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Treatment of Epstein Barr virus-induced haemophagocytic lymphohistiocytosis with rituximab-containing chemo-immunotherapeutic regimens

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Summary

Haemophagocytic lymphohistiocytosis (HLH) is a life threatening complication of Epstein–Barr virus (EBV) infection. The anti-CD20 antibody rituximab depletes B cells, leading to improved outcomes for patients with EBV-associated B-lymphoproliferative disorders. To gather data on the use of rituximab in EBV-HLH, we performed a retrospective investigation involving 42 EBV-HLH patients who had received treatment with rituximab-containing regimens. On average, patients received 3 rituximab infusions (range 1–10) at a median dose of 375 mg/m^2. In all patients, rituximab was administered with other HLH-directed medications, including steroids, etoposide and/or ciclosporin. Rituximab-containing regimens appeared well tolerated and improved clinical status in 43% of patients. Examination of laboratory data obtained prior to and within 2–4 weeks after the first rituximab dose revealed significant reductions in EBV load (median load pre-rituximab: 114 200 copies/ml, median post-rituximab: 225 copies/ml, $P = 0{\cdot}0001$) and serum ferritin levels (median ferritin pre-rituximab: 4260 μg/l, median post-rituximab: 1149 μg/l, $P = 0{\cdot}001$). Thus, when combined with conventional HLH-directed therapies, rituximab improves symptoms, reduces viral load and diminishes inflammation. These data support the incorporation of rituximab into future prospective clinical trials for patients with EBV-HLH.

Keywords: Epstein-Barr virus, haemophagocytic lymphohistiocytosis, macrophage activation syndrome, rituximab, x-linked lymphoproliferative disease.

Haemophagocytic lymphohistiocytosis (HLH) comprises a rare group of disorders typified by activation of CD8+ T cells and macrophages and secretion of high levels of pro-inflammatory cytokines (Lykens *et al*, 2011; Janka, 2012; Risma & Jordan, 2012). HLH occurs as a hereditary condition caused by germline mutations that impair lymphocyte cytotoxic function or as a nonhereditary disorder triggered by infection, malignancy or autoimmune disease (Coffey *et al*, 1998; Nichols *et al*, 1998; Stepp *et al*, 1999; Feldmann *et al*, 2003; Zur Stadt *et al*, 2005, 2009; Rigaud *et al*, 2006; Cote *et al*, 2009; Janka, 2012). Currently, a two-tiered approach is used to treat HLH: chemo-immunotherapeutic agents are administered to dampen inflammation and targeted therapies are given to eliminate HLH trigger(s) (Janka, 2012).

Epstein–Barr virus (EBV) is a common trigger of HLH, particularly in Asian individuals (Kawaguchi *et al*, 1993; Imashuku, 2002; Yachie *et al*, 2003) and in patients with congenital or acquired immunodeficiencies (McClain *et al*, 1988; Pasic *et al*, 2003; Rezaei *et al*, 2011). EBV is poorly responsive to antiviral agents; however, it resides in B lymphocytes, which can be rapidly depleted using the B cell-targeting monoclonal antibody rituximab. Based on its efficacy in lowering disease burden in patients with B-lymphoproliferative disorders (DiNardo & Tsai, 2010; Maloney, 2012), some investigators are using rituximab to treat EBV-HLH. It is not known whether this is an effective strategy for this disorder.

To understand current practice and prepare for the possible incorporation of rituximab into future HLH protocols, we

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performed this retrospective investigation, which describes 42 patients with EBV-induced disease, who were treated with regimens containing rituximab, steroids, etoposide and/or ciclosporin. Rituximab-containing regimens improved clinical status in most patients, exhibited no toxicities beyond those normally encountered and significantly reduced EBV load and serum ferritin levels. These data suggest that rituximab can be safely added to standard therapies and provide the evidence needed to move forward with a prospective clinical trial.

Materials and methods

Data collection

Survey documents were developed, approved by the Institutional Review Board at The Children's Hospital of Philadelphia and distributed to members of the Histiocyte Society.

