CASE REPORT

An uncommon presentation of EBV-driven HLH. Primary or secondary? An ongoing dilemma

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SUMMARY

Haemophagocytic lymphohistiocytosis (HLH) is a potentially fatal syndrome, mainly characterised by dysregulated immune activation. The syndrome is related to a genetic cause, in the classic primary form, or to identified triggers such as infections, malignancy or rheumatological processes, in the classic secondary form. Epstein-Barr virus (EBV) is the most common agent implicated in hereditary and non-hereditary conditions. We describe a 23-month-old girl who experienced severe clinical deterioration with respiratory distress due a bilateral pleural effusion within the first week of primary EBV infection. Fever, generalised oedema and hepatosplenomegaly, along with a pruritic morbilliform erythematous rash, were the first clinical signs. Respiratory impairment followed with hypoxaemia and the patient was admitted to the intensive care unit for thoracentesis. Further investigation showed persistent bicytopenia, hypertriglyceridaemia, hyperferritinaemia and elevated α chain of interleukin-2 receptor (sCD25). Diagnostic criteria for HLH were fulfilled. Therapy was instituted with dexamethasone, ciclosporin A and intravenous immunoglobulin 6 days after admission with progressive clinical recovery.

BACKGROUND

Haemophagocytic lymphohistiocytosis (HLH) is a rare, life-threatening disease, defined as a syndrome of inappropriate activation of the immune system with impaired function of natural killer (NK) and cytotoxic T cells, macrophage hyperactivation and overexpression of cytokines.^{1 2}

The syndrome is disclosed by the criteria for HLH-2004, with a unique pattern of clinical and laboratory findings.^{3 4} Prompt assessment and recognition of the signs are crucial, but HLH is still often underdiagnosed and suboptimally managed in infants and young children.^{4 5} The clinical presentation is usually severe, but respiratory involvement with pleural effusion is not a common finding. Moreover, prognosis remains unclear particularly for paediatric, relapsing patients or those masked by identified triggers, who present a higher risk for underdiagnosed primary forms, with higher risk for recurrence and need for haematopoietic stem cell transplantation.

We present the case of a previously healthy 23-month-old girl with acute infectious mononucleosis followed by a rapidly deteriorating clinical course, with pleural effusion, resulting from Epstein-Barr virus (EBV)-induced HLH.

CASE PRESENTATION

A healthy 23-month-old girl came to medical attention with 3 days of high fever and diarrhoea. Familial and personal medical history were irrelevant. Physical examination was normal, chest X-ray was unremarkable and laboratorial findings showed white cell count 2 300 cells/ μ L and platelet count $68 \times 10^3/\mu$ L. Therefore, she was admitted for further investigation.

The patient's clinical condition deteriorated and she appeared acutely ill, with malaise. Physical examination progressively showed cervical adenopathies, hepatosplenomegaly, generalised oedema and an intermittent non-pruritic morbilliform erythematous rash on the trunk and extremities. Neurological examination was normal. The patient remained haemodynamically stable, with no fever, but on the fourth day, respiratory impairment with hypoxaemia disclosed a bilateral pleural effusion, and she was transferred to the intensive care unit. Thoracentesis was performed on fourth and ninth days, with drainage of 307 mL and 236 mL fluid, respectively. Pleural fluid examination showed characteristics of an exudate with 3662 neutrophil cells/ μ L, proteins 3.1 g/dL, glucose 98 mg/dL and lactate dehydrogenase (LDH) 1088. Abdominal ultrasound and CT demonstrated mild hepatomegaly, splenomegaly and acalculous gallbladder thickening with a normal bile duct.

INVESTIGATIONS

Laboratory findings from the third day showed pancytopenia; hypertriglyceridaemia (291 mg/dL), hyperferritinaemia ($>1650 \mu$ g/L) and hypofibrinogenemia (112 g/L); with levels of alanine transaminase and LDH progressively elevated, and sCD25 >5000 IU/mL (table 1). Bone marrow aspiration showed some haemophagocytic cells. Urinalysis and cerebrospinal fluid examination were normal. Later, NK-cell evaluation showed low activity.

Immunophenotype of peripheral blood lymphocytes showed at presentation: leucocytes 2000 cells/ μ L; lymphocytes 1400 cells/ μ L; CD3+1065 cells/ μ L (93%); CD3+CD4+259 cells/ μ L (24%); CD3+CD8+757 cells/ μ L (69%); CD4+CD8+0.34; CD19+60 cells/ μ L (5%) and CD16+CD56+CD3-8 cells/ μ L (1%; table 1). Whole exome sequencing (WES) is currently ongoing.

