Viral Etiology, Clinical, and Laboratory Features of Adult Hemophagocytic Lymphohistiocytosis

Jinghong Chen,1,2 Xuehua Wang,3 Ping He,2 Yazhen Li,2 Mengya Si,2 Zhichen Fan,2 Xiaolan Chang,2 Qindong Xie,2 and Xiaoyang Jiao2*

1Shantou Center for Disease Control and Prevention, Shantou, Guangdong, China
2Cell Biology and Genetics Department of Shantou University Medical College, Shantou, Guangdong, China
3Hospital of Shantou University Medical College, Shantou, Guangdong, China

Secondary hemophagocytic lymphohistiocytosis (SHLH) is a potentially fatal hyperinflammatory syndrome with a heterogeneous etiology and has nonspecific clinical and laboratory findings. The diagnosis and treatment of adult SHLH is challenging because the etiology of the disease is difficult to identify, and the majority of reported cases are pediatric patients. The aim of this study was to describe the etiology, clinical characteristics, and outcomes of adult SHLH. Fifty-four adult patients who fulfilled the criteria of SHLH were enrolled in the study. Viral etiology, blood biomarkers, and clinical manifestations of SHLH were analyzed in these patients. Twenty-four SHLH patients had viraemia, whereas 30 SHLH patients were secondary to other diseases. Epstein-Barr virus (EBV) was the most common virus that associated SHLH among all viruses studied. Severe SHLH patients with EBV-viraemia presented significantly high levels of ferritin, lactate dehydrogenase, aspartate transaminase (AST), and alanine transaminase (ALT). Positively relationships existed between EBV DNA titers and levels of AST and ALT (P < 0.05). The prognosis of SHLH patients with EBV viraemia was worse than that of non-EBV SHLH and non-viral SHLH. Our data reveal that EBV is the major pathogen in virus-associated SHLH, and EBV load influence disease development in SHLH patients with EBV infection that prognosis is worse than other viruses associated SHLH. J. Med. Virol. 88:541–549, 2016. © 2015 Wiley Periodicals, Inc.

KEY WORDS: hemophagocytic lymphohistiocytosis; Epstein-Barr virus; virus

INTRODUCTION

Hemophagocytic lymphohistiocytosis (HLH) is characterized by the activation of the mononuclear phagocytic system, histiocyte proliferation, and hemophagocytosis. In the clinic, HLH is a rare but potentially fatal hyperinflammatory syndrome, the typical findings of which are high fever, hepatosplenomegaly, cytopenia, hypertriglyceridemia, hyperferritinemia, hypofibrinogenemia, and hemophagocytosis in bone marrow (BM) or other organs [Henter et al., 2007]. The diagnosis of HLH in adults is challenging, not only because HLH occurs in many disease entities and results in symptoms similar to these diseases, but also because the etiology cannot be identified in many patients. The heterogeneous etiology and nonspecific clinical and laboratory findings of HLH often lead to delayed diagnosis or misdiagnosis, and thus, the mortality rate resulting from this disease is quite high.

HLH may be inherited (i.e., primary or familial, generally occurring in infants) or secondary to other diseases. Contrary to primary HLH, secondary HLH (SHLH) can occur at any age, which is associated with a wide spectrum of underlying conditions. [Park et al., 2012]. Infection as the main trigger of SHLH constitutes 50.4% of reported cases, which include viral, bacterial, fungal, and parasitic infections. Presently, viral infection is the most frequent trigger of SHLH, either as a primary infection in healthy people or reactivated infections in patients with immunosuppressed diseases [Ramos-Casals et al., 2014]. Viral infections may trigger SHLH by lymphocytes activation or by proinflammatory cytokines secretion. In some countries, viruses are the most common...
infectious agents associated with SHLH, followed by bacteria, parasites, and fungi [Tib et al., 2000; Deane et al., 2010]. Because some subtypes of SHLH confer a higher mortality rate than those seen in primary HLH [Rouphael et al., 2007], a thorough investigation of underlying etiology is beneficial for the proper diagnosis and timely treatment of the disease.

