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Requirement for etoposide in the initial treatment of Epstein-Barr virus–associated haemophagocytic lymphohistiocytosis

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Summary

Haemophagocytic lymphohistiocytosis (HLH) is a severe, even fatal, inflammatory condition. Epstein–Barr virus (EBV) infection-associated HLH (EBV-HLH) is one of the most common types of secondary HLH. Etoposide is a key drug in HLH-94/04 regimen. We sought to identify the importance of etoposide in the initial treatment of EBV-HLH. Ninety-three patients with EBV-HLH of all ages admitted to our centre in 2017 were divided into two groups according to whether the initial treatment contained etoposide or not. The survival of the group whose initial therapy included etoposide (Group 1, 52 patients; 6-month survival rate 0·769) was significantly better than that of the group whose initial therapy did not include etoposide (Group 2, 41 patients; 6-month survival rate 0·269) ($P < 0·001$). In patients aged <18 years old, the survival of Group 1 patients was not significantly better than that of Group 2 patients ($P = 0·447$), in contrast to patients aged ≥18 years, where the survival of Group1 patients was significantly better than that of Group 2 patients ($P < 0·001$). We concluded that including etoposide in the initial treatment of EBV-HLH patients can improve their prognosis, especially adult patients. This may be because that adult patients are recognized as "higher risk" patients.

Keywords: haemophagocytic lymphohistiocytosis, Epstein-Barr virus, etoposide.

Haemophagocytic lymphohistiocytosis (HLH) is a severe, even fatal, inflammatory condition caused by a hereditary or acquired immunoregulatory abnormality, non-malignant proliferation of lymphocytes and tissue cells, and secretion of a large number of inflammatory cytokines (Henter *et al*, 2007). HLH is divided into two categories: primary and acquired. Acquired HLH is often associated with and caused by infections, malignant tumours and autoimmune diseases (Maakaroun *et al*, 2010). Among the infections associated with HLH, Epstein–Barr virus (EBV) -associated HLH (EBV-HLH) is one of the most common. The use of rituximab has improved the prognosis of EBV-HLH patients in western countries (Chellapandian *et al*, 2013). However, in Asian countries, EBV-HLH patients exhibited much poorer prognoses compared with other types of infection associated with HLH, especially in adult HLH patients (Imashuku, 2011; Tseng *et al*, 2011). A previous study of 61 cases of EBV-HLH reported a 1-year overall survival (OS) of only 25·0% (Zeng *et al*, 2015). The current mainstream treatment strategy for EBV-HLH is the HLH-94/04 regimen, of which etoposide is a key drug (Imashuku, 2011). However, some physicians do not use etoposide in the initial treatment due to complications and adverse reactions. In the study reported by Imashuku *et al* (2001) the probability of long-term survival was significantly increased when etoposide treatment was initiated less than 4 weeks from diagnosis. In this study, no significant difference in long-term survival was noted between the patients who received etoposide in their initial treatment *versus* those whose initial treatment did not include etoposide [treated with corticosteroids, intravenous immunoglobulin (IVIG), and ciclosporin instead] (Imashuku *et al*, 2001). As the previous study was limited to children, the same group performed a subsequent study that focused on young adults. The results clearly demonstrated that patients who received etoposide within 4 weeks exhibited a more favourable prognosis compared with those were not treated with etoposide until after 4 weeks or did not use etoposide at all (Imashuku *et al*, 2003). However, these two studies were limited to

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patients under 35 years of age and was based on a literature review. Although the HLH-94/04 regimen is widely used, the diagnosis and treatment of EBV-HLH are complicated. Once HLH is diagnosed, etoposide is often considered as first-line treatment. However, different findings suggest that greater than 60% of EBV-HLH patients do not need cytotoxic drugs (Shiraishi *et al*, 2012). Other opinions suggest that other therapeutic drugs can be tried within 4 weeks. If no improvement is observed, then etoposide should be considered (Imashuku, 2011). What is the significance of using etoposide as soon as possible in the initial treatment? Considering the poorer prognosis of adult EBV-HLH patients in previous reports (Tseng *et al*, 2011; Arca *et al*, 2015), is it possible that the importance of initial treatment with etoposide differs in different age groups? This study retrospectively collected 93 patients with EBV-HLH admitted to our centre in 2017. By comparing whether or not the initial treatment regimen included etoposide, whether or not etoposide was used within 4 weeks, and different age groups, we hoped to determine the importance of etoposide in the treatment of EBV-HLH.