DIFFERENTIAL DIAGNOSIS

On aetiological investigation, serum antibodies were negative against several viruses/bacteria (including cytomegalovirus, HIV, adenovirus, parvovirus, herpes simplex I/II and hepatitis A, B

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Table 1 Laboratory findings

Laboratory test	On admission	Before treatment (D3)	Partial remission (D21)	Total remission (D33)
Laboratory HLH criteria				
Haemoglobin (g/dL)	12.4	8.7	9.7	11
Platelets ($10^9/L$)	68 000	43 000	442 000	521 000
Neutrophils ($10^9/L$)	307.8	336.4	423.5	5025
Triglyceridaemia (mg/dL)	—	291	476	195
Fibrinogenaemia (mg/dL)	—	112	223	225
Ferritin (ng/mL)	—	>1650	614	82
CD25s (U/mL)	—	>5000	—	784 (D61)
Other laboratory findings				
Leucocyte ($10^9/L$)	2300	2900	7000	11 000
Protein (g/dL)	—	4.53	7.48	—
Albumin (g/dL)	—	3.66	3.73	4.14
AST (U/L)	—	213	58	28
ALT (U/L)	—	376	135	55
LDH (U/L)	919	938	—	—
Immunophenotype of peripheral blood				
	D2	D48		2 years after
Leucocyte (cells/ μ L)	2 000	9100		5400
Lymphocytes (cells/ μ L)	1400	5600		3000
CD3+(cells/ μ L)	1065 (93%)	4034 (82%)		2091 (79%)
CD3+CD4+(cells/ μ L)	259 (24%)	1614 (36%)		1119 (43%)
CD3+CD8+(cells/ μ L)	757 (69%)	1951 (43%)		613 (23%)
CD4+CD8+(cells/ μ L)	0,34	0,83		1,83
CD19+(cells/ μ L)	60 (5%)	690 (13%)		229 (9%)
CD16+CD56+CD3– (cells/ μ L)	8 (1%)	153 (3%)		175 (7%)

ALT, alanine transaminase; AST, aspartate transaminase; HLH, haemophagocytic lymphohistiocytosis; LDH, lactate dehydrogenase.

and C, as well as *Rickettsia conori*), except for an acute EBV infection (serum antibodies against EBV were positive for IgG viral capsid antigens—VCA, weakly positive for IgM VCA (25.7 U/mL) and negative for Epstein-Barr virus-determined nuclear antigen (EBNA)). Blood PCR for EBV was positive with 51 700 000 copies/mL. Genetic testing including familial HLH was negative (PFR-1, STX-11, UNC13D, STXBP2 and ITK).

TREATMENT

The clinical and laboratory findings—fever, splenomegaly, bicytopenia, hypertriglyceridaemia, hyperferritinaemia, low NK-cell activity and elevated sCD25—fulfilled the diagnostic criteria for HLH, and the patient was treated according to HLH-2004 protocol including ciclosporin A (CSA), started on day 6. A haematopoietic stem cell (HCT) donor search began early with the diagnosis.

OUTCOME AND FOLLOW-UP

There was a positive response to treatment. Criteria for clinical response and resolution (non-active disease) were fulfilled at day 13 and 25 post-treatment; EBV viral load lowered to 195 copies/mL 2 months after and the patient was discharged on day 75 fully recovered.

A month later EBV viral load rose to 11 610 000 copies DNA/mL. Despite no associated clinical findings, prophylactic treatment with acyclovir was instituted and maintained for 6 months. The patient remained without clinical symptoms; NK-cell-mediated cytotoxicity was repeated and normalised; and EBV viral load was definitively negative after a year of an undulating course.

At present—2 years after the HLH syndrome—the patient remains symptom free, with normal laboratory results including immunoglobulins and negative blood PCR for EBV.

DISCUSSION

We report a case of HLH presenting with bilateral pleural effusion during a primary EBV infection in an otherwise healthy 23-month-old child.

