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CASE REPORT

A case of T-cell-Epstein–Barr virus-haemophagocytic lymphohistiocytosis and sustained remission following ruxolitinib therapy

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Abstract

Objectives. Epstein–Barr virus (EBV) is a common cause of secondary haemophagocytic lymphohistiocytosis (HLH). While B cells are reservoirs for EBV, infection within T cells and NK cells in this disease can be difficult to treat. **Methods.** A 19-year-old female presented with a 6-week history of coryzal symptoms on a background of Crohn's disease. On examination, she was febrile and tachycardic with mild tonsillar enlargement and splenomegaly. New trilineage cytopenias and elevation in liver enzymes were detected, with acute EBV subsequently confirmed on whole blood PCR. A diagnosis of EBV-associated HLH was supported further with elevated serum ferritin, triglycerides and soluble CD25, low fibrinogen and the presence of haemophagocytosis in the bone marrow. **Results.** Corticosteroids, IVIG and rituximab were given, and anakinra was subsequently added due to ongoing fevers. EBV infection was then demonstrated within $CD8^+$ T cells on EBER Flow-FISH assay. Ruxolitinib was commenced and her fevers abated on day 5, with improvement in other HLH parameters. She was discharged after a 39-day hospital admission. To date, she has remained in remission of HLH, despite developing COVID-19 infection during the convalescence phase of HLH. **Conclusion.** EBV viraemia requires adequate treatment to control EBV-associated HLH as rituximab may be insufficient, and corticosteroid resistance can result in continued EBV infection in $CD8^+$ T cells. This entity is known as T-cell-EBV-HLH. Ruxolitinib is a novel treatment strategy in this specific context and has several advantages, including inhibition of corticosteroid resistance to promote apoptosis of EBV-infected T cells.

Keywords: EBV infection, haemophagocytic lymphohistiocytosis, ruxolitinib, T-cell-EBV-HLH

INTRODUCTION

Haemophagocytic lymphohistiocytosis (HLH) is a complex disease with high morbidity and mortality. Primary HLH is due to genetic defects in the natural killer (NK) cell cytotoxicity pathway.[1] Secondary HLH is triggered by many causes including lymphoproliferative disorders, autoimmune rheumatic diseases, and infections.[1] Epstein–Barr virus (EBV) is most frequently associated with secondary HLH and can lead to a prolonged disease course.[2] More recently, coronavirus disease 2019 (COVID-19) has been associated with the development of secondary HLH and also is implicated in HLH recurrence from other causes.[3,4]

We present a case of T-cell-EBV-HLH responsive to ruxolitinib, followed by acute COVID-19 in the convalescence phase that did not result in HLH recurrence.

CASE REPORT

A 19-year-old female presented with a 6-week history of coryzal symptoms in addition to worsening fevers, shortness of breath and new-onset abdominal pain. Her background history included Crohn's disease controlled on infliximab, allergic rhinitis, asthma and depression.

On presentation, she was febrile (temperature: 40°C) and tachycardic (heart rate: 122 beats per minute). Mild tonsillar enlargement was present without lymphadenopathy. There was right upper quadrant abdominal tenderness and palpable splenomegaly.

Laboratory parameters showed new trilineage cytopaenia: haemoglobin 109 g L^{-1} (115–155 g L^{-1}), platelet counts 84 $\times$ 10^9 L^{-1} (150–450 $\times$ 10^9 L^{-1}) and neutrophil count 1.48 $\times$ 10^9 L^{-1} (1.80–7.50 $\times$ 10^9 L^{-1}). There was elevation of liver enzymes: aspartate aminotransferase (AST) of 527 U L^{-1} (0–45 U L^{-1}), alanine aminotransferase (ALT) of 1188 U L^{-1} (0–55 U L^{-1}) and gamma-glutamyl transferase (GGT) of 132 U L^{-1} (0–60 U L^{-1}) (Figure 1b). Inflammatory markers C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) were normal, and autoimmune serology was not contributory.

