Hemophagocytic lymphohistiocytosis syndrome associated with Epstein-Barr infection in an immunocompetent patient. A case study

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Abstract

Introduction Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening rare disease resulting from the uncontrolled activation of the immune system, leading to unrestrained cytokine release and macrophage activation. It can be either hereditary or acquired due to infections, hematological disease or malignancy.

Case report We present the case of a 19-year old woman that presented with high fever and acute cholestatic hepatitis. She was initially admitted to the Gastroenterology department and the following days she developed respiratory distress and multiorgan insufficiency that necessitated intubation and support in the Intensive Care Unit. Fever, splenomegaly, hypertriglyceridemia, increased ferritin levels and hemophagocytosis in the bone marrow were found, thus, fulfilling the criteria of hemophagocytic lymphohistiocytosis. Laboratory examination was notable for positive serology (IgM and IgG) and PCR for EBV in the serum. An extensive workup including virology and immunologic workup, blood cultures, a CT of the thorax and the abdomen and a bone marrow biopsy did not reveal any cause of secondary HLH other than the EBV infection. The patient was treated with high dose corticosteroids and intravenous immunoglobulins with slow resolution of her symptoms.

Conclusions In patients with EBV infection who exhibit persistent high fever and unresponsiveness to antibiotics, the possibility of HLH should be considered. Early diagnosis and rapid initiation of appropriate treatment may avert an unfavorable outcome.

Keywords Hemophagocytic lymphohistiocytosis, macrophage activation syndrome, EBV, Epstein-Barr

Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a rare, life-threatening disease that results from uncontrolled immune activation leading to unrestrained cytokine release and macrophage

activation that can lead to multiorgan dysfunction.¹ HLH can present in either the primary or hereditary type, which is most commonly seen in children and young adults, or the secondary or acquired type, which is most

Received: 27 April 2020; revised: 04 July 2020; accepted: 16 July 2020.

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Article downloaded from www.germs.ro

Published September 2020

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ISSN 2248 - 2997

ISSN - L = 2248 - 2997

commonly encountered in adults and is often triggered by bacterial, viral, fungal or protozoal infections, autoimmune diseases or malignancies.^{1,2} If left untreated, HLH carries a high mortality rate of 58% - 75% in adults.^{1,3} Its treatment includes identification and treatment of the underlying cause, and in some cases, specific treatment for HLH depending on the etiology, such as dexamethasone, intravenous immunoglobulin (IVIG), etoposide cyclosporine A, and rituximab for infection-associated HLH, cyclosporine, IVIG, and etoposide for malignancy-associated HLH, cyclosporine and anakinra for autoimmune-associated HLH, and cyclosporine and tocilizumab for drug-induced HLH.^{4,5} We present the case of a young woman that presented with HLH associated with a concurrent EBV infection.

Case report

A 19-year old Caucasian woman with no significant past medical history presented to the Emergency Department with fever up to 39°C for 10 days and vomiting. The clinical examination was notable for jaundice and splenomegaly, while laboratory examination revealed abnormal liver chemistry, with an SGOT of 628 U/L (normal range (nr) 0-35 U/L), SGPT of 432 U/L (nr 0-35 U/L), γ GT of 382 U/L (nr 0-38 U/L), ALP 117 (nr 30-120 U/L), total bilirubin of 18.59 mg/dL (nr 0.3-1.2 mg/dL) and direct bilirubin of 10.05 mg/dL (nr 0-0.2 mg/dL), while LDH was 2006 U/mL (nr 0-247 U/L). Hemoglobin was 10.6 g/dL (nr 12-16 g/dL), white blood cells were 14.1 K/ μ L (nr 3.8-10.5 K/ μ L) and platelets were 160,000 K/ μ L (nr 150-450 K/ μ L). An ultrasound of the upper abdomen revealed mild splenomegaly of 14 cm without any other abnormalities. The patient was admitted and treatment with intravenous levofloxacin and oral doxycycline was initiated and continued for three days. Further laboratory work-up revealed positive IgM and IgG for EBV viral capsid antigen and CMV, while PCR for CMV DNA in the serum was negative and PCR for EBV DNA in the serum was positive with a titer of 5.13×10^5 copies/mL. HAV IgM & IgG, HIV, HBsAg, IgM & IgG HBcAb, anti-HBs, anti-HCV, HCV RNA, HEV RNA, HSV-1 IgM &