Patients and methods

Patients

In total, 93 patients with EBV-HLH of all ages admitted to our centre in 2017 were retrospectively reviewed. All patients met the HLH-04 diagnostic criteria with EBV infection (cellular EBV-DNA > 5·0E + 02 copies/ml) and no evidence of primary HLH and tumours (Henter *et al*, 2007).

Methods

The patients were divided into two groups according to the initial treatment regimen. Group 1: etoposide was administrated in the initial treatment, and Group 2: the initial treatment plan didn't include etoposide. The dose of etoposide was determined according to the treatment plan, such as 150 mg/m^2 for the HLH-94 regimen and 100 mg/m^2 for the DEP regimen (doxorubicin, etoposide, methylprednisolone).

Evaluation criteria and observed indicators

The age, gender and initial clinical symptoms as well as clinical data, initial treatment regimen, response rate, transplantation and outcome of each patient were identified.

The assessment of the efficacy of treatment of EBV-HLH followed the criteria proposed by Marsh *et al* (2013) and Wang *et al*, 2015). A complete response (CR) was defined as the normalization of all quantifiable symptoms and laboratory markers of HLH, including levels of soluble CD25, ferritin, triglyceride and haemoglobin, neutrophil and platelet counts; and alanine aminotransferase (ALT) levels. A partial response (PR) was defined as improvement in two or more of the following quantifiable symptoms and laboratory markers by 2 weeks: 1·5-fold decrease in soluble CD25 response; ferritin and triglyceride decreases of at least 25%; an increase of at least 100% to >0·5 × 10^9/l in patients with an initial neutrophil count of <0·5 × 10^9/l; an increase of at least 100% to >2·0 × 10^9/l in patients with an initial neutrophil count of 0·5–2·0 × 10^9/l; and a decrease of at least 50% in patients with initial ALT levels >400 u/l. Additionally, the body temperature must have reverted to normal ranges for either CR or PR to be diagnosed. Failure to achieve PR was defined as no response.

Survival time

Survival times were calculated from the date of diagnosis of EBV-HLH. All patients were followed up until death or 1 May 2018, whichever occurred first. Patients undergoing stem cell transplantation were censored on the date of that procedure.

Statistical analysis

SPSS 22.0 (IBM, New York, NY, USA) statistical software was adopted, and data that did not fit a normal distribution are presented as median and range. Kaplan–Meier survival curves were used to analyse the patients' survival. Multi-factor analysis using regression model Cox risk analysis and the factors included the age of onset (divided into two groups: ≥18 years and <18 years), patient gender, number of EBV-DNA copies, whether the patient was in Group 1 or Group 2, and whether etoposide was used as within 4 weeks of diagnosis. $P < 0·05$ was considered to denote a significant difference, and $P < 0·01$ was considered very significant.

Results

Patients characteristics

Of the 93 patients, 54 were male, and 39 were female. The male to female ratio was 1·4:1. The median age of the patients was 26 years (1–70 years), including 35 patients (37·6%) aged <18 years and 58 patients (62·4%) aged >18 years.

Treatment and outcome

The patients were divided into two groups according to their initial treatment regimen. Group 1 ($n = 52$): initial treatment after diagnosis included etoposide. The specific treatment options included: single drug etoposide, HLH-94 regimen, HLH-04 regimen, DEP, L-DEP (pegylated-aspargase and DEP), E-CHOP (etoposide, cyclophosphamide, doxorubicin, vincristine, prednisone); Group 2 ($n = 41$): the initial treatment plan after diagnosis did not contain etoposide. The specific treatment methods included corticosteroids alone,

corticosteroids + ciclosporin, corticosteroids + pegylated-aspargase, rituximab, CVAD chemotherapy regimen (cyclophosphamide, vincristine, doxorubicin, dexamethasone). 64 patients were treated with etoposide within 4 weeks, and 29 patients received etoposide after 4 weeks or did not receive etoposide. As some of the patients were diagnosed and managed in other hospitals, and many other centres do not fully understand the treatment options for HLH, the decision to treat patients with etoposide or not was only based on the clinician's experience. Comparing the baseline data between two groups, there was no significant differences ($P > 0{\cdot}05$; detailed data are summarized in Table I.

The response rate of the 93 patients was 62·4%, with a CR rate of 25·8% (24/93) and PR rate of 36·6% (34/93). In Group 1, a total of 44 cases achieved remission, and the remission rate was 84·6% (CR 45·1%; PR 41·2%). 14 cases of Group 2 achieved remission, and the remission rate was 34·1% (CR 7·3%; PR 26·8%). A significant difference was noted between the two groups ($P < 0{\cdot}01$).