Acute EBV infection was confirmed on whole blood nucleic acid test (NAT) with a viral load of 1 304 893 copies mL^{-1}. Computer tomography (CT)

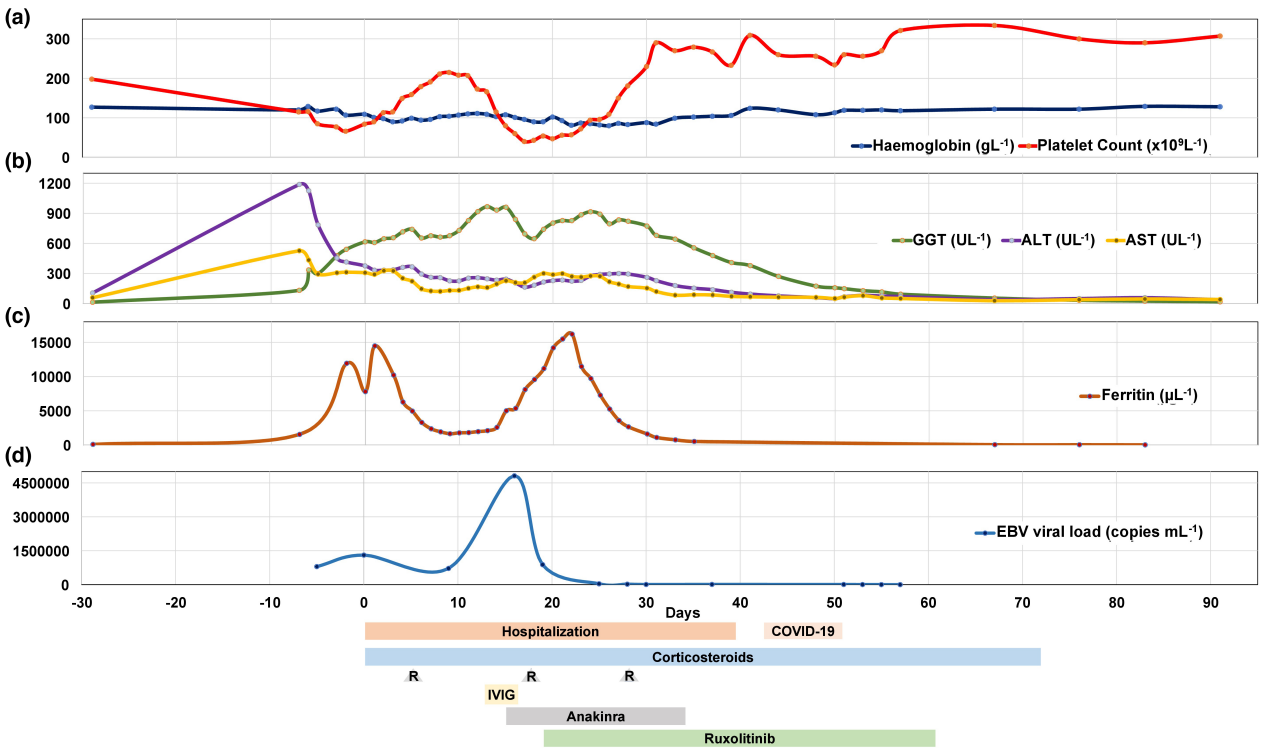

Figure 1. Graphical representation of some important laboratory parameters and treatment. **(a)** Haemoglobin and platelet count, **(b)** liver enzymes, **(c)** ferritin and **(d)** EBV viral load. ALT, alanine transaminase; AST, aspartate transamine; EBV, Epstein–Barr virus; GGT, gamma-glutamyl transferase; R, rituximab.

of the abdomen demonstrated splenomegaly measuring 17 cm and features of acute cholecystitis.

Iron studies revealed an elevated ferritin of 11 937 μg L^{-1} (30–250 μg L^{-1}) and HLH secondary to EBV infection was suspected. Further investigations supported this diagnosis: elevated triglycerides (2.6 mmol L^{-1}; 0.3–2.0 mmol L^{-1}) and soluble CD25 (sCD25) (30 625 pg mL^{-1}; < 2678 pg mL^{-1}), reduced fibrinogen (1.05 g L^{-1}; 1.5–4.0 g L^{-1}) and presence of haemophagocytosis in the bone marrow (Figure 2). Collectively, her *H* score was 258, indicating a > 99% probability of HLH.[5] A genetic cause for HLH has not identified on trio next-generation whole-exome sequencing of the patient and her parents. This included phenotyping analysis using PanelApp UK Primary Immunodeficiency v2.527 panel and PanelApp Australia Immunological disorders_superpanel v8.51.