Patients

This study included 42 patients with EBV-HLH who received treatment with rituximab-containing regimens between June 1, 2000 and October 31, 2011 (Table I). HLH was diagnosed based on established criteria (Henter *et al*, 2007). The median age at diagnosis was 6·75 years (range 1·2–44) and there was a predominance of males ($n = 30$; 71%). Positive EBV status was documented by monospot ($n = 9$), serological evidence of acute infection ($n = 28$) and/or evidence of EBV DNA in the blood by real time polymerase chain reaction [$n = 41$; median viral load 114 200 copies of EBV genome/ml (range 500–4 $\times$ 10^7)]. Thirty-seven patients underwent mutational analysis for at least 1 HLH gene, and in 16 patients, mutations were identified. The gene most commonly mutated was *SH2D1A* ($n = 8$), followed by *PRF1* ($n = 2$, each monoallelic), *XIAP* ($n = 2$), *UNC13D* ($n = 2$) and *STXBP2* ($n = 2$). Both patients with heterozygous *PRF1* mutations developed disease recurrence, suggesting a genetic form of HLH.

Clinical and laboratory manifestations

All patients exhibited fever, hepatomegaly and/or splenomegaly and were cytopenic in one or more lineages (Table I). Natural killer (NK) cell cytotoxicity was examined in 20 patients, and in 12 (60%), activity was reduced or absent. Among these patients, 3 harboured HLH-associated mutations. Twenty-three patients (96% of those tested) exhibited elevated levels of soluble IL2 receptor (sIL2RA; median 12 412 u/ml; range 1891–206 567 u/ml). Similarly, ferritin levels were high in all 41 patients for whom data were provided (median 6334 μg/l; range 210–121 379 μg/l). Haemophagocytosis in the bone marrow was present in 30 patients (71%).

Table I. Laboratory Features at diagnosis with EBV-HLH.

Number of patients with positive result/number of patients tested (%)	
HLH genetic testing	
PRF1	2/28 (7)
SH2D1A	8/24 (33)
XIAP	2/21 (10)
UNC13D	2/25 (8)
STX11	0/21 (0)
STXBP2	2/4 (50)
RAB27A	0/3 (0)
ITK	0/1 (0)
Assay to detect EBV	
Monospot	9/12 (75)
EBV serology	28/30 (93)
EBV PCR	41/41 (100)
	Median (range)
Complete blood count (normal values)	
WBC (4·5–13 $\times$ 10^9/l)	2·85 $\times$ 10^9/l (0·1–85·2 $\times$ 10^9/l)
ANC ($\geq 1 \times 10^9$/l)	1·25 $\times$ 10^9/l (0·014–11·3 $\times$ 10^9/l)
Hb (130–160 g/l)	89 (44–146)
Plt (150–450 $\times$ 10^9/l)	69 $\times$ 10^9/l (9–31 000 $\times$ 10^9/l)
Hepatic panel (normal values)	
AST (15–45 u/l)	410 (46–7334)
ALT (10–40 u/l)	205 (14–5227)
Total bilirubin (10–24 μmol/l)	62 (6·8–282)
Total protein (62–81 g/l)	52 (40–71)
Albumin (37–56 g/l)	23 (16–38)
Other HLH tests (normal values)	
Soluble interleukin 2 receptor (<2000 u/ml)	12 412 (1891–206 567)
Fibrinogen (1·7–4·7 g/l)	1·5 (0·5–7·9)
Ferritin (10–70 μg/l)	6334 (210–121 379)
Triglycerides (0·3–1·4 mmol/l)	3·3 (0·9–14)
NK function (Normal/depressed)	Depressed in 12 pts
Haemophagocytosis (Present/absent)	Present in 30 pts

EBV, Epstein–Barr virus; HLH, haemophagocytic lymphohistiocytosis; PCR, polymerase chain reaction; WBC, white blood cells; ANC, absolute neutrophil count; Hb, haemoglobin concentration; Plt, platelets; AST, aspartate transaminase; ALT, alanine transaminase; NK, natural killer cell.