A total of 40 deaths occurred in 93 patients with a total mortality rate of 43·0%. Of these fatal cases, 24 died of active disease, 11 of bacterial or fungal infections (sepsis or pulmonary), 2 died of severe haemorrhage, and 3 of transplant-related preconditioning complications. There were 12 deaths in Group 1 (mortality rate 23·1%) and 28 deaths in Group 2 (mortality rate 65·1%). A significant difference in mortality was noted between the two groups ($P < 0{\cdot}05$).

Survival

The 6-month survival rate of Group 1 was $0{\cdot}769 \pm 0{\cdot}061$ [95% confidence interval (CI) 0·649, 0·889], and that of Group 2 was $0{\cdot}269 \pm 0{\cdot}074$ [95% CI 0·124, 0·414]. A significant difference in 6-month survival rate was noted between the two groups ($P < 0{\cdot}001$). The survival curve was drawn by K-M method. (Fig 1).

Multivariate analysis of patient gender, age, number of EBV-DNA copies, whether the initial treatment plan included etoposide (Group 1 or Group 2), whether etoposide was used within 4 weeks and patient prognosis, demonstrated that age group ($P = 0{\cdot}017$, ExpB = 0·393) and initial treatment group ($P < 0{\cdot}001$, ExpB = 0·170) were associated with prognosis (Fig 2). The number of EBV-DNA copies was not associated with prognosis ($P = 0{\cdot}163$).

On the basis of "whether the usage of etoposide is within 4 weeks" and "whether etoposide was used as initial treatment" are relevant as two factors and that previous literature confirmed the significance of the use of etoposide within

Table I. Initial clinical characteristics of the patients according to group

Features	Group 1† ($n = 52$)	Group 2‡ ($n = 41$)	*P* value
Age, years			
Median	22·5	30	0·088
Range	[1–70]	[4–70]	
Gender (*n*)			
Male	31	23	0·83
Female	21	18	
Fever (>38·5°C)	52	41	
Splenomegaly (*n*)	45 (86·5%)	35 (85·4%)	1·00
Hepatomegaly (*n*)	22 (42·3%)	15 (36·6%)	0·67
Haemophagocytosis (*n*)	38 (73·1%)	21 (51·2%)	0·49
WBC ($\times 10^9$/l)	1·8 [0·37–40·65]	2·33 [0·2–10·1]	0·097
Hb (g/l)	98 ± 21·41	104 ± 18·61	0·105
PLT ($\times 10^9$/l)	49 [2–255]	62 [13–365]	0·070
ALT (u/l)	122 [10·0–2142·3]	101 [26–998]	0·623
AST (u/l)	132·5 [17·1–1744·0]	135·3 [23·3–695·8]	0·952
Total bilirubin (μmol/l)	17·99 [4·85–254·08]	27·53 [7·15–367·24	0·074
Creatinine (μmol/l)	52·7 [23·0–291·9]	70·53 ± 40·36	0·080
Triglycerides (mmol/l)	3·05 ± 1·66	2·92 [0·59–16·77]	0·891
Fibrinogen (g/l)	1·62 ± 0·95	1·53 [0·21–7·69]	0·607
Ferritin (μg/l)	8·40 [4·48–12·56]	7·68 [5·32–11·23]	0·070
sCD25 (pg/ml)	9·07 [7·13–10·69]	10·24 [7·11–10·69]	0·623
EBV-DNA (lg copies/ml)	5·13 ± 0·92	4·90 ± 1·51	0·406

Values are given as median [range] or mean ± standard deviation. ALT, alanine aminotransferase; AST, aspartate aminotransferase; EBV-HLH, Epstein–Barr virus-associated haemophagocytic lymphohistiocytosis; Hb, haemoglobin concentration; PLT, platelet count; WBC, white blood cell count.

†Patients whose initial therapy included etoposide.

‡Patients whose initial therapy did not include etoposide.

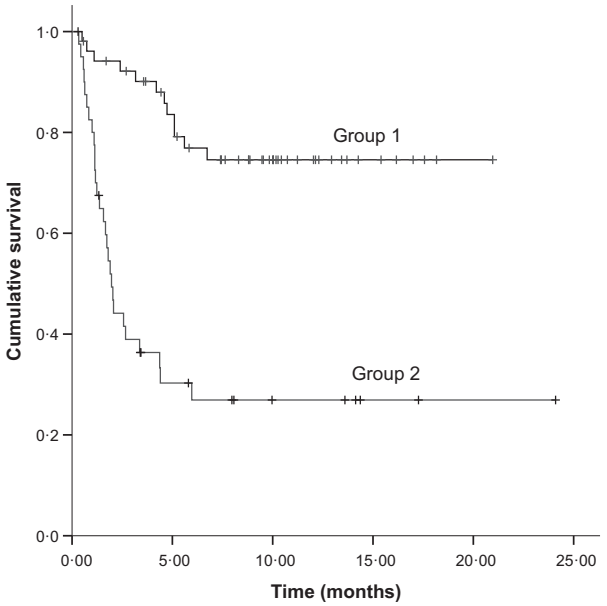

Fig 1. The survival curves of the two groups. Group 1: patients whose initial therapy included etoposide. Group 2: patients whose initial therapy did not include etoposide.