Bone marrow and peripheral blood flow cytometry showed an activated T-cell immunophenotype (HLA-DR^+) on 15% of $CD8^+$ T cells as well as an aberrant population of $CD8^+$ T cells ($CD2^+CD5^{dim/-}CD7^{dim}$) comprising 22% of total lymphocytes (Figure 3).

Intravenous methylprednisolone (1 g) was given for 3 days, followed by oral prednisolone at 1 mg per kg per day. She received 3 days of intravenous immunoglobulin (IVIG) therapy, in addition to antibiotic therapy for acute cholecystitis. Infliximab was withheld as Crohn's disease was quiescent with no symptoms and normal faecal calprotectin (FCP).

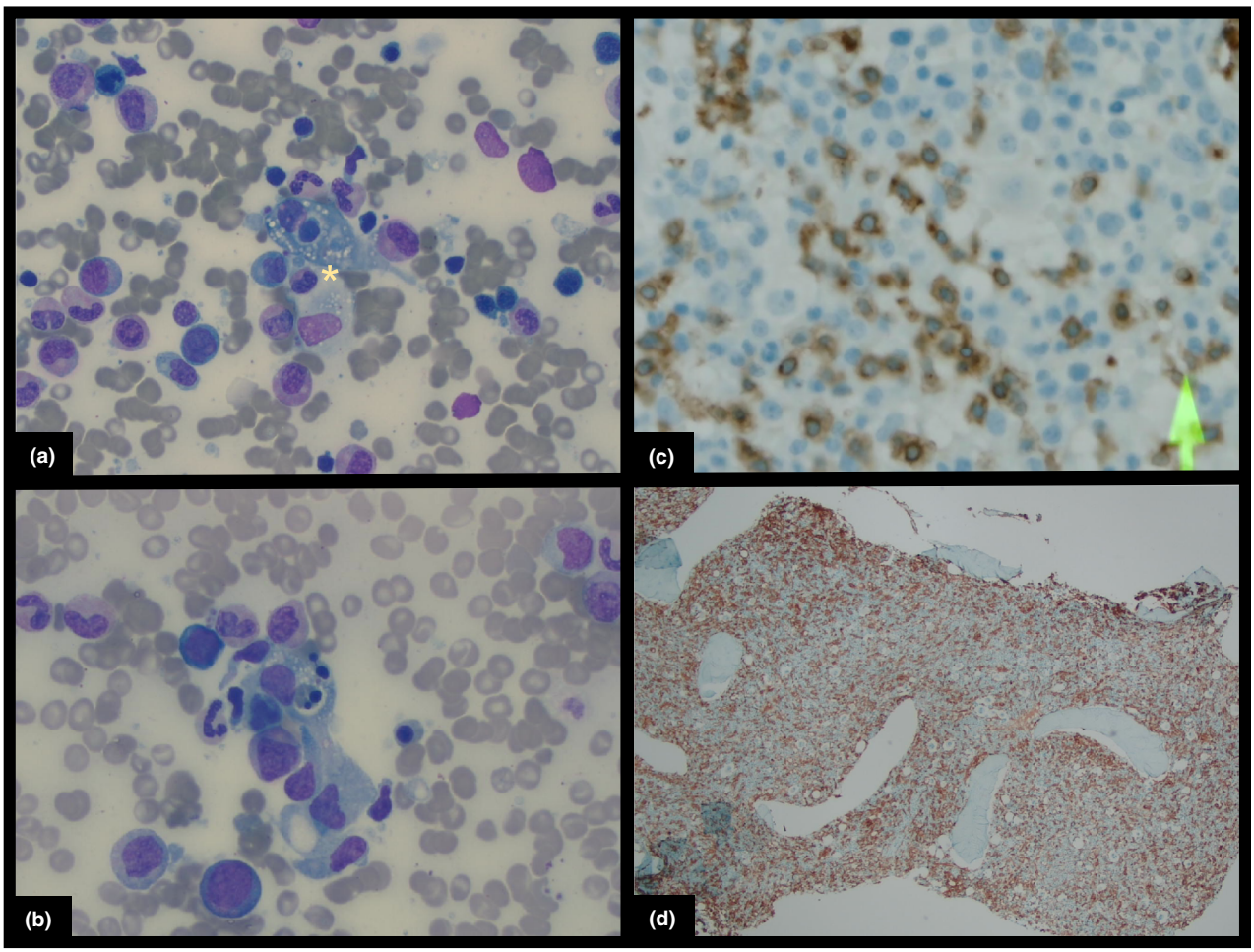

Figure 2. Morphology and immunohistochemistry of bone marrow biopsy. **(a)** Haemophagocytosis, one metamyelocyte within a histiocyte (*), **(b)** erythrophagocytosis (arrow), **(c)** CD3-staining indicative of prominent T cells of intermediate size with occasional large cells (arrow) and **(d)** CD163-staining demonstrating increased histiocytes. *Immunohistochemistry method*: Formalin-fixed, paraffin-embedded bone marrow trephine processed on automated Ventana BenchMark ULTRA® platform with CD3 and CD163 monoclonal antibodies and presence identified by *ultra*View Universal DAB Detection Kit™.