Management of HLH

All patients received treatment with rituximab-containing regimens (Table II). The median dose was 375 mg/m^2 (range 175–412) and the average number of infusions was 3 (range 1–10). Twenty-eight patients (67%) received the first dose of rituximab within 1 month of HLH diagnosis (median 5·5 d; range 1–28), while the remaining patients received it later (median 73·5 d; rage 30–210). Nine patients received the first dose of rituximab as therapy for recurrent, not primary, HLH (median 90 d; range 53–210). No rituximab infusions

Table II. Dosing and schedule of rituximab administration.

	Patients (*n*)	Median, range
Dosage information		
Dose (mg/m^2)	42	375 (175–412)
Days between diagnosis and first rituximab	42	13 (1–210)
<30 d	28	5·5 (1–28)
≥30 d	14	73·5 (30–210)
Number of doses*	42	3 (1–10)

	Patients (*n*)	% of total cohort
Schedule		
Weekly	26	62
Monthly	1	2
Other†	15	36
Other medications		
Chemotherapy	38	90
Steroids	41	98
IVIG	33	79
Ciclosporin	32	76
SCT	20	48
Antiviral therapies		
Acyclovir	13	31
Ganciclovir	14	33
Other‡	7	17

IVIG, intravenous immunoglobulin; SCT, stem cell transplantation.
*11 patients received 1 dose, 24 pts received between 2–5 doses and 7 patients received 6 or more doses.
†11 patients received 1 dose, 1 received 2 doses 10 days apart and 3 patients had an unspecified schedule.
‡Cidofovir (*n*=2), Foscarnet (*n*=3).

followed haematopoietic stem cell transplantation (HSCT). Twenty-six patients (62%) received rituximab weekly, while one patient received it monthly. Eleven patients received a single dose, one patient received two doses given 10 d apart, and for three patients the schedule was not specified.

All patients received concomitant therapy with etoposide (n = 38), dexamethasone (n = 41) and/or ciclosporin (n = 32). Twenty-seven patients (64%) received anti-viral medications and 33 (79%) received intravenous immunoglobulin. Twenty patients (48%) underwent allogeneic (HSCT) and among these, 11 (55%) harboured HLH gene mutations. The remaining nine patients had at least one reactivation, suggesting a genetic form of disease. Consistent with the presence of a verified or suspected diagnosis of familial HLH, the median age at presentation was 3·5 years for those going on to HSCT and 14·5 years for the non-transplanted patients.

Statistical analysis

Comparisons of laboratory parameters before and after the first rituximab dose were completed using the Wilcoxon matched-pairs signed rank test. Univariate associations between clinical and laboratory features at diagnosis and response to rituximab were analysed using the log-rank test. Kaplan–Meier curves were created using STATA 11.1 (StataCorp, College Station, TX, USA) software with time to last follow-up or death as endpoints. Statistical significance was declared at $P \leq 0{\cdot}05$.

Results

Clinical effects of rituximab-containing regimens

After initiation of a rituximab-containing regimen, 18 of the 42 patients (43%) improved clinically, with resolution of fever and reduction in hepatosplenomegaly and fluid retention. To assess quantitatively whether rituximab-containing regimens improved HLH manifestations, laboratory data were collected prior to and within 2–4 weeks after the first course of rituximab-based chemo-immunotherapy (Fig 1). Among 36 patients for whom serial data were available, treatment was associated with a 500-fold reduction in EBV load (median load pre-rituximab: 114 200 copies/ml; median load post-rituximab: 225 copies/ml; P = 0·0001). In 22 patients (61%), the EBV load dropped to <1000 copies/ml (n = 14) or fell below the limits of detection (n = 8). Similarly, ferritin, a surrogate for HLH activity (Allen *et al*, 2008; Lin *et al*, 2011), exhibited a 3·7-fold reduction (median ferritin pre-rituximab: 4260 μg/l; median post-rituximab: 1149·5 μg/l; P = 0·0001). Other changes included a significant increase in platelet count and reduction in aspartate transaminase (AST), and a lowering of alanine transaminase (ALT) that neared but did not reach statistical significance.