IgG, HSV-2 IgM & IgG, parvovirus IgM & IgG, adenovirus IgM & IgG, varicella zoster virus IgM & IgG, *Leptospira* antibodies, PCR for respiratory pathogens were all negative. Blood cultures were positive only twice, however, the patient was febrile during the whole time of her hospitalization, despite treatment with appropriate antimicrobials and sterilization of the blood cultures. IgG against the Epstein-Barr nucleic antigen (EBNA) was negative, thus suggesting acute rather than chronic infection. Table 1 shows the laboratory workup and the antimicrobial treatment of the patient.

On the second hospital day a drop of the hemoglobin level was noted (down to 5 g/dL), and a direct Coombs test was positive (IgM $\pm$, C3d+) while reticulocytes were not increased (1.01%). A peripheral blood smear revealed the presence of activated lymphocytes and aggregation of red cells. Cryoglobulin was positive with a titer of 1:128. On the third hospital day the patient suddenly developed severe hypoxemia refractory to oxygen supplementation and was transferred to the Intensive Care Unit (ICU). An ultrasound of the lungs revealed the presence of bilateral pleural fluid that was tapped and was proved to be an exudate. Due to worsening respiratory function she was intubated on the third hospital day and was treated with high doses of norepinephrine due to fluid resistant hypotension. The fourth hospital day she became anuric and a urine sediment microscopic examination revealed the presence of muddy brown casts, suggesting acute tubular necrosis. Thus, continuous veno-venous hemofiltration was initiated. Further laboratory examination revealed increased triglycerides up to 1,722 mg/dL that persisted despite the discontinuation of propofol infusion. Furthermore, ferritin was increased, with a value of 18,685 ng/mL. During her 19-day stay in the ICU she remained febrile with a fever up to 40°C and was treated with acyclovir and several courses of antibiotics including tigecycline, daptomycin, meropenem, colistin and micafungin, without improvement. She was also treated with intravenous prednisolone in a dose of 0.7 mg/kg/day, but despite that she developed a maculopapular rash on the face, the torso and the extremities that

Table 1. Laboratory course and antimicrobial treatment of the patient

Day	CRP (mg/dL)	WBC (/μL)	Hb (g/dL)	Antimicrobial therapy	Blood cultures	PCR EBV (copies /mL)	EBV VCA IgM (U/mL)	EBV VCA IgG (U/mL)
ED		13,100	12.6	Doxy+levofl				
1	15	14,100	8.8	Doxy+levofl			12.4	1.42
2		26,100	5	Doxy+levofl				
3	19	26,100	8	Tigecycline		5.13x10 ⁵		
4		36,000	7.2	Tigecycline				
5		20,600	7.6	Meropenem				
6	10	15,700	8.1	Meropenem				
7		12,700	8.8	Meropenem				
8		8300	8.3	Meropenem				
9	1.88	10,100	8.1	Meropenem				
10		8,000	7.6	Meropenem	<i>A. baumannii</i>			
11		11,800	8.5	Colistin				
12		18,000	8	Colistin				
13	13.7	22,000	8	Colistin			64.6	38.8
14		22,300	6.2	Colistin				
15		16,400	7.2	Colistin				
16	19.8	18,100	7.8	Colistin	<i>S. epidermidis</i>			
17		19,100	7	Dapto+colistin+mica		4.73x10 ⁵		
18		16,700	8.7	Dapto+colistin+mica				
19		17,100	9.2	Dapto+colistin+mica				
20		13,500	7.3	Dapto+colistin+mica				
21	12.2	11,000	7.5	Dapto+colistin+mica				
22	28	12,600	9.1	Dapto+colistin+mica				
23		11,700	8.8	Dapto+colistin+mica				
24	6.65	14,400	9.2	Dapto+colistin+mica				
25		13,800	8.8	Dapto+colistin+mica				
26		16,800	8.6	Dapto+colistin+mica				
27	3.55	13,900	8.2	Dapto+colistin+mica				
28		19,200	8.1	Dapto+colistin+mica				
29				Dapto+colistin+mica				
30	2.68	22,200	8.7	Dapto+colistin+mica				

