CHALLENGES IN DIAGNOSING FAMILIAL HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS: THE ROLE OF T-CELL BIOMARKERS IN THE EARLY DIAGNOSIS OF HEREDITARY HLH

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ABSTRACT

Introduction: Hemophagocytic lymphohistiocytosis (HLH) is a rare and potentially life-threatening syndrome. In this article, we describe a case of hereditary HLH in an infant, highlighting the challenges in diagnosis and the significance of the biomarker $CD38^{high}$/HLA-DR^+ cells among $CD8^+$ T cells for the early diagnosis of HLH. **Case Presentation:** A 1.5-month-old male infant presented with persistent fever and hepatosplenomegaly. Initial laboratory tests revealed pancytopenia, hyperferritinemia. Despite the similarity in clinical manifestations between HLH and sepsis, distinguishing the two is crucial due to their differing treatment approaches. **Discussion:** The infant's condition was complicated by acute T-cell activation, a finding characteristic of HLH but absent in sepsis. Specifically, $CD38^{high}$/HLA-DR^+ effector cells were identified with prominent $CD8^+$ T-cell activation. These findings underscore the importance of specific biomarkers in the early diagnosis of HLH. **Conclusion:** The identification of $CD38^{high}$/HLA-DR^+ T cells may serve as a valuable diagnostic tool for clinicians in recognizing HLH early, ultimately guiding appropriate treatment.

Keywords: *Children, Hemophagocytic lymphohistiocytosis, EBV.*

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I. INTRODUCTION

Hemophagocytic lymphohistiocytosis (HLH) is a rare, life-threatening syndrome caused by abnormal activation of the immune system. Clinical manifestations of HLH include high fever and hepatosplenomegaly. Laboratory tests typically reveal pancytopenia, hyperferritinemia, hypertriglyceridemia, or hypofibrinogenemia, along with macrophage activation syndrome (MAS) featuring hemophagocytosis (1). The clinical presentation of HLH resembles the inflammatory response seen in other diseases, and the non-specific manifestations pose significant challenges in diagnosing HLH (2). HLH and sepsis have similar clinical manifestations, making diagnosis uncertain, but distinguishing HLH from sepsis is important because of the different treatment approaches. An important finding is acute T-cell activation in HLH but not in sepsis. T cell activation in HLH patients is characterized by $CD38^{high}$/HLA-DR^+ effector cells, $CD8^+$ activation being the most prominent (3). In a recently published study of 43 pediatric HLH patients, a threshold of > 7% $CD38^{high}$/HLA-DR^+ cells among $CD8^+$ T cells had a strong negative and positive predictive value in distinguishing HLH and sepsis (3). HLH can affect children of any age, but the incidence is highest in children < 3 months of age. And there is no difference between male and female (4). HLH can be

hereditary or secondary to many different conditions including infection, the most common cause is EBV or can be related to immunosuppressive diseases (eg, autoimmune disease, malignancy) (5). Genetic HLH may be associated with a genetic syndrome in immunology or it may be familial hemophagocytic lymphohistiocytosis (FHL), which is an autosomal recessive disorder (6). Based on genetic defects, FHL can be divided into 4 types, including FHL-2, FHL-3, FHL4 and FHL-5. The most common mutation is PRF1, found in 30% of cases and specific for FHL-2. In addition, the criteria of HLH-2004 cannot distinguish between hereditary HLH and secondary HLH (7). HLH is often associated with genetic abnormalities that affect lymphocyte cytotoxicity. The HLH-2004 diagnostic criteria can be useful in guiding diagnosis, but it has certain limitations. During the early stages of the disease, some patients present with nonspecific symptoms and do not satisfy five of the eight criteria, especially those with atypical presentations such as CNS disease or ALF (8).