SHLH has a variety of prognoses ranging from mild to life-threatening. The natural course of SHLH can be rapidly fatal within a few days or weeks. The patient’s clinical manifestations, disease course, and treatment response are affected not only by SHLH itself, but also by its possible underlying conditions [Park, Kim et al., 2012]. Until now, the formal guideline for evaluating patients with infection-associated SHLH has not been established [Fisman, 2000]. Although many viruses, such as Epstein-Barr virus (EBV), human herpes virus 8 (HHV8), cytomegalovirus (CMV), influenza virus, hepatitis virus, and parvovirus B19 (PVB19), have been identified as triggers of SHLH [Fardet et al., 2003; Henter et al., 2006; Yilmaz et al., 2006; Rouphael, Talati et al., 2007; Titze et al., 2009; Demircioglu et al., 2013], many previous studies were based on sporadic case reports. In EBV-associated SHLH, a high viral DNA load has been associated with poor outcomes [Rouphael, Talati et al., 2007]. Direct molecular virologic assays may help for better detection of this potentially underdiagnosed disease, although diagnosis after considering all the viruses associated with SHLH would not be practical currently.

In order to elucidate the relationship between viral infection and incidence of SHLH, patients with SHLH were selected for this study, and viruses that are frequently epidemic in the local area were measured in patients’ serum samples. Viral etiology, demographic, clinical features, and outcomes of SHLH patients were analyzed.

METHODS

The study protocol was approved by the Shantou University Medical College Ethics Committee, and informed consents were obtained from each subject prior to our study.

Patients

All patients fulfilled five out of eight criteria for SHLH: (1) fever >38.5˚C; (2) splenomegaly; (3) cytopenia of two or more cell types: absolute neutrophil count (ANC) < 1 x 10^9/L, hemoglobin level < 90 g/L, and/or platelet count < 100 x 10^9/L; (4) hypertriglyceridemia (triglyceride level ≥ 3.0 mmol/L) or hypofibrinogenemia (fibrinogen [FIB] level ≤ 1.50 g/L); (5) hemophagocytosis in the BM (SHLH’s typically feature is activated macrophages with engulfed leukocytes, erythrocytes or their precursor cells, and platelets); (6) hyperferritinemia (ferritin level, ≥ 500 μg/L); (7) elevated soluble CD25 (interleukin-2Ra chain ≥ 2400 IU/ml), and (8) low or absent natural killer (NK) cell cytotoxicity. All patients were adults with no family history of HLH.

Laboratory tests included a complete blood count (CBC); blood cell morphology; C-reactive protein (CRP); serum IgG, IgM, and IgA. Biochemical markers included aspartate transaminase (AST), alanine transaminase (ALT), gamma-glutamyl transpeptidase (GGT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), FIB, ferritin, triglycerides, and cholesterol. In order to distinguish between patients with infections and other malignant diseases, BM aspiration and tumor biomarker tests were performed in all patients. Except for lymphoma and leukemia, no solid tumors were found in our patients.

Patients were treated according to the HLH-2004 protocol, which consists of initial therapy with dexamethasone (10 mg/m² per day), etoposide (150 mg/m² twice weekly), and cyclosporine, followed by dexamethasone intravenous pulses [Henter, Horne et al., 2007].

Pathogen Measurement

To identify the viral etiology of SHLH, viruses that were hyperendemic epidemic infection in local area were measured. These included EBV; influenza virus (Flu A, FluB); parainfluenza virus types 1–4 (hPIV-1, -2, -3, and -4); respiratory syncytial virus (RSV); adenovirus (ADV); coronavirus (OC43, 229E, NL63, and HKU1); human metapneumovirus (MPV); bocavirus (HBoV); rhinovirus (HRV); human papillomavirus (HPV); herpes simplex virus-1 (HSV-1); and parvovirus B19 (PVB19). Human immunodeficiency virus (HIV) was also measured. All viruses were detected using quantitative real-time polymerase chain reaction (Q-PCR) on the plasma samples. DNA/RNA extraction and Real-time polymerase chain reaction was according to the manufacture’s instructions (QIAgen, Hilden, Germany). DNA was extracted from serum using the QIAamp DNA Blood Mini Kit, and RNA was extracted using the QIAamp Viral RNA Mini Kit. The highly purified DNA was amplified using the PCR Kit (Qiagen), which includes viurs-specific primers. DNA was detected by Real-time PCR using the Path-IDTM qPCR Master Mix (Ambion, Carlsbad, CA) with a program consisting of one cycle of 95˚C for 10 min, followed by 40 cycles of 95˚C for 15 sec, 60˚C for 60 sec. RNA was detected using the AgPath-IDTM One-Step RT-PCR Kit (Ambion, Carlsbad) with a program consisting of one cycle of 45˚C for 10 min, 95˚C for 10 min, followed by 40 cycles of 95˚C for 15 sec, 60˚C for 45 sec. In order to confirm the co-infection, all of them were examined using specific primers and probes. The reaction was performed in ABI 7500-Fast system (Applied Biosystems). Primers were provided by the Center for Disease Control and Prevention of Guangdong province. Purified water was used as a negative control. Viral load was expressed as number of EBV DNA copies/ml. The presence of a viral load supported the diagnosis of virus-associated SHLH.