Side effects of rituximab

Fifteen patients (36%) experienced immediate (within 1 d) or later (all other times) side effects. Of these, eight patients developed one or more infusion-related toxicities, including fever (n = 7), chills (n = 2) or other symptoms (n = 5; respiratory distress, facial flushing/swelling, myalgia, urticaria). Two patients developed tachycardia and hypotension, requiring a decrease in the rate of infusion or discontinuation of the rituximab. Later side effects included hypogammaglobulinaemia (n = 5), neutropenia (n = 4) and transaminitis (n = 1). Of the five patients with hypogammaglobulinaemia, 4 underwent genetic analysis and 3 harboured mutations (one each in *XIAP*, *SH2D1A*, *STXBP2*). No patients died within 24 h after receiving a dose of ritumximab or experienced reactivation of hepatitis B, hepatitis C, cytomegaolvirus, herpes simplex virus, parvovirus, varicella zoster virus or West Nile virus.

Overall survival

Twenty-six (62%) patients were alive at the time of analysis with a median duration of survival of 1120 d since EBV-HLH diagnosis (range 230–3750). Sixteen patients died at a median of 97·5 d (range 22–900) due to HLH/multi-sys-

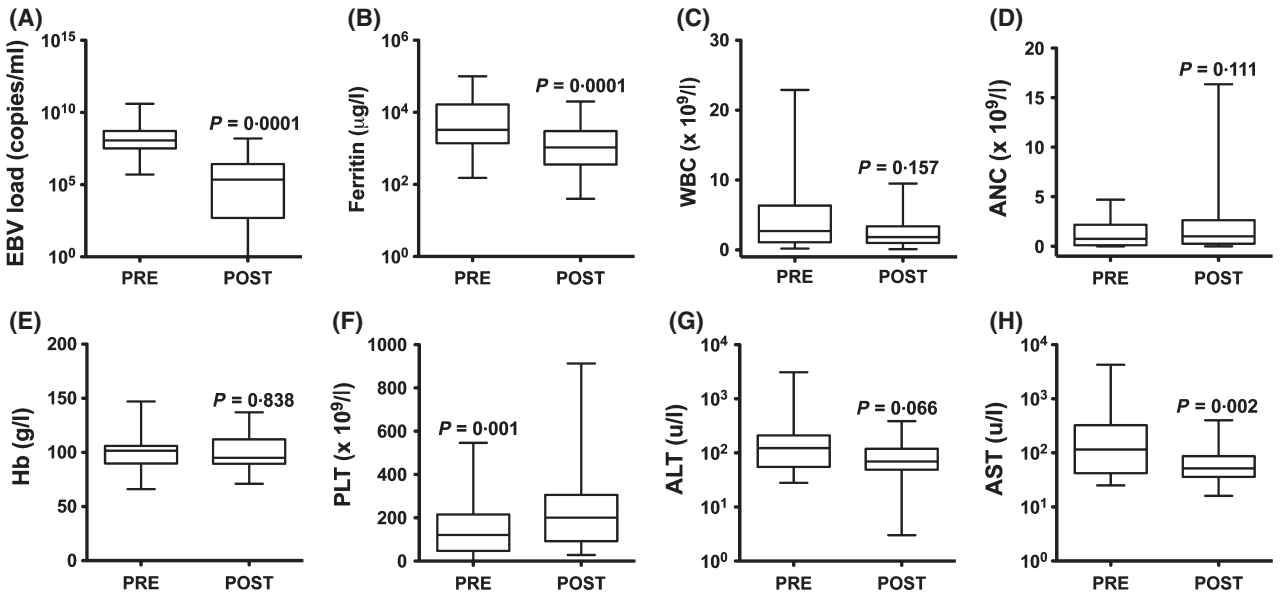

Fig 1. Laboratory parameters before and after administration of the first dose of rituximab: Box plots representing laboratory values prior to and within 2–4 weeks after delivery of the first dose of rituximab. The central line of the box plots represents the median value with the whiskers representing the minimum and maximum levels. Laboratory parameters included: Epstein-Barr virus (EBV) DNA levels (n = 36; A), ferritin (n = 35; B), white blood cell count (WBC, n = 37; C), absolute neutrophil count (ANC, n = 36; D); haemoglobin concentration (Hb, n = 37; E), platelet count (PLT, n = 37; F), alanine transaminase (ALT, n = 36; G), aspartate transaminase (AST, n = 36; H). Statistical associations between pre- and post-rituximab values were calculated using the Wilcoxon matched-pairs signed rank test.