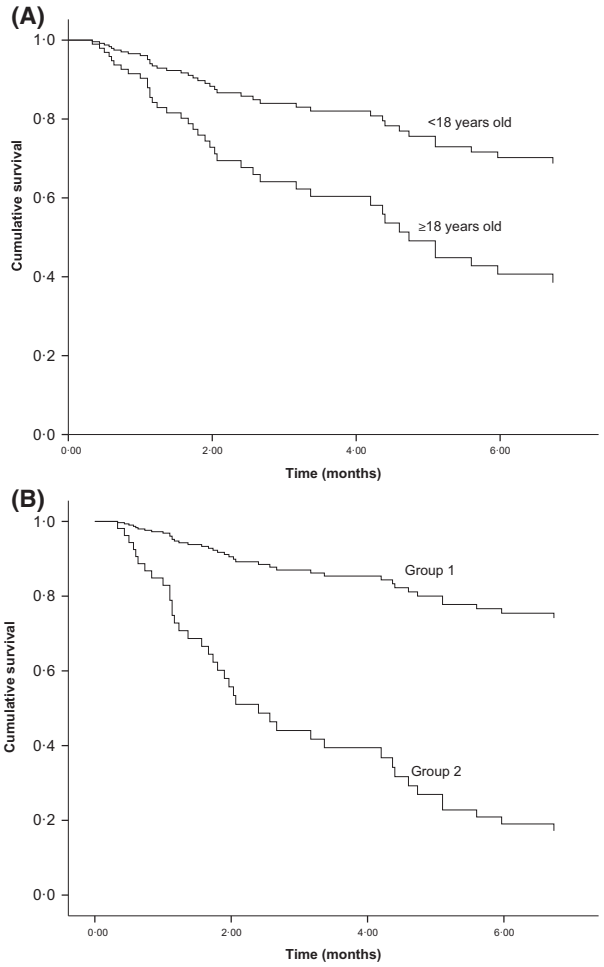

Fig 2. Cox analysis: analysis of the patient's gender, age and EBV-DNA copies numbers, according to whether the initial treatment plan included etoposide (Group1) or not (Group2), whether etoposide was used within 4 weeks and the prognosis of the patient. Age group (A) ($P = 0{\cdot}017$, ExpB = 0·393) and the initial treatment group (B) ($P < 0{\cdot}001$, ExpB = 0·170) were associated with prognosis.

4 weeks for the prognosis of patients with EBV-HLH (Imashuku *et al*, 2001, 2003), this study divided the patients into juvenile (<18 years old) and adult groups (≥18 years old). Whether the initial treatment involved etoposide and whether etoposide was used within 4 weeks of diagnosis were investigated in both age groups. In juvenile patients, whether etoposide was used within 4 weeks was associated with prognosis ($P = 0{\cdot}002$) (Fig 3), whereas Group 1/2 had no significant effect on prognosis ($P = 0{\cdot}447$). In the adult group, Group 1/2 had a significant effect on prognosis ($P < 0{\cdot}001$) (Fig 4); however, whether etoposide was given within 4 weeks minimally affected the prognosis ($P = 0{\cdot}163$).

For those patients who used etoposide ≥4 weeks or no etoposide, or the initial treatment included etoposide were not associated with prognosis ($P = 0{\cdot}292$), but the survival curve showed that the etoposide group had a tendency to have better prognosis than the without-etoposide group (Fig 5).