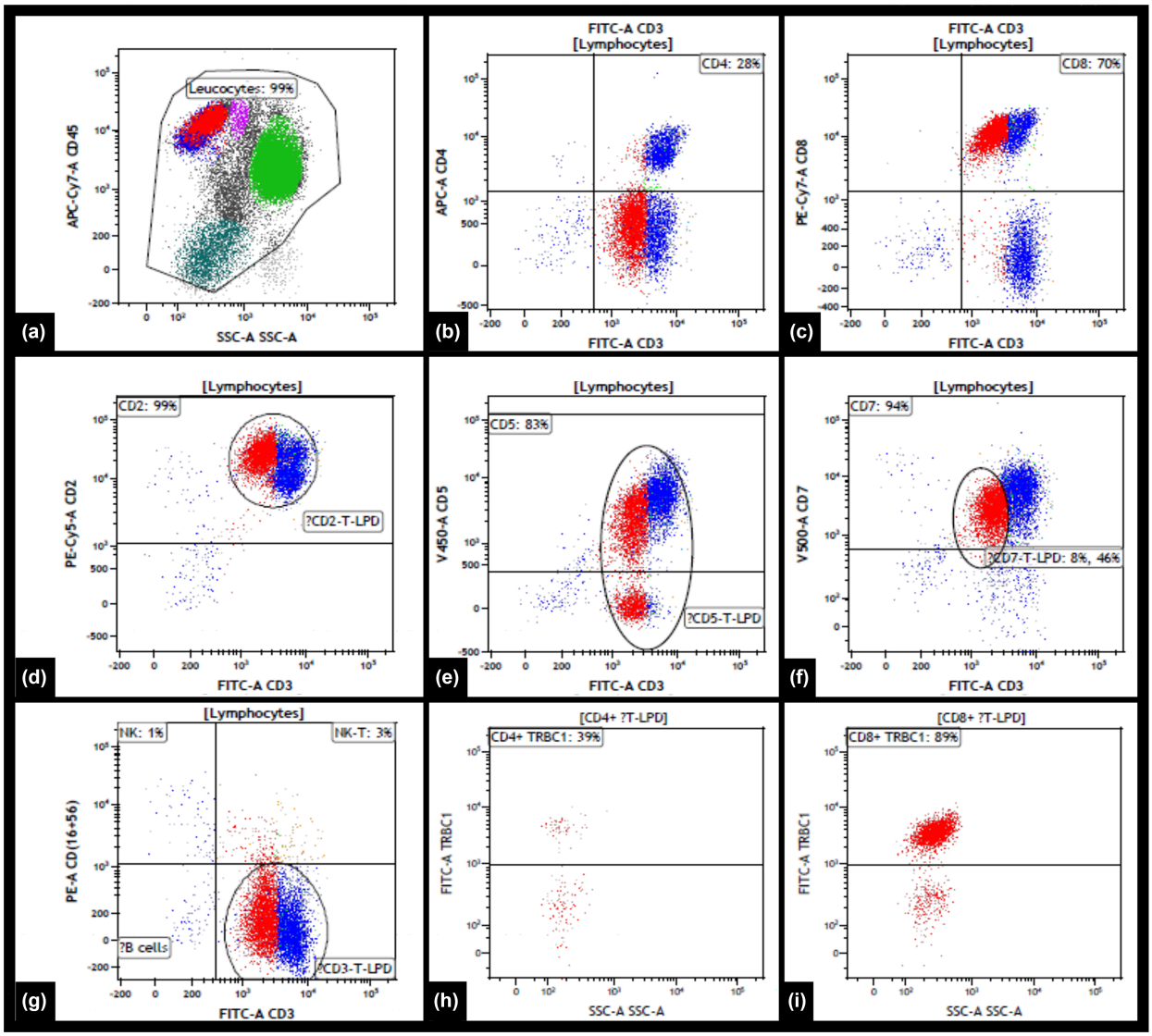

Figure 3. Flow cytometry. Immunophenotyping of bone marrow, indicating lymphocytes (blue) with aberrant $CD8^+$ T cells (red) expressing CD2 **(d)**, dim to negative CD5 **(e)**, dim CD7 **(f)** and TRBC1 expression **(i)**. *Flow cytometry method*: Bone marrow aspirate sample is lysed, washed, reconstituted in RPMI medium and incubated with monoclonal antibodies (CD45, CD3, CD2, CD5, CD7, CD16/56 and TRBC1) tagged with fluorescent dyes. Data were acquired through BD FACSCanto II™ flow cytometer and analysed on Beckman Coulter Kaluza C software. The gating strategy identifies lymphocytes by CD45 and side scatter **(a)** followed by gating CD3 against $CD4^+$ T cells **(b)** and $CD8^+$ T cells **(c)**, T-cell surface markers on these $CD4^+$ and $CD8^+$ T cells; CD2 **(d)**, CD5 **(e)**, CD7 **(f)**, CD16/56 **(g)** and TRBC1 on $CD3^+$ $CD4^+$ T cells **(h)** and $CD3^+$ $CD8^+$ T cells **(i)**.

DIFFERENTIAL DIAGNOSIS AND TREATMENT

There was an initial improvement in fevers and laboratory parameters; however, these were transient (Figure 1). Although rituximab was given with subsequent B-cell depletion confirmed on peripheral blood immunophenotyping, she had ongoing viraemia (Figure 1d). Anakinra was added and oral prednisolone switched to oral dexamethasone on day 20 of admission. She continued to have high-grade fevers but remained haemodynamically stable. There were no features to suggest an alternate cause for fevers such as another infective process or a malignancy as a cause for secondary HLH.

Trilineage cytopaenias and liver enzymes continued to worsen, accompanied by rising EBV viral load (Figure 1d). EBV-encoded small RNA (EBER) flow cytometric *in situ* hybridisation (Flow-FISH) assay demonstrated EBER present in 16% of $CD8^+$ T cells (Figure 4).

Escalation in treatment options at this stage included chemotherapy using the HLH-2004 protocol and haematopoietic stem cell transplant (HSCT).[6] The HLH-2004 protocol was considered; however, its significant adverse effect profile in this young patient and overall high morbidity