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Below is primers of virus that detected from SHLH patients with viremia:

- **HSV-1**: Primers Forward 5’-TTCGACTTTGCCGACCTGTG-3’, Reverse 5’-CAGGGAGGCTGGTCGAAG-3’
  - Probe FAM 5’-AGCATCATTCCAGCCACAACCTGTG-3’
- **PIV-1**: Primers Forward 5’-GCTGAAGCTGAGACTTGTTCTTCAATT-3’, Reverse 5’-GATGAGAGTCCTCAACATTC-3’
  - Probe: FAM 5’-CTTCCTGGCGCTCTGATGC-3’
- **EBV**: Primers Forward 5’-AGCATCATCCAGGCCCACAACCTGTG-3’, Reverse 5’-CTCGATTGTCTGAAAAAYTGC-3’
  - Probe: FAM 5’-GAGCASGAGCCCCCTCCTTACATTGGTT-3’
- **MPV**: Primers Forward 5’-TGAGGCTCTTCCATCAGTAC-3’, Reverse 5’-TTCGACTTTGCCGACCTGTG-3’
  - Probe: FAM 5’-CTGCATTGTCTGAAAAYTGCCG-3’

In order to exclude other infectious factors that could induce SHLH, other pathogens were measured. These included *Chlamydia* species, *Rickettsia* species, *Orientia tsutsugamushi*, *Campylobacter*, *Fusobacterium*, *Mycoplasma*, *Legionella*, *typhoid*, *Brucella*, Lyme’s disease, and tick-borne encephalitis (TBE).

Anaplasma phagocytophilum, *Ehrlichia chaffeensis*, and *Borrelia burgdorferi* s.l. None of these pathogens were detected in the patients.

### Statistical Analysis

Continuous variables were reported as medians with interquartile ranges (IQRs). The Kruskal-Wallis H test, Mann–Whitney U test, Wilcoxon signed-rank test, and χ² test were used to evaluate differences among each group. Event-free survival (EFS) was calculated from the date of complete remission until the first event (i.e., relapse or death from any cause).

Survival curves were calculated using the Kaplan–Meier method, and survival comparisons were made using Mantel’s log-rank test. Statistical analyses were performed using SPSS version 13.0 (SPSS Inc., Chicago, IL). A P value < 0.05 was considered to be statistically significant.

### RESULTS

#### Viral Etiology of SHLH

This study included 54 adult patients with SHLH; of these, 24 patients were identified as having viremia. Among all viruses measured, EBV was the most common virus (seen in 14 of 24 patients) among patients with viral SHLH. Previous EBV infection can be confirmed with an antibodies test. Accordingly, we analyses EBV antibodies in patients with positive EBV viremia. Our data showed that the antibody is positive in all patients with EBV viremia, indicating these patients having a previous EBV infection.

The next most common virus was HSV-1 (five cases). There were two cases of co-infection with EBV and HSV-1, two cases of MPV, and one case of hPIV-1. Thirty patients with SHLH did not find vireaemia. We then divided the 54 patients into two groups: negative vireaemia and positive vireaemia. In 24 cases of positive vireaemia, there were 10 server cases, while in 30 cases of negative vireaemia, there were eight server cases, more severe cases fell in the viral group than in non-viral group (incidence: 41.6% vs. 26.6%, respectively). Among 10 severe SHLH patients with vireaemia, eight were induced by EBV, and two cases were induced by EBV and HSV-1 co-infection. Among 30 cases of non-viral SHLH, the primary etiologies were lymphoma (six cases), pulmonary infection (nine cases), immune thrombocytopenia (four cases), and alcoholic hepatitis (three cases). Four cases of SHLH were induced by Still’s disease, encephalitis, lymphnoditis, and diabetes (one case each). Four cases were induced by myelodysplasia, myeloid proliferation disorders, multiple myeloma, and acute leukemia (one case each).