CRP – C reactive protein; **Dapto** – daptomycin; **doxy** – doxycycline; **EBV** – Epstein-Barr virus; **ED** – Emergency Department; **Hb** – hemoglobin; **levofl** – levofloxacin; **mica** – micafungin; **VCA** – viral capsid antigen; **WBC** – white blood cell count.

resolved the following days. Considering the possibility of hemophagocytic lymphohistiocytosis associated with EBV infection, the patient was treated with corticosteroid pulses and intravenous gamma-globulin at a dose of 0.4 gr/kg/day for 5 days. A bone marrow biopsy revealed the presence of hyperplastic bone marrow with many macrophages and evidence of

red blood cell phagocytosis, thus confirming the diagnosis of hemophagocytic lymphohistiocytosis. The patient remained intubated for 11 days, and after 19 days of ICU stay, she was transferred to the Internal Medicine Department where she was treated with corticosteroids and intermittent hemodialysis until her renal function was restored and fever subsided, while ferritin and

triglycerides were reduced. A computerized tomography (CT) of the thorax and the abdomen was performed and was unrevealing. The patient was discharged after one month of hospitalization and was followed up on an outpatient basis for one year, with no subsequent evidence of relapse or any sign of hematologic, malignant or autoimmune disease.

Discussion

The pathogenesis of secondary HLH involves gene polymorphisms as in primary HLH, as well as factors that trigger the development of the syndrome. EBV infection has been previously described to be a trigger for secondary HLH. In a series of 73 patients with HLH, EBV infection was identified as the causative factor in 5 cases.¹ EBV can lead to macrophage activation due to the increased production of TNF- α , IFN- γ , and other cytokines by the EBV-infected T-cells.⁶ On the other hand, EBV-associated HLH could be more frequent in patients with genetic predisposition, such as in patients with ITK deficiency, CD70 deficiency, CD27 deficiency and magnesium transporter 1 (MAGT1) deficiency.⁷

In our case, the patient developed fever, splenomegaly, hypertriglyceridemia, increased levels of ferritin and hemophagocytosis in the bone marrow, as revealed by bone marrow biopsy, fulfilling the diagnostic criteria for diagnosis of HLH,¹ as indicated below - five of the following 8 criteria must be fulfilled:

1. Fever
2. Splenomegaly
3. Bicytopenia or pancytopenia (absolute neutrophil count $<1 \times 10^9/L$, hemoglobin <9 g/dL, platelet count $<100 \times 10^9/L$)
4. Hypertriglyceridemia (>265 mg/dL) and/or hypofibrinogenemia (<150 mg/dL)
5. Hemophagocytosis in the bone marrow, spleen or lymph nodes
6. Hyperferritinemia (>500 mg/L)
7. Elevated soluble IL-2 receptor ($>2,400$ U/mL)
8. Absent or decreased NK cell function

Alternatively, a molecular diagnosis consistent with HLH suffices to establish the diagnosis.