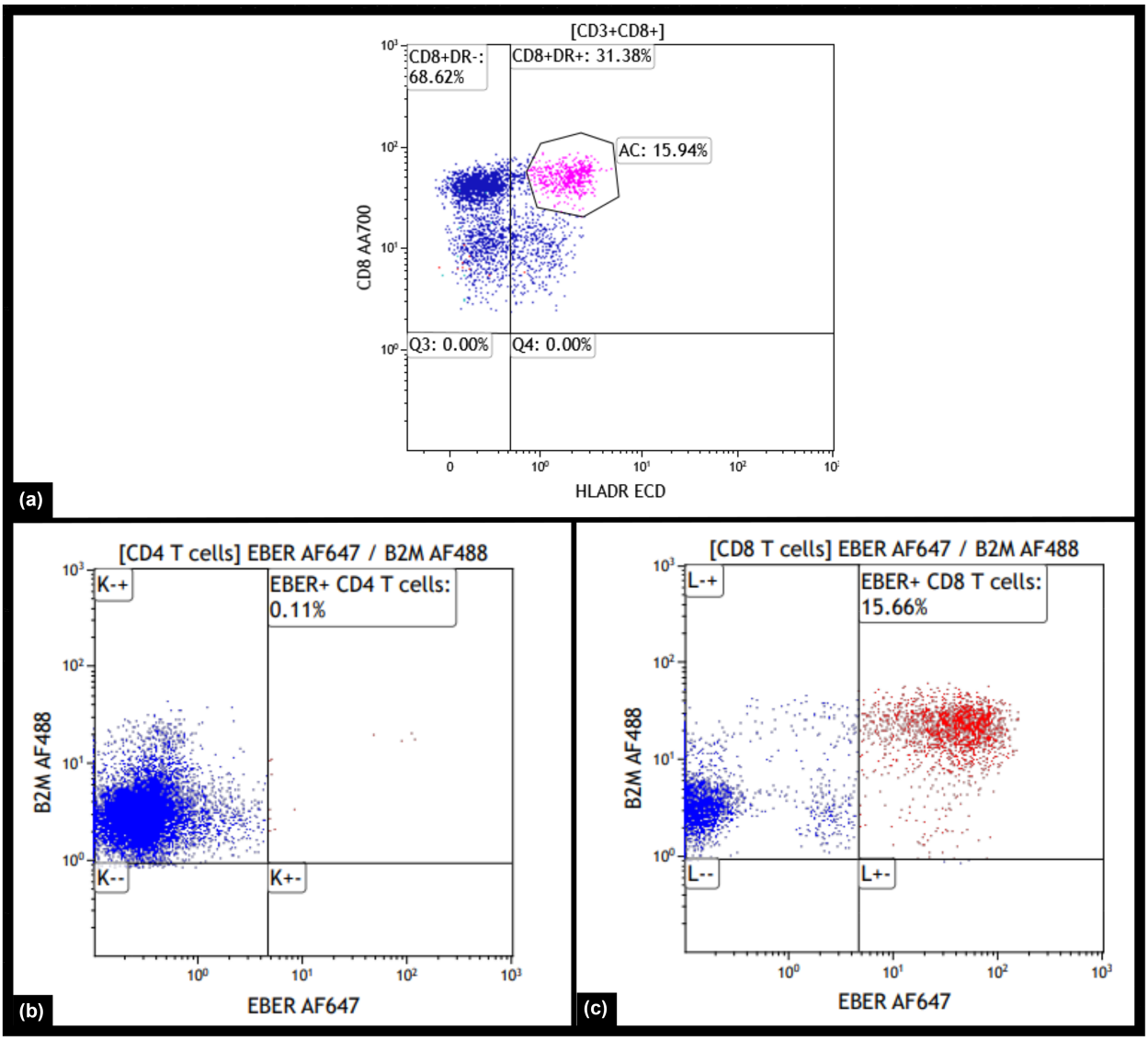

Figure 4. Flow cytometry. Immunophenotyping of whole blood demonstrating an activated $CD8^+$ T-cell population (pink) comprising 15.94% of all $CD8^+$ T cells **(a)**. EBER Flow-FISH on PBMCs demonstrating EBER-positive $CD8^+$ T cells (red) comprising 15.66% of all $CD8^+$ T cells **(c)**, while no EBER positivity detected on $CD4^+$ T cells **(b)**. *Flow cytometry method*: Whole blood incubated with DURAClone IM TCRs pre-formulated antibody panel. The gating strategy includes the identification of lymphocytes *via* CD45 and side scatter and activated $CD8^+$ T cells defined as $CD3^+$ $CD8^+$ $HLA\text{-}DR^+$ lymphocytes. *EBER Flow-FISH method*: Surface staining of PBMCs using CD45, CD3, CD4, CD8, CD19 and CD56. The PrimeFlow™ RNA Assay kit was used to fix and permeabilize cells and perform *in situ* hybridization with EBER 1/2 Type 1 AF647 and B2M Type 2 AF488 probes. The gating strategy includes the identification of lymphocytes using CD45 and side scatter and $EBER^+$ $CD8^+$ T cells defined as $CD3^+$ $CD8^+$ $B2M^+$ $EBER^+$ lymphocytes.

made it an option only if the patient were to develop haemodynamic instability or signs of end-organ compromise. Similarly, HSCT would only be pursued if no improvement given the immediate and long-term complications of this therapy. Therefore, the novel JAK inhibitor, ruxolitinib, was selected as the next treatment given its emerging role in the treatment of T-cell-EBV-HLH. As the patient had haemodynamic stability and was being managed in a regular hospital ward rather than an intensive care unit setting, a trial of ruxolitinib was pursued as the next step, and if no improvement, then HLH-2004 protocol and HSCT were to be considered.

Ruxolitinib 10 mg twice daily (BD) was started with dose escalation to 40 mg BD after 48 h. Her fevers abated on day 5 of this higher dose, and over the following 2 weeks, there was a decline in ferritin and liver enzymes as well as improvements in cytopaenias (Figure 1). Anakinra was ceased and dexamethasone changed back to prednisolone on day 34 of admission. Her laboratory parameters continued to improve and ruxolitinib dose was reduced to 20 mg BD. She was discharged after a 39-day hospital admission.