#### Clinical Manifestations and Laboratory Biomarkers in SHLH Patients

In this study, patients experienced various clinical presentations. High fever presented in all cases, especially in severe ones, with a mean temperature of 39.5°C (range: 38.9–40.5°C). SHLH patients’ body temperature was significantly higher in severe cases than in mild cases. In severe patients, the illness was rapidly fatal within a few days or weeks, and the majority of patients had to be transferred into intensive care unit for treatment. Mortality rate was 37.5% in viral patients and 20.6% in non-viral SHLH patients.

In order to compare the differences between viral and non-viral cases of SHLH, as well as severe and non-severe SHLH cases, patients were divided into four groups: severe (or fatal) cases with positive or negative vireaemia, and non-severe cases with positive or negative vireaemia. Accordingly, the demographics, clinical characteristics, and lab data were analyzed. No difference was observed in age among the four groups (range: 18–85 years). Male patients were more common than female patients, accounting for 70.4%. The laboratory biomarkers for patients of four groups were presented in (Table I).

Very high ferritin levels (≥1500 ng/ml) were observed in patients with virus-associated SHLH (both severe and non-severe), which could jump by up to 1500 ng/ml (the upper limit of measurement) in 70% of severe and 50% of non-severe virus-associated SHLH cases. In severe patients, ferritin levels were higher in the positive than in negative viraemia group, though the difference did not reach statistically significant (P > 0.05). In negative viraemia group, ferritin levels in severe cases were significantly higher than in the mild cases (P < 0.01).
### Comparative Laboratory Data for EBV- and Non-EBV SHLH

The laboratory data for SHLH patients with EBV- and non-EBV viraemia were shown in (Table II). The levels of ferritin, LDH, AST, ALT, and GGT in the EBV-SHLH group were significantly higher than those in the non-EBV-SHLH group ($P < 0.05$). Liver enzymes, especially AST and ALT, were significantly higher in patients with severe SHLH than in non-severe SHLH, regardless of etiology ($P < 0.01$). AST and ALT in patients with viraemia were significantly higher than in non-viraemia group ($P < 0.05$). Furthermore, in non-severe cases of SHLH, both viral and non-viral, AST and ALT levels remained at lower levels. Our data reveal that AST and ALT are biomarkers reflecting diseases severe status in SHLH. CRP levels were significantly higher in severe SHLH cases than in non-severe cases, in both viral and non-viral SHLH ($P < 0.01$). However, the amplitude of variation of CRP is not significantly higher than the ferritin, indicating ferritin may be a more sensitive marker than CRP in reflecting infectious status in SHLH. Our data revealed that serum ferritin, LDH, AST, and ALT are valuable biomarkers reflecting disease severity of SHLH.

Serum FIB levels were within normal limits in non-severe SHLH, whereas FIB was lower in severe than in non-severe SHL patients with viraemia ($P < 0.05$). In non-viral SHLH, no difference was observed in FIB levels between severe and non-severe groups. Cholesterol levels were normal in all patients. Triglycerides were significantly higher in the severe SHLH group than in the non-severe SHLH group, but only in the non-viral subgroup, a difference was statistically significant ($P < 0.05$).

Peripheral blood and BM samples were obtained from patients at the time of disease onset. Pancytopenia was found in all severe-SHLH patients. White blood cells (WBCs) and platelets reduced significantly in all patients. The nadir WBC and platelet were found in severe viral SHLH. Hemophagocytosis was found in the BM of all patients: enlarged histiocytes, engulfing red blood cells, granulocytes, and lymphocytes, engulfing plasma cells could be observed occasionally. Hemophagocytosis consisted mainly of erythropagocytosis (Fig. 1a,b). Cytological findings revealed hypercellular BM with no evidence of malignancy (except in a few patients with acute leukemia, myelodysplasia, myeloid proliferation disorders, and lymphoma). Normal erythropoiesis, granulopoiesis, and maturing megakaryopoiesis were found in all patients. In patients with positive viraemia, increased lymphocytes were observed in blood and BM.