Discussion

HLH is a serious or even fatal inflammatory condition caused by the secretion of a large number of inflammatory factors due to the non-malignant proliferation of lymphocytes and histiocytes (Henter *et al*, 2002, 2007). Secondary HLH is often associated with malignancy, infections, rheumatism and other factors. The most common type of infection-related HLH is EBV, especially in Asian populations. In western countries, the use of rituximab, has improved the prognosis of EBV-HLH (Chellapandian *et al*, 2013). However, in Asian countries, compared with other types of HLH, EBV-HLH exhibits a low remission rate, high recurrence and mortality rates, poor prognosis and unsatisfactory treatment outcomes (Imashuku *et al*, 2004; Zeng *et al*, 2015). The current standard treatment, the HLH94/04 regimen, is mostly aimed at children. However, adult patients are not uncommon in the clinical setting. In the present study, adult patients constitute a larger proportion of all EBV-HLH patients compared with child patients. Considering the different immune status and basal status between adult and child HLH patients, no standard treatment strategy is currently available for adult EBV-HLH. According to previous literature, age is a negative factor for the prognosis of EBV-HLH, and adult EBV-HLH patients suffer poorer prognoses compared to children (Tseng *et al*, 2011; Kogawa *et al*, 2014; Arca *et al*, 2015). Our centre uses L-DEP as a salvage treatment for relapsed and refractory EBV-HLH, which can effectively improve the remission rate and achieve the goal of bridging to allogeneic haematopoietic stem cell

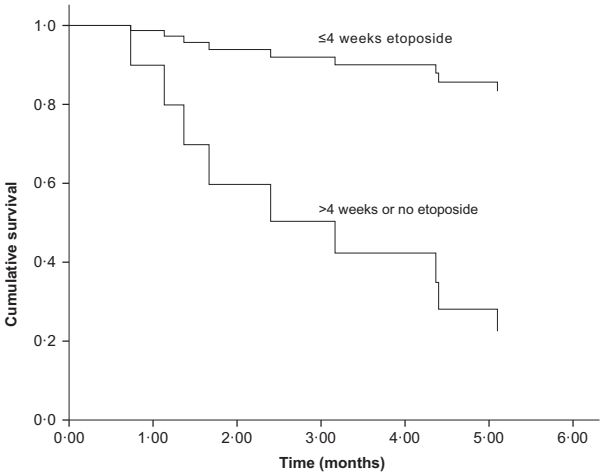

Fig 3. In patients aged <18 years, the survival of those who received early etoposide (≤4 weeks) was significantly better than those who received late (>4 weeks) or no etoposide ($P = 0{\cdot}002$).

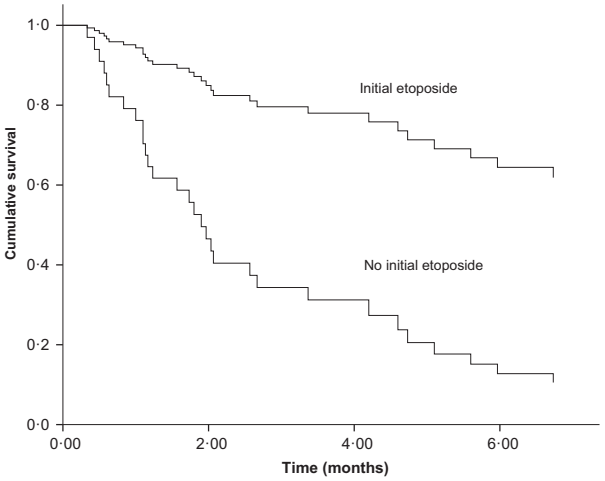

Fig 4. In patients aged ≥18 years old, the survival of patients whose initial therapy included etoposide (initial etoposide) was significantly better than patients whose initial therapy did not include etoposide (no initial etoposide) ($P < 0{\cdot}001$).

transplantation (allo-HSCT) (Wang *et al*, 2016). However, some controversies remain regarding the initial treatment strategy in EBV-HLH. Etoposide is the core drug in the HLH-94/04 standard protocol, and its important position in HLH treatment has been confirmed. In one study of EBV-HLH treatment, it was found that in children and young adult patients, the use of etoposide within 4 weeks of diagnosis can improve the prognosis, and whether etoposide is used for the initial treatment is not relevant (Imashuku *et al*, 2003). There are many controversies regarding the treatment of EBV-HLH, such as the use of immunomodulatory treatments (corticosteroids, IVIG, etc.) within 4 weeks of diagnosis, and those patients who cannot achieve remission should be administered etoposide (Imashuku, 2011; Janka &