The differential diagnosis of HLH can be broad and includes viral infections, fungal and bacterial infections, hematologic malignancy, autoimmune diseases, primary immunodeficiency, and post solid organ transplantation, but in many cases, no cause is found and HLH is then termed idiopathic.^{1,8} In the present case, the patient had an EBV infection, as proved by the positive EBV DNA PCR in the serum. An extensive workup including virology and immunologic workup, blood cultures, a CT of the thorax and the abdomen and a biopsy of the bone marrow did not reveal any other possible cause of secondary HLH. Furthermore, the patient was followed up for one year after discharge and no sign of malignancy or autoimmune disease developed, suggesting that EBV was the only factor that could be associated with the development of secondary HLH. On the other hand, it could be that the patient developed primary HLH that led to reactivation of EBV infection and persistent EBV viremia, even though this scenario seems unlikely due to the negative IgG EBNA that implies that EBV infection was acute, rather than chronic.⁷ To that end, even though the patient had not had an episode of HLH beforehand, the follow-up period after the episode had been one year, thus, the possibility of a relapse of HLH exists, even though a relapse of HLH would not necessarily mean that HLH was primary, but could also be secondary to another stimulus.

The mortality of HLH, if left untreated, is 95% with a very poor survival of less than 2 months; thus, early recognition of this fatal disorder is critical in order to reduce mortality.² The HLH-94 protocol recommended treatment with intravenous dexamethasone and etoposide, while treatment with cyclosporine is also suggested from the HLH-2004 in order to reduce the possibility of relapse.⁹ However, according to newer recommendations, dexamethasone, etoposide and cyclosporine are reserved for primary HLH and for cases of secondary HLH that does not respond to treatment of the underlying cause.⁵ Our patient was treated with intravenous corticosteroids and immunoglobulin based on reports of cases that were successfully treated with intravenous immunoglobulin.¹⁰⁻¹⁴

Although acyclovir has not been associated with a clear clinical benefit in patients with infectious mononucleosis, treatment with intravenous acyclovir was added to the immunosuppressive therapy in order to reduce the viral load of EBV.^{15,16}

This case study has some limitations that should be acknowledged. First of all, the patient was considered to be immunocompetent based on the lack of a past medical history suggesting immunosuppression, the lack of previous immunosuppressive treatment, and the absence of a concurrent diagnosis that could suggest impaired immune responses. However, it could be that the patient had defective cytotoxic lymphocyte activity that could have been diagnosed based on natural killer (NK) cell assays; however, these assays were not performed in our patient. Thus, the possibility of a concealed immunosuppression due to defective cytotoxic cell activity cannot be ruled out. Furthermore, the possibility of genetic (primary) HLH leading to reactivation of an EBV infection cannot be definitively ruled out, since genetic testing was not performed, even though the negative IgG EBNA makes this scenario unlikely. Thus, the diagnosis of secondary HLH due to EBV infection is not the only possible diagnosis in this case.

Conclusions

We have presented a rare case of HLH leading to multiple-organ failure that was associated with EBV and was successfully treated with acyclovir, high dose corticosteroids and intravenous immunoglobulin. In patients with EBV infection who exhibit persistently high fever and unresponsiveness to antibiotics, the possibility of HLH should be considered. Early diagnosis and rapid initiation of appropriate treatment may avert an unfavorable outcome.

Consent: Written informed consent was obtained from the patient for publication of this case report.

Authors' contributions statement: PI, EA, KA, AP and ES collected the data and wrote the manuscript. MP, MK, EK, and DPK critically reviewed the manuscript. All authors read and approved the final version of the manuscript.

Conflicts of interest: All authors – none to declare.

Funding: None to declare.

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Please cite this article as:

Ioannou P, Akoumianaki E, Alexakis K, Proklou A, Psyllaki M, Stamatopoulos E, Koulentaki M, Kondili E, Kofteridis DP. Hemophagocytic lymphohistiocytosis syndrome associated with Epstein-Barr infection in an immunocompetent patient. A case study. *GERMS*. 2020;10(3):266-271. doi: 10.18683/germs.2020.1216