HLH diagnosis criteria (HLH-2004)³ are well established but could be incomplete in the early stages of the disease. Most patients present with fever, hepatosplenomegaly, and coagulation abnormalities and cytopenias,⁴ accompanied by low fibrinogen levels, and high serum triglycerides and ferritin levels. Impaired NK-cell activity, increased plasma concentrations of the α chain of interleukin-2 receptor (sCD25; >2400 U/mL) and ferritin level >10 000 μ L have high specificity and contribute to earlier diagnosis as they correlate with current disease activity.¹ The histopathological signs include tissue evidence of haemophagocytosis (bone marrow, spleen and lymph nodes).

Besides, non-specific abnormalities are regularly detected on a chest X-ray or a CT scan.⁶ Hepatosplenomegaly, ascites and gall-bladder wall thickening are common findings at ultrasonography performed within the first week but pleural effusion, as was found in our patient, is not common.^{7 8} Although PCR for EBV in the pleural fluid was not performed, the authors believe EBV played a possible role in the pathogenesis of the pleural effusion, either by a direct cytopathic effect or indirectly via a dysregulated immunological response. The fact that it was a bilateral pleural effusion with a prominent lymphocytosis (3975 cells/ μ L 92% mononuclear cells) that only improved with immunosuppressive therapy supports that hypothesis.

Two different conditions have been classically described: a primary and a secondary form. Difficult to distinguish from one another, the primary form usually involves younger children with a clear familial inheritance or genetic cause; while the secondary form presents with concurrent infections, malignancy or rheumatological

disorders as triggers.^{6–9,13} Nowadays, several authors have begun to consider this classification as unsettled, as primary forms have been reported at any age; gene mutations, assigned to the primary condition, are found in association with secondary forms; and primary and secondary forms can both be precipitated by infections with a substantial risk of mortality. Our report strengthens this position as it fits an uncertain field for classification: infant age at diagnosis points to a primary cause, although genetic research was negative; WES is currently ongoing. The child remains symptom free with no need for HCT 2 years after diagnosis.

EBV is the leading viral cause of HLH in hereditary and non-hereditary conditions, especially in immunocompetent individuals,^{14–18} children, adolescents living in Asia and during reactivation of infection.^{5–19–24} As this was the only positive aetiological finding in our patient, other primary immunodeficiencies that predispose to EBV-driven haematological diseases were considered: ITK was excluded while SAP, XIAP and MAGT1 deficiency were not studied as they appeared unlikely. CD27 deficiency was also not researched as it is associated with combined immunodeficiency and persistent symptomatic EBV viraemia.²⁵ Genetic defects of lymphocyte cytotoxic function (familial HLHs) and the HLH syndromes associated with albinism were excluded. Looking at the favourable evolution, with clinical stabilisation for over 2 years, other causes also do not seem to fit, and a responsible gene has not yet been found.

Recurrence of HLH in the absence of autoimmune disease or malignancy is generally considered to be good evidence that a patient has primary HLH,¹ but the risk of recurrence in cases of secondary HLH is poorly defined. Hence, authors cannot exclude a primary form. Beside, clinicians should always be on alert to consider an underlying primary immunodeficiency when patients suddenly fail to control the EBV or other herpes viruses without obvious iatrogenic reasons.²⁶

Timely therapy can lead to increased survival. Treatment is centred on the inhibition of the overactive T and NK-cell responses, and corticosteroids remain the most important anti-inflammatory drugs, followed by immunomodulatory drugs—CSA and immune globulin intravenous (IGIV)—(HLH-2004).^{27–29} Despite, some authors continue to question the need to add CSA to the induction therapy, as the risk and benefits are not yet clearly defined.¹ HCT is the treatment resort for refractory forms of EBV-HLH, and the only cure for EBV infection occurring in genetic forms of HLH.⁴ Mortality is significant (median survival of untreated HLH primary patients is 2 months), but optimal treatment strategies, including HCT, demonstrate a good survival rate (>75%).

Learning points

- Our patient survived despite her severe clinical condition. We concluded that the favourable outcome was attributable to the early institution of diagnosis and prompt therapy. We also speculate about the existence of a milder genetic defect resulting in a temporary failure to control Epstein-Barr virus replication that possibly does not need correction by a full immunological reconstitution. Nonetheless, according to reports that show risk of recurrence, prognosis remains unclear.
- Early diagnosis is essential but frequently difficult in pediatric HLH patients.
- Prompt therapy is essential to favourable prognosis.
- A genetic cause is not always easy to determinate. Long term follow up is essential.

Contributors First and last authors contributed in the design and interpretation of the data. First author drafted the manuscript while the second author performed a critical revision for important intellectual content. Finally, all reviewed and approved the final version to be published.

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