tem organ dysfunction (n = 10), with or without infection (n = 5), toxicities of stem cell (n = 3) or multi-visceral transplantation (n = 1), cerebral bleeding (n = 1), lymphoma (n = 1) or EBV-associated lymphoproliferative disease (n = 1). Of the 26 surviving patients, 24 exhibited no evidence of active disease. Fourteen of these 24 (58%) had received an allogeneic HSCT. Within the 10 non-transplanted patients, two experienced $\geq$1 HLH reactivation and in both patients, repeat treatment with rituximab-containing regimens was sufficient to control disease.

Prognostic factors

To ascertain whether specific clinical or laboratory factors at diagnosis predicted response to the first course of a rituximab-containing regimen, we examined the relationship between presenting parameters [age, gender, EBV load, ferritin, white blood cell count, absolute neutrophil count (ANC), haemoglobin, platelet count, sIL2RA level, NK function, presence of HLH mutations, bilirubin, AST, ALT, albumin, number of days until the first rituximab dose] and reduction in EBV load and/or ferritin (defined as a drop in viral load to $\leq$1500 copies/ml and/or ferritin to $\leq$1000 μg/l). Although none of the features examined were predictive of a combined response, higher diagnostic ferritin levels inversely correlated with a drop in ferritin to $\leq$1000 μg/l post-rituximab [odds ratio 0·38, 95% confidence interval (CI) 0·19–0·75; P = 0·005]. Conversely, a higher ANC at presentation significantly correlated with a drop in viral load to $\leq$1500 copies/ml post-rituximab (odds ratio 2·14, 95% CI 1·14–4·00; P = 0·018). To determine whether the degree of response to the first course of a rituximab-containing regimen impacted upon long-term outcomes, Kaplan Meier analyses were completed and revealed that patients whose EBV load dropped to $\leq$1500 copies/ml (alone or combined with a drop in ferritin) were significantly more likely to survive compared to those whose viral loads remained >1500 copies/ml (Fig 2).

Discussion

The management of EBV-HLH remains challenging, with $\geq$30% patients dying of the disease or its complications (Imashuku, 2011; Qin *et al*, 2012; Shiraishi *et al*, 2012). It is proposed that outcomes in infection-associated HLH are improved if one targets the inciting pathogen. We hypothesized that the depletion of B cells, which serve as a reservoir for EBV, might ameliorate the signs and symptoms and improve the survival rate for patients with EBV-HLH. To test this hypothesis, we analysed data from 42 patients who had received treatment with rituximab-containing regimens. Chemo-immunotherapeutic approaches incorporating rituximab appeared well tolerated and, in most patients, significantly reduced EBV load and/or serum ferritin levels. In addition, patients whose viral load dropped to $\leq$1500 copies/ml were significantly more likely to survive their disease than those whose viral loads remained elevated. These data lend credence to the potential beneficial effects of rituximab and they concur with prior reports linking a drop in