### Table I. Clinical and Lab Parameters of Four SHLH Groups

| Parameter | A (EBV-SHLH group) | B (non-EBV SHLH group) | C (EBV-SHLH group with virus negative) | D (non-EBV SHLH group with virus negative) |
|-----------|--------------------|------------------------|----------------------------------------|---------------------------------------------|
| Gender (M/F) | 7/3 (23.75–33.75) | 11/3 (34.75–25.00) | 6/2 (29.25–75.25) | 14/8 (25.00–77.50) |
| Age (years) | 64.00 (23.50–77.25) | 53.50 (23.75–60.25) | 65.00 (63.25–75.25) | 49.50 (24.75–71.25) |
| Fe (ng/ml) | 1500.00 (1125.75–1500.00) | 1500.00 (758.50–1500.00) | 1180.50 (578.00–1500.00) | 371.00 (216.75–920.25) |
| WBC (10^9/L) | 2.96 (1.86–10.46) | 4.71 (2.80–7.69) | 11.74 (6.01–14.35) | 5.04 (2.66–9.62) |
| HB (g/L) | 86.50 (80.75–102.25) | 103.00 (78.75–135.50) | 85.50 (63.50–113.75) | 103.50 (81.00–121.25) |
| LDH (U/L) | 1620.00 (752.75–2668.00) | 360.00 (190.25–694.50) | 842.00 (317.50–1194.50) | 139.00 (36.25–300.75) |
| AST (U/L) | 374.50 (151.25–536.75) | 31.00 (22.25–112.75) | 36.50 (21.75–84.38) | 68.00 (12.00–103.50) |
| ALT (U/L) | 193.00 (65.50–218.50) | 35.50 (16.75–104.75) | 53.00 (30.00–233.00) | 29.50 (15.75–94.75) |
| GGT (U/L) | 150.00 (53.75–266.50) | 31.50 (20.25–76.25) | 115.00 (82.75–165.75) | 93.50 (67.50–143.00) |
| ALP (U/L) | 184.00 (105.75–361.50) | 64.00 (48.25–122.00) | 183.00 (105.75–361.50) | 64.00 (48.25–122.00) |
| IgG (g/L) | 10.60 (10.29–11.85) | 9.73 (7.27–13.50) | 12.05 (8.93–17.80) | 13.90 (11.83–18.20) |
| IgM (g/L) | 0.80 (0.39–1.01) | 1.22 (0.62–1.94) | 1.79 (0.94–2.60) | 1.75 (1.38–2.62) |
| IgA (g/L) | 1.75 (1.38–2.62) | 1.79 (0.94–2.60) | 2.58 (1.05–3.63) | 2.44 (1.66–4.20) |
| FIB (g/L) | 1.83 (0.95–2.48) | 2.63 (1.81–4.44) | 2.65 (0.97–3.61) | 2.32 (1.87–3.45) |

A, severe SHLH group with virus positive; B, non-severe SHLH group with virus positive; C, severe SHLH group with virus negative; D, non-severe SHLH group with virus negative.
Immunoglobulin (Ig) G, IgM, and IgA levels in the EBV-positive group were significantly lower than those in the non-EBV SHLH group (P < 0.05). Lower levels of FIB and WBCs, as well as higher levels of CRP and triglycerides, were found in the EBV-induced SHLH group than in the non-EBV group, but the difference did not reach statistical significance (P > 0.05). Positive correlations were found between EBV DNA load and age (r = 0.497, P = 0.05), AST (r = 0.515, P = 0.041), and ALP (r = 0.529, P = 0.035) (Fig. 2). This point was further confirmed by our findings that mean EBV load was 9.0 × 10^5 copies/ml in server SHLH patients with EBV viraemia, while mean EBV load was 3.2 × 10^2 copies/ml in non-server SHLH patients with EBV viraemia, indicating that EBV load was associated with diseases severe status. Our results indicated that EBV DNA load influence disease development in SHLH patients with EBV viraemia.