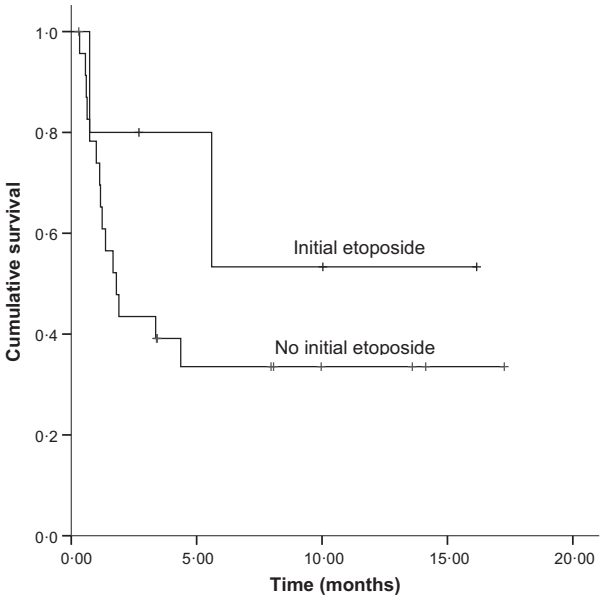

Fig 5. For those patients who those who received late etoposide (≥4 weeks) or no etoposide, whether the initial treatment included etoposide was not associated with prognosis ($P = 0{\cdot}292$).

Lehmberg, 2013). Other authors suggest that more than 60% of EBV-HLH cases do not require the use of cytotoxic drugs (Shiraishi *et al*, 2012). However, the results of this study found that the use of etoposide in the initial treatment is important for prognosis; however, its importance may differ in different age groups.

The present, study found that for patients under the 18 years old, the use of etoposide within 4 weeks of diagnosis and whether it is used in initial treatment have no significant relationship with the prognosis, which is also consistent with the current international opinion of EBV-HLH, namely, that within 4 weeks, observation or conservative treatment can be performed first (Imashuku, 2011; Janka & Lehmberg, 2013). However, for adult patients, initial treatment with etoposide is strongly related to prognosis. According to the results of previous studies (Kogawa *et al*, 2014) and the findings of this study, the prognoses of adult patients with EBV-HLH are significantly poorer than those of children with EBV-HLH. This finding may explain why the importance of initial etoposide is different between two age groups. For adult EBV-HLH patients, the inflammatory storm is more severe and the disease may progress more rapidly than children affected by EBV-HLH. Therefore, the sooner etoposide is involved, the sooner the patient's inflammatory response is controlled, and the better the prognosis. Although Shiraishi *et al* (2012) reported that >60% of EBV-HLH may not require cytotoxic drugs (including etoposide) and only early administration of an immunomodulatory regimen can achieve remission; however, the subjects were limited to children. Ahn *et al* (2010) studied 15 adult EBV-HLH patients, all of whom were treated with immunomodulation therapy, and only 2 survived. This finding demonstrates the poor

prognosis of adult EBV-HLH compared with children and the importance of the early use of etoposide. Indeed, current international opinion agrees that when EBV-HLH is identified as high risk, the early use of etoposide is recommended (Ehl *et al*, 2018). This information also indicates that age is a risk factor for EBV-HLH. However, for those patients who used etoposide ≥4 weeks or no etoposide, the prognosis is not significantly improved when the initial treatment includes etoposide even though there is a tendency that initial etoposide results in a better outcome. This may suggest that early treatment with etoposide is always recommended, but further study is still needed.

Etoposide is a chemotherapy medication used for the treatment of a number of cancers. It forms a ternary complex with DNA and the topoisomerase II enzyme (that aids DNA unwinding), preventing re-ligation of the DNA strands. Thus, etoposide causes DNA strands to break (Pommier *et al*, 2010). Etoposide has also been widely used in the treatment of histiocytic diseases in recent years, and is one of the core drugs in the HLH-94 and HLH-04 regimens. Its shows efficacy within 24–48 h and, in HLH, far outweighs the risk of and transient worsening of the cytopenia and reactive leukaemia (Henter *et al*, 2007; Arca *et al*, 2015). It can selectively ablate over-activated T cells to inhibit mononuclear-macrophage activation, thereby reducing the production of inflammatory factor storms to control HLH without compromising quiescent phase and memory T cells (Johnson *et al*, 2013). This is different from the immunosuppressive effects of corticosteroids and the immunomodulatory effects of IVIG. At present, the boundary between EBV-HLH and EBV+ lymphoproliferative disease is not clear, and the opinion exists that EBV+ LPD is actually a continuation of EBV-HLH. Some studies have identified monoclonal CD8+ T cell proliferation in EBV-HLH, and these patients respond very well to etoposide (Qin *et al*, 2012; Smith *et al*, 2014). This finding indicates that etoposide is superior to immunomodulatory drugs because it has a more pronounced effect on the presence of clonal, proliferative EBV-HLH; however, etoposide may not have this advantage for other types of HLH. Etoposide is very important in the treatment of EBV-HLH. Early effective control of inflammatory reactions provides good conditions for subsequent therapy and allo-HSCT. Therefore, for adult EBV-HLH patients, initial treatment with etoposide is essential for improving the prognosis. However, for children, considering the side effects of etoposide, including myelosuppression, teratogenesis, etc., immunomodulatory treatment (including hormones, IVIG, ciclosporin, etc.) within 4 weeks is recommended. If this treatment is not effective or if the patient experiences early recurrence, etoposide should be added to the treatment regimen.