Diagnostic criteria HLH-2004 (9). If either A or B is fulfilled, the diagnosis of HLH can be established:

A. A molecular diagnosis consistent with HLH
B. Any 5 of 8 following criteria: 1. Fever > 38.5°C 2. Splenomegaly 3. Cytopenia (≥ 2 of 3 lineages in peripheral blood): • Hemoglobin < 9g/dL • Platelets < 100 x 10^9/L • Neutrophils < 1 x 10^9/L 4. Hypofibrinogenemia and/or hypertriglyceridemia • Triglycerides > 3 mmol/L (> 265 mg/dL) or • Fibrinogen ≤ 1.5 g/L 5. Hemophagocytosis in bone marrow, liver, lymph nodes, spleen or other tissues 6. Serum ferritin ≥ 500 µg/L 7. Low or absent NK cell activity 8. Soluble CD25 ≥ 2400 U/mL

Immunology of T-cell activation in HLH

The term “cytokine storm” refers to a pathological inflammatory response in which acute immune activation causes more harm than good. Cytokine storms can occur in a variety of conditions, including sepsis (10) and HLH (11). Activated CD8+ T cells play an important role in animal models of HLH and are more useful than $CD4^+$ T cell in distinguishing HLH from sepsis. They found that the CD8:CD4 ratio was only slightly increased in HLH patients, and that activated $CD8^+$ T cell were fivefold more abundant than $CD4^+$ T cell in most HLH patients (3). In contrast to previous reports that primary and secondary HLH could be distinguished based on the degree of T-cell activation (12), they found no differences between $CD38^{high}$/HLA-DR^+ $CD8^+$ and $CD4^+$ T cells in HLH patients with and without mutations, infections, or mortality (3). To better

understand the characteristics of activated T cells in HLH, further studies were conducted on the differentiation and function of $CD38^{high}$/HLA-DR^{+} $CD8^{+}$ T cells. Notably, these cells were not present in healthy children or patients with sepsis (3).

II. CASE REPORT

A 1.5-month-old male infant with hepatosplenomegaly and pancytopenia was transferred from the Neonatal Intensive Care Unit (NICU) to the Infectious Department for further evaluation. He had a history of fever and progressive enlarged abdomen for two weeks, treated with antibiotics with some clinical improvement but unchanged of pancytopenia and hepatosplenomegaly, and had fever again after stop antibiotics for 1 day.

He was the third child of non-consanguineous parents, with no significant perinatal history (normal pregnancy, C-section at gestational age 39 weeks, birth weights 2800-gram, normal neonatal development). He was breastfeeding with good tolerance and weight gain, no history of other acute illnesses). The first child is a 10-year-old boy, and the second child is a 5-year-old boy, both are healthy. The family history is unremarkable.

On the physical examination, he presented good tone and reactivity. He had a high fever, cutaneous and mucous pallor, and a few petechiae on the skin. Respiratory and cardiovascular system examination was normal. His abdomen was enlarged due to hepatosplenomegaly.

The laboratory examination, as show in Table 2, revealed pancytopenia, with anemia: Hb 6-8 g/dL, neutropenia: 300 – 900/µL (always below 1), thrombocytopenia: 30,000/ µL, and progressive hyperferitinemia: 1700 – 2600 µg/L, hypertriglyceridemia: 260 – 430 mg/dL, and hypofibrinogenemia: 100 mg/dL. The liver enzymes and kidney function were within normal range. The inflammatory markers were slightly increased: CRP 16 mg/dL, procalcitonin 0.4 ng/mL and IL-6 45 pg/mL.

Table 2. **Case study. 1.5-month-old male infant with HLH. Laboratory results**

	Patient's result	**Normal Range**
Complete blood count		
Leukocytes	5000 – 8000/ µL	5.5 - 15.5 x 10^3/µL
Neutrophils	300 – 900/ µL	1.5 - 8.5 x 10^3/ µL
Lymphocytes		2 - 8 x 10^3/ µL
Hemoglobin	6 – 8 g/dL	11 - 14 g/dL
Platelet	30,000/ µL	150,000 - 450,000/ µL
Inflammatory markers		
CRP	16.6 mg/L	0 - 5 mg/L
Procalcitonin	0.4 ng/mL	< 0.05 ng/mL
IL-6	45 pg/mL	< 7 pg/mL
LDH		120 – 300 U/L
Ferritin	1700 – 2600 µg/L	4 – 67 µg/L
Coagulation		
PT		11.3 - 15.6 s

	Patient's result	Normal Range
INR	1 – 1.2	0.84 - 1.2
APTT	40 s	24 - 37s
Fibrinogen	100 mg/dL	160 - 390 mg/dL
D-dimers	2 - 5 µg/mL	0 - 0.5 µg/mL
Liver transaminase		
AST	50	2 - 48 U/L
ALT	13	2 - 29 U/L
Kidney function		
BUN		< 39 mg/dL
Creatinