosis because previous studies reported that the majority of deaths occurred in the first month after diagnosis.[1,5,9] We believe that clinical manifestations and laboratory investigations in the first month are the most helpful predictors that should be assessed upon diagnosis, allowing early intensive treatment and guiding the clinician's evaluation of the outcome of HLH children.

The 30-day OS rate of the HLH children in our study was 45.8%, which is lower than the 30-day OS rates in two recent studies[1,5] of 86.0% and 83.2%. There are two factors which we believe can explain our lower rate. First, because our center is the major referral center in the south of Thailand, we get cases from other hospitals so their disease is already more progressed when we see them, leading to lower survival. Second, all HLH children in our study died from infections, while in the other studies most deaths were from HLH-related causes.[1,5] In our institution in a developing country during the study period, we were faced with various limitations concerning intensive treatment such as lack of a positive pressure room and modern antibiotic drugs.

In multivariate analysis, three factors were associated with poorer survival in our study. The first was CNS involvement at diagnosis, which was also identified as significant in recent studies.[3,6,7] The incidence of CNS involvement in our patients was lower than in other studies[6,11,12] which reported 20.0-75.0% CNS involvement, and all three patients in this study who had CNS involvement at diagnosis died within 30 days. The second was ANC less than 1,000.0 cells/μL, again as in other studies.[1,13] Patients with leukopenia are prone to develop severe opportunistic infections and we found all 13 patients who had early death in this study had severe infections, including pneumonia and sepsis, so early empirical antibiotics and use of a positive pressure room may improve outcomes. And we also found that renal insufficiency at diagnosis was related to poorer survival, a factor which was not reported in previous studies. These earlier studies[8,14] evaluated the use of creatinine level as a survival predictor, and found this was not a significant factor, but our study used creatinine clearance which depends on the age and height of the patient. The importance of creatinine clearance for clinicians is its usefulness in predicting HLH outcome.

EBV infection was found in 40.9% of our patients, similar to studies conducted in other East Asian countries such as China, Korea, and Japan[5,7,12,14,17] which reported EBV in 25.0-70.0% of HLH children. Most recent studies[3,7,15-17] have not found a connection between EBV infection and survival outcome of HLH children. However, one study from Japan[14] found children with an EBV infection had a higher 3-year OS than children who did not have an EBV infection. In our study, the survival outcomes were not statistically different between patients with and without EBV-HLH.

Our study had some limitations. First, this was a single center retrospective study. Second, gene sequencing was not performed in our patients, so the proportion of FHL patients was uncertain. Third, inflammatory markers analysis was not performed. Thus, multi-center prospective study with a larger number of cases, gene sequencing and inflammatory marker testing is needed to give more concrete findings.

Conclusion

CNS involvement, low absolute neutrophil count and renal insufficiency at diagnosis were independent risk factors for early death in HLH children.

Acknowledgement

We would like to thank Mr. Dave Patterson of the Office of International Affairs, Faculty of Medicine, Prince of Songkla University, for his English editing.

Conflict of interest

This research did not receive any specific grants from any funding agencies in the public, commercial, or not-for-profit sectors.

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