Conclusion

As one of the secondary HLHs, EBV-HLH has poor therapeutic responses and poor prognosis. However, no clear and unified initial treatment plan is currently available, especially for adult patients. The study found that initial treatment with etoposide can improve the prognosis of EBV-HLH patients; however, the importance of etoposide may be different in different age groups. In juvenile patients, the use of etoposide within 4 weeks of diagnosis can effectively improve the prognosis; thus, 4 weeks can be used as a window to try other initial treatments. Because adult patients are recognized as higher risk patients, initial treatment with etoposide is essential to improve prognosis.

Author contributions

ZW contributed to the design of the study. YNW helped with the study design and data analyses. YS conducted the data analysis and wrote the manuscript. All authors approved the final manuscript.

References

Ahn, J.S., Rew, S.Y., Shin, M.G., Kim, H.R., Yang, D.H., Cho, D., Kim, S.H., Bae, S.Y., Lee, S.R., Kim, Y.K., Kim, H.J. & Lee, J.J. (2010) Clinical significance of clonality and Epstein-Barr virus infection in adult patients with hemophagocytic lymphohistiocytosis. *American Journal of Hematology*, **85**, 719–722.

Arca, M., Fardet, L., Galicier, L., Riviere, S., Marzac, C., Aumont, C., Lambotte, O. & Coppo, P. (2015) Prognostic factors of early death in a cohort of 162 adult haemophagocytic syndrome: impact of triggering disease and early treatment with etoposide. *British Journal of Haematology*, **168**, 63–68.

Chellapandian, D., Das, R., Zelley, K., Wiener, S.J., Zhao, H., Teachey, D.T. & Nichols, K.E. (2013) Treatment of Epstein Barr virus-induced haemophagocytic lymphohistiocytosis with rituximab-containing chemo-immunotherapeutic regimens. *British Journal of Haematology*, **162**, 376–382.

Ehl, S., Astigarraga, I., von Bahr Greenwood, T., Hines, M., Horne, A., Ishii, E., Janka, G., Jordan, M.B., La Rosee, P., Lehmberg, K., Machowicz, R., Nichols, K.E., Sieni, E., Wang, Z. & Henter, J.I. (2018) Recommendations for the use of etoposide-based therapy and bone marrow transplantation for the treatment of HLH: consensus statements by the HLH steering committee of the histiocyte society. *The Journal of Allergy and Clinical Immunology. In Practice.*, **6**, 1508–1517.

Henter, J.I., Samuelsson-Horne, A., Arico, M., Egeler, R.M., Elinder, G., Filipovich, A.H., Gadner, H., Imashuku, S., Komp, D., Ladisch, S., Webb, D., Janka, G. & Histocyte, S. (2002) Treatment of hemophagocytic lymphohistiocytosis with HLH-94 immunochemotherapy and bone marrow transplantation. *Blood*, **100**, 2367–2373.

Henter, J.I., Horne, A., Arico, M., Egeler, R.M., Filipovich, A.H., Imashuku, S., Ladisch, S., McClain, K., Webb, D., Winiarski, J. & Janka, G. (2007) HLH-2004: diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. *Pediatric Blood & Cancer*, **48**, 124–131.

Imashuku, S. (2011) Treatment of Epstein-Barr virus-related hemophagocytic lymphohistiocytosis (EBV-HLH); update 2010. *Journal of Pediatric Hematology/Oncology*, **33**, 35–39.

Imashuku, S., Kuriyama, K., Teramura, T., Ishii, E., Kinugawa, N., Kato, M., Sako, M. & Hibi, S. (2001) Requirement for etoposide in the treatment of Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis. *Journal of Clinical Oncology*, **19**, 2665–2673.

Imashuku, S., Kuriyama, K., Sakai, R., Nakao, Y., Masuda, S.-I., Yasuda, N., Kawano, F., Yakushijin, K., Miyagawa, A., Nakao, T., Teramura, T., Tabata, Y., Morimoto, A. & Hibi, S. (2003) Treatment of Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis (EBV-HLH) in young adults: a report from the HLH study center. *Medical and Pediatric Oncology*, **41**, 103–109.

Imashuku, S., Teramura, T., Tauchi, H., Ishida, Y., Otoh, Y., Sawada, M., Tanaka, H., Watanabe, A., Tabata, Y., Morimoto, A., Hibi, S. & Henter, J.I. (2004) Longitudinal follow-up of patients with Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis. *Haematologica*, **89**, 183–188.

Janka, G.E. & Lehmberg, K. (2013) Hemophagocytic lymphohistiocytosis: pathogenesis and treatment. *Hematology/the Education Program of the American Society of Hematology*, **2013**, 605–611.

Johnson, T.S., Terrell, C.E., Millen, S.H., Katz, J.D., Hildeman, D.A. & Jordan, M.B. (2013) Etoposide selectively ablates activated T cells to control the immunoregulatory disorder hemophagocytic lymphohistiocytosis. *The Journal of Immunology*, **192**, 84–91.

Kogawa, K., Sato, H., Asano, T., Ohga, S., Kudo, K., Morimoto, A., Ohta, S., Wakiguchi, H., Kanegane, H., Oda, M. & Ishii, E. (2014) Prognostic factors of Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis in children: report of the Japan Histiocytosis Study Group. *Pediatric Blood & Cancer*, **61**, 1257–1262.

Maakaroun, N.R., Moanna, A., Jacob, J.T. & Albrecht, H. (2010) Viral infections associated with haemophagocytic syndrome. *Reviews in Medical Virology*, **20**, 93–105.

Marsh, R.A., Allen, C.E., McClain, K.L., Weinstein, J.L., Kanter, J., Skiles, J., Lee, N.D., Khan, S.P., Lawrence, J., Mo, J.Q., Bleesing, J.J., Filipovich, A.H. & Jordan, M.B. (2013) Salvage therapy of refractory hemophagocytic lymphohistiocytosis with alemtuzumab. *Pediatric Blood & Cancer*, **60**, 101–109.

Pommier, Y., Leo, E., Zhang, H. & Marchand, C. (2010) DNA topoisomerases and their poisoning by anticancer and antibacterial drugs. *Chemistry & Biology*, **17**, 421–433.

Qin, Q., Xie, Z., Shen, Y., Yang, S., Liu, C., Huang, Z., Xu, J., Al, J. & Shen, K. (2012) Assessment of immunochemotherapy and stem cell transplantation on EBV-associated hemophagocytic lymphohistiocytosis in children: a systematic review and meta analysis. *European Review for Medical and Pharmacological Sciences*, **16**, 672–678.

Shiraishi, A., Ohga, S., Doi, T., Ishimura, M., Takimoto, T., Takada, H., Miyamoto, T., Abe, Y. & Hara, T. (2012) Treatment choice of immunotherapy or further chemotherapy for Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis. *Pediatric Blood & Cancer*, **59**, 265–270.

Smith, M.C., Cohen, D.N., Greig, B., Yenamandra, A., Vnencak-Jones, C., Thompson, M.A. & Kim, A.S. (2014) The ambiguous boundary between EBV-related hemophagocytic lymphohistiocytosis and systemic EBV-driven T cell lymphoproliferative disorder. *International Journal of Clinical and Experimental Pathology*, **7**, 5738–5749.

Tseng, Y.T., Sheng, W.H., Lin, B.H., Lin, C.W., Wang, J.T., Chen, Y.C. & Chang, S.C. (2011) Causes, clinical symptoms, and outcomes of infectious diseases associated with hemophagocytic lymphohistiocytosis in Taiwanese adults. *Journal of Microbiology, Immunology, and Infection*, **44**, 191–197.

Wang, Y., Huang, W., Hu, L., Cen, X., Li, L. & Wang, J. (2015) Multicenter study of combination DEP regimen as a salvage therapy for adult refractory hemophagocytic lymphohistiocytosis. *Blood*, **126**, 2186–2192.

Wang, J., Wang, Y., Wu, L., Zhang, J., Lai, W. & Wang, Z. (2016) PEG-aspargase and DEP regimen combination therapy for refractory Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis. *Journal of Hematology & Oncology*, **9**, 84.

Zeng, X., Wei, N., Wang, Y., Wang, J., Zhang, J., Wu, L., Huang, W., Gao, Z., Pei, R., Chen, J., Jin, Z. & Wang, Z. (2015) Treatment outcomes and prognostic analysis of 61 Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis. *Zhonghua xue ye xue za zhi = Zhonghua Xueyexue Zazhi*, **36**, 507–510.