**Prognosis of Virus-Associated SHLH and Non-Virus-Associated SHLH**

No patients (even those with virus-associated SHLH) received any specific antiviral treatment, as viral infection was not taken into consideration at diagnosis. Instead, most patients were treated with the conventional protocol for the primary disease, or symptomatic treatment. Survival information was collected from March 2012 to 2014. The median time from initial disease onset to death varied according to the severity of the case. Nine SHLH patients with viraemia and seven patients without viraemia died. Survival time in fatal cases ranged from 6–48 days. In viraemia cases, five patients died of multi-organ failure, two patients died of respiratory failure, one patient died of disseminated intravascular coagulation (DIC), and one patient died of severe pneumonia. The mortality rate was higher in patients with EBV viraemia than in non-EBV viraemia, especially in high viral load (>10^5 copies/ml) (Table III)  In non-viraemia cases, two patients died of multi-organ failure, two patients died of primary disease (lymphoma), 1 patient died of DIC, and two patients died of severe infection. Thirty-eight patients survived long-term.

Survival curve revealed that survival time was significantly shorter in patients with EBV-associated SHLH than in those with non-EBV-associated SHLH.

**TABLE II. Biomarkers of SHLH Patients With EBV Viraemia/Non-EBV Viraemia**

| Parameters        | EBV positive | EBV negative |
|-------------------|--------------|--------------|
| Gender (M/F)      | 10/4         | 8/2          |
| EBV (copies/ml)   | 5.67E+5 (range:1.24E+1–3.62E+6) | —          |
| Age (year)        | 56.50 (23.25–71.25) | 59.50 (29.75–69.50) |
| Fe (ng/ml)        | 1500.00 (1252.50–1500.00)** | 518.00 (312.75–1500.00) |
| WBC (10E9/L)      | 3.98 (2.50–7.34) | 5.25 (2.85–12.55) |
| HB (g/L)          | 92.00 (61.25–127.00) | 100.50 (79.00–120.25) |
| PLT (10E9/L)      | 48.50 (13.75–67.75) | 46.00 (19.00–99.50) |
| LDH (U/L)         | 842.50 (470.00–2286.75)** | 293.00 (175.50–597.75) |
| AST (U/L)         | 153.00 (70.00–469.50)** | 36.50 (23.00–117.00) |
| ALT (U/L)         | 99.00 (46.75–199.50)** | 29.35 (13.00–89.50) |
| GGT (U/L)         | 78.50 (32.25–240.75)* | 34.00 (17.75–89.75) |
| ALP (U/L)         | 127.50 (63.25–318.00) | 92.00 (64.25–143.00) |
| IgG (g/L)         | 10.55 (8.53–11.53)** | 13.55 (10.30–17.65) |
| IgM (g/L)         | 0.84 (0.52–1.26)* | 1.41 (0.94–2.01) |
| IgA (g/L)         | 1.67 (1.15–2.28)* | 2.45 (1.37–3.54) |
| CRP (mg/L)        | 55.85 (16.65–113.75) | 35.80 (11.65–94.10) |
| CH (mmol/L)       | 2.66 (1.99–3.24) | 3.34 (2.62–3.97) |
| TG (mmol/L)       | 1.92 (1.33–4.51) | 1.50 (0.93–2.42) |
| FIB (g/L)         | 2.01 (1.43–2.49) | 2.42 (1.86–3.61) |

*P < 0.05 when EBV positive group compared with EBV negative group.

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Similarly, EFS was significantly shorter in patients with severe SHLH than in those with non-severe SHLH, in both the viral and non-viral groups (Fig. 3). Our data demonstrated that patients with EBV-associated SHLH have poorer outcomes than those with non-EBV-associated SHLH, and SHLH patients with viraemia have poorer short-term survival rates than patients without viraemia.

**DISCUSSION**

SHLH is a life-threatening disease. Patients may deteriorate very rapidly after disease onset and die of multi-organ dysfunction and other complications. In children, the outcomes of EBV-associated SHLH have been reported, including a 90% overall response rate to multi-agent therapy that includes corticosteroids, etoposide, and cyclosporine. In contrast, adult patients with infectious SHLH die within days or months [Sharp et al., 2014]. The factors affecting disease prognosis remain incompletely understood. Diagnosis and treatment of SHLH in adults is challenging not only because the majority of reported data were from pediatric patients and there is no well-established network for adult SHLH, but also because SHLH occurs in many disease entities [Janka and Lehmberg 2014; Otrock and Eby 2015]. Presently, the incidence of virus-associated SHLH is increasing; however, no systematic study has been conducted on the incidence and clinical features of this subtype of SHLH [Rouphael, Talati et al., 2007].

EBV belongs to the group of B lymphotropic human γ-herpes viruses. EBV infects more than 95% of the global population with prevalence varying by socio-demographics and region [Svahn et al., 2006]. EBV is also one of the most frequent pathogens found in infection-associated SHLH [Fox et al., 2010]. In 52–62% of reported herpes virus-infected cases of SHLH, 43% are caused by infection with EBV and 9% are caused by CMV infection [Deane, Selmi et al., 2010]. Although the predominant cause of infection-associated SHLH differs in each country because of the population’s specific genetic backgrounds and differences in suspected triggering agents, the majority of virus-associated SHLH cases in Asian countries are caused by EBV infection [Imashuku 2002]. This phenomenon possibly due to more pathogenic EBV strains presenting in this region [Rouphael, Talati et al., 2007; Janka and Lehmberg, 2014]. Our study is consistent with these findings: not only the

| TABLE III. Clinical Outcomes in Severe Patients With EBV Viraemia |
|---------------------------------------------------------------|
| Patients | Gender/age | EBV copies/ml | Survival time | Cause of death |
|-----------|------------|---------------|---------------|----------------|
| 1         | F/85       | 3.10E + 06    | 6 days        | DIC            |
| 2         | F/67       | 7.38E + 05    | 6 days        | Multi-organ failure |
| 3         | M/21       | 6.14E + 05    | 20 days       | Multi-organ failure |
| 4         | M/61       | 3.28E + 04    | 27 days       | Respiratory failure |
| 5         | F/21       | 3.62E + 06    | 17 days       | Respiratory failure |
| 6         | F/78       | 3.60E + 04    | 48 days       | Multi-organ failure |
| 7         | M/24       | 2.90E + 05    | 12 days       | Severe pneumonia |
| 8         | M/72       | 3.43E + 04    | 7 days        | Multi-organ failure |
| 9         | M/22       | 1.57E + 05    | Survival      | Multi-organ failure |
| 10        | M/55       | 4.54E + 05    | 32 days       | —              |
| 11        | M/69       | 1.57E + 02    | Survival      | —              |
| 12        | M/55       | 2.10E + 02    | Survival      | —              |
| 13        | M/45       | 2.72E + 01    | Survival      | —              |
| 14        | M/18       | 1.24E + 01    | Survival      | —              |

F, female; M, male.
Survival time indicate the time from disease onset to die.

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incidence of EBV-associated SHLH was higher than other virus-associated SHLH cases, but also EBV was main virus found in the fatal cases, moreover, mean EBV load was significantly higher in server viral SHLH patients than in non-server viral SHLH patients, indicating that EBV load could influence disease development in SHLH patients with EBV infection. EBV induced SHLH confers the worst prognosis [Rouphael, Talati et al., 2007].

EBV infects via the oropharyngeal route, and is transmitted primarily through saliva. Primary EBV infection is mostly asymptomatic, following primary infection, EBV DNA can be detected in blood (mainly in the B-lymphocyte) from most individuals, and is rapidly cleared in 1–3 weeks [Bauer et al., 2005; Zoufaly et al., 2009]. EBV establishes lifelong latency at low copy numbers (1–50 copies among 10^6 cells) in the B-cell memory compartment [Heslop 2005]. The incidence of potentially life-threatening complications depends on the EBV viral load, in our study, EBV viral titers was significantly higher in severe SHLH patients than in non-severe patients, moreover, patients with worse prognosis had higher EBV copies than those of good prognosis (>10^4 copies/ml vs. 10^1-2 copies/ml). Clinically, EBV DNAemia is an infectious complication following hematopoietic stem cell or solid organ transplantation or HIV infection, a significant proportion of patients with EBV DNAemia can be life-threatening [Ocheni et al., 2008; van Esser et al., 2001; Zoufaly, Stellbrink et al., 2009]. There is scarce data of EBV viremia existing in patients with SHLH. As EBV antibody were detected in our patients, EBV reactivation is suspected to these patients. EBV reactivation is likely due to the over immunosuppression and persistent EBV infection [Bamoulid et al., 2013].