Four days following discharge, she developed acute COVID-19 infection. Nirmatrelvir/ritonavir (Paxlovid) was prescribed, but an interaction with ruxolitinib was present when using COVID-19 Drug Interactions Checker (www.covid19-druginteractions.org) in which Paxlovid, a strong CYP3A inhibitor, and ruxolitinib which metabolises through this enzyme, had a theoretical risk of increasing ruxolitinib levels.[7] Therefore, ruxolitinib dose was reduced to 5 mg BD. Her laboratory parameters were monitored closely, with only transient neutropaenia present in this period. HLH parameters were normalised including an undetectable EBV viral load a fortnight later, and ruxolitinib was ceased. Prednisolone was weaned to 5 mg and then ceased 2 weeks later.

Infliximab was restarted 4 weeks after COVID-19 diagnosis and is being well tolerated. The patient continues to be in treatment-free remission 6 months later, with normalisation of splenomegaly and no further detection of the aberrant $CD8^+$ T-cell population in peripheral blood.

DISCUSSION

Our patient had many of the classical features of HLH: fever, organomegaly, trilineage cytopaenia, elevated ferritin, triglycerides, liver enzymes, low fibrinogen and haemophagocytosis in the bone marrow.[8] Although she remained haemodynamically stable throughout her admission, she had high-grade fevers and abnormal laboratory parameters which did not respond to IVIG, rituximab and anakinra. While there is extensive evidence on the utility of rituximab to clear EBV infection in B cells, further treatment strategies need to be considered if EBV infection involves other immune cells such as T cells or NK cells.[9,10] Infections within these cells have been associated with a poorer prognosis for EBV-associated HLH, and regarding T cells, this subset is termed T-cell-EBV-HLH.[10] The persisting infection within T cells can also lead to clonal proliferation.[11–13] As observed in our patient, infection within $CD8^+$ T cells was responsible for ongoing EBV viremia despite B-cell depletion and was confirmed by EBER Flow-FISH demonstrating EBV-infected $CD8^+$ T cells (Figure 4).