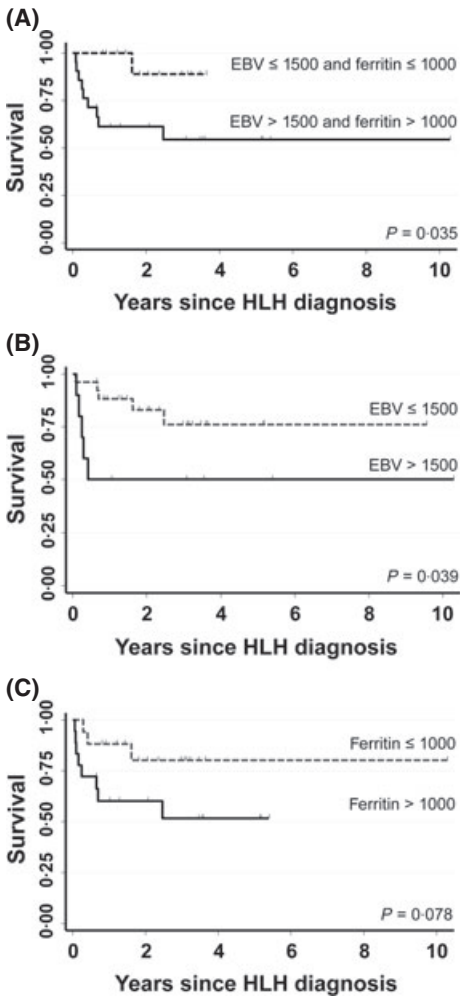

Fig 2. Probability of survival. Kaplan–Meier estimates of the probability of survival for the 42 patients in this cohort based on a combined reduction in viral load to $\leq$1 500 copies/ml and ferritin to $\leq$1 000 μg/l (A), reduction in viral load alone (B) or reduction in ferritin alone (C). Statistical differences between groups were evaluated using the log-rank test.

viral load with improved survival in EBV-HLH (Teramura *et al*, 2002).

Epstein–Barr virus infects B as well as non-B cell populations in HLH patients (Kawaguchi *et al*, 1993; Beutel *et al*, 2009; Yang *et al*, 2012) and there has been concern that a B cell depleting agent might not be effective in HLH. Nonetheless, physicians reported an immediate improvement in the signs and symptoms of HLH in 43% of patients. From a molecular perspective, treatment with the initial course of a rituximab-containing regimen led to a significant drop in viral load, and in 78% of patients for whom serial data were available, viral DNA fell within the normal range or below the limits of detection. As rituximab does not deplete T or NK cells, we presume that EBV was residing within a significant proportion of the B cells in responding patients. Alternatively, the administration of etoposide and/or dexamethasone could have reduced T and NK cell numbers and facilitated the clearance of virus residing in these cell populations.

Immediate toxicities of rituximab include infusion-related fever, chills, hypotension and bronchospasm (Kimby, 2005). Surprisingly, only seven patients (17%) in the current cohort were reported to develop a new fever while receiving rituximab and only two (5%) developed hypotension. After treatment with rituximab, B cell numbers are depleted for months, but immunoglobulin levels generally remain within the normal range (Kimby, 2005). Consistent with these data, only five patients in this cohort developed hypogammaglobulinaemia. Notably, of these individuals, three harboured mutations in *SH2D1A*, *XIAP* or *STXBP2*, genes associated with the development of humoral immune defects (Meeths *et al*, 2010; Booth *et al*, 2011; Pachlopnik Schmid *et al*, 2011). As a result, it is not possible to determine whether the low immunoglobulin levels in these patients resulted from the rituximab itself or instead from an underlying genetic predisposition. Other rare yet significant side effects of rituximab include development of thrombocytopenia, neutropenia and anaemia (Kimby, 2005; Wolach *et al*, 2010), and reactivation of specific viral infections (Aksoy *et al*, 2007). Within this cohort, four patients developed neutropenia; however, none experienced reactivation of hepatitis B, hepatitis C or other viral infections. Collectively, these data suggest that rituximab is safe, even when given to HLH patients who are often critically ill and receiving concomitant cytotoxic and/or immunosuppressive agents.

There are several limitations of this study that warrant consideration. First, the sample size is small, which limits power to detect associations between diagnostic parameters or variations in treatment and response. By completing this investigation on an international level, we attempted to capture as many EBV-HLH patients as possible. Second, this cohort is retrospective and contains patients with familial as well as non-familial HLH. While all patients received rituximab, it was given at varying schedules and dosages and always along with other medications. Thus, there are potential reporting biases and confounding factors, which make interpretation of these data challenging. Nonetheless, this study demonstrates that rituximab-containing regimens significantly reduce EBV load and signs of inflammation. The retrospective nature of this study does not allow us to determine the efficacy of rituximab as a treatment for EBV-HLH; however, it provides the evidence needed to move forward with a prospective clinical trial to address this important and clinically relevant, but currently unanswered question.

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Author contributions

D.C. designed the research, analysed data and wrote the manuscript. S.W. and K.Z. collected and analysed data. R.D. assisted with data interpretation and preparation of figures. H.Z. completed statistical analyses. K.E.N. oversaw all analyses, and edited and revised the manuscript.

Conflict of interest

The authors declare that there are no conflicts of interest.

Appendix I

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