In our study, diagnosed patients were treated using conventional therapy. Because viral infection was seldom considered as the etiology for SHLH, treatment for viral infection was lacking. Thus, we cannot exclude whether the high mortality rates among patients with EBV-associated SHLH resulted from ineffective therapy though there is no effective treatment for these viral infections. We can conclude that the prognosis for severe cases of non-EBV SHLH was better than the cases of EBV-associated SHLH. Compared with EBV, other viral SHLH, including HSV-1, MPV, and hPIV-1, had better prognoses.

The molecular mechanisms that underlie virus SHLH are unclear. A cytokine-driven condition triggered by viral replication has been postulated, and it has been speculated that hypercytokinemia impairs the normal functioning of cytotoxic T-lymphocytes, NK cells, or both. In SHLH, hemophagocytosis and cytokine overproduction may result in cellular damage and multi-organ dysfunction [To et al., 2001; Muralitharan et al., 2007]. Cytokine storm in SHLH is more pronounced in EBV-associated disease, causing hemophagocytosis and organ dysfunction. This might explain why the majority of fatal cases were found in EBV-associated SHLH. If SHLH were a manifestation of an overwhelming hypercytokinemia, then treatment of the infection might not have been sufficient [Han et al., 2012].

SHLH can clinically mimic an infection and obscure its coexistence [Rouphael, Talati et al., 2007]. Thus, it is of major importance for physicians to be aware that the clinical picture of SHLH, sepsis, and MODS can share common features. These syndromes cannot be reliably discriminated by using diagnostic criteria devised for SHLH alone. Therefore, the diagnosis of SHLH must rely on other clinical, laboratory, and histopathological findings [Henter, Horne et al., 2007]. Phagocytosis of blood cells is a hallmark of SHLH [Goel et al., 2012]. During inflammation, macrophages may engulf erythrocytes, leukocytes, platelets, and their precursor cells, enacting a unique and evolving aspect of macrophage biology [Risma and Jordan, 2012]. In this study, hemophagocytosis was found in the BM smears of all patients, indicating that BM hemophagocytosis has a high sensitivity for diagnosing SHLH. There is significant overlap in the clinical and laboratory findings between SHLH and malignant diseases (particularly

Fig. 3. Kaplan–Meier survival curve for overall survival stratified by severe or non-severe outcome. a: survival curve between severe and non-severe SHLH patients (P < 0.01). b: show significantly different survival rate existed in patients with EBV viraemia and without EBV viremia (P < 0.05).
lymphomas or tumor metastasis), sepsis, and multiple organ dysfunction syndrome (MODS), which makes it difficult to differentiate these patients [Castillo and Carcillo, 2009]. Under these circumstances, hemophagocytosis would help to differentially diagnose these patients, especially those who do not fulfill the criteria for SHLH and are overlooked. Among the various biomarkers available for diagnosing SHLH, the presence of hemophagocytosis is what most practitioners continue to rely on to timely and differentially diagnose the patients [Gupta et al., 2008]. Hemophagocytosis is triggered specifically by interferon gamma (IFN-γ), and the direct action of IFN-γ on macrophages leads to the development of severe consumptive anemia and cytopenia [Zoller et al., 2011]. Indeed, unexplained acute cytopenia is commonly observed and has been associated with worse outcomes in critically ill patients [Risma and Jordan 2012]. Although hemophagocytosis has high sensitivity for differential diagnosis, it has been observed that patients’ prognosis is various, even when the same hemophagocytosis is observed in BM [Gupta et al., 2008]. Serum ferritin is a biomarker of a final common pathway of systemic inflammatory response, and hyperferritinemia is frequently used as a marker of SHLH severity. In our study, higher levels of ferritin, LDH, AST, and ALT were found in the severe SHLH group compared with the non-severe SHLH group, indicating that ferritin, LDH, AST, and ALT are all sensitive biomarkers that reflect disease severity for SHLH.

In our study, SHLH patients with EBV viremia had inadequate disease control and died within days or weeks of disease onset, whereas a significant fraction of SHLH patients with lower virus load or without viremia responded adequately to conventional therapy and the majority experienced a complete response. Although it is not possible to elucidate the mechanism, our data reveal that patients with high EBV DNA titers have worse prognoses.

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