Treatments for HLH were derived from the original HLH-1994 and revised in the HLH-2004 study protocol.[6,14] These still portend a poor prognosis, with a 5-year survival rate of 61% and are associated with a significant risk of myelosuppression and infection.[6] Recently, ruxolitinib, an oral selective Janus kinase (JAK) 1/2 inhibitor, was shown to be effective in mouse models of HLH.[15] Subsequently, ruxolitinib therapy has been reported as a treatment modality in both first-line and salvage treatment strategies in HLH.[16–18] In a small pilot trial in eight paediatric patients with EBV-HLH, ruxolitinib provided an initial 100% response rate, and in one patient, persistent EBV infection required myeloablative treatment.[17] In another study, 54 patients were treated with ruxolitinib as a first-line agent, and all responded within 3 days, with 69.2% with sustained remission by day 28.[19] The doses used in this study were determined according to weight, with those above 20 kg given 10 mg BD and it is possible that the lower dose and individual differences in the pharmacokinetic response to ruxolitinib could attribute to the lower rates of sustained remission.[19] In refractory cases, the sustained responses are less durable, possibly due to chronicity of the disease process; in a case series of nine patients, only three achieved sustained remission.[20] Therefore, considerations with ruxolitinib institution include dosage and early administration.

Given ruxolitinib's mechanism of action in suppressing proinflammatory cytokines (IFN-γ, IL-10 and IL-6) and its rapid on-and-off characteristics, it is an attractive treatment option

in HLH. Furthermore, in the presence of high levels of STAT5-dependent cytokines, activated murine $CD8^+$ T cells become resistant to dexamethasone-induced cell death.[18] By blocking STAT5-dependent cytokine signalling, ruxolitinib restores corticosteroid-mediated apoptosis.[18]

In our patient with T-cell-EBV-HLH, rituximab therapy for EBV clearance was insufficient. Ruxolitinib treatment resulted in a reduction of both EBV viral load and aberrant $CD8^+$ T-cell population. Recently, alemtuzumab, a monoclonal anti-CD52 antibody, has been used in this subset of HLH in addition to traditional chemotherapy, with an aim to reduce the burden of activated mature T cells.[9] Of the three patients, one had a response, but only after HSCT.[9] Overall, this highlights that the T-cell-EBV-HLH entity carries with it a high morbidity, and the utility of ruxolitinib in this cohort needs to be explored in larger studies. This is also important as this treatment may have fewer adverse effects compared with alternate therapies such as chemotherapy and alemtuzumab.

Our patient tolerated the higher dose of ruxolitinib with improvement in laboratory parameters and fevers. There was a delay in the resolution of anaemia which improved following the reduction in ruxolitinib dose. Anaemia is a common adverse effect of ruxolitinib, particularly at higher doses, while other less commonly reported adverse effects include thrombocytopenia, increased risk of infection and non-melanoma skin cancer.[17,21,22]

Our case is unique as she developed acute COVID-19 during the convalescence phase of HLH, while on ruxolitinib therapy. This patient had two COVID-19 vaccines, with her last more than 8 months prior. It is possible that the patient may have recovered without Paxlovid, as it is recognised that COVID-19 infection can be mild even when risk factors for severe disease are present, such as receiving rituximab treatment. Alternatively, it is also possible that concurrent ruxolitinib and Paxlovid during acute COVID-19 infection prevented the severity of the disease. This potential effect may be reflected in the routine use of baracitinib for those with moderate to severe COVID-19 infection, in addition to corticosteroids.

CONCLUDING REMARKS

EBV-associated HLH can be difficult to treat when EBV viraemia is persistent. Prompt recognition of HLH is required to initiate early treatment and enable chances of remission. Ruxolitinib as a first-line treatment in HLH is emerging as a useful treatment modality, and in those with EBV-associated HLH, should be considered. In the convalescence phase of HLH, patients who develop symptoms of viral infections, such as COVID-19, must be screened with relevant testing and treated accordingly, to ensure no reactivation of HLH.

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AUTHOR CONTRIBUTIONS

Syed Ali: Conceptualization; writing – original draft. **Sharon Choo:** Methodology; writing – review and editing. **Laine Hosking:** Methodology; writing – review and editing. **Anthony Smith:** Writing – review and editing. **Tiffany Hughes:** Conceptualization; writing – review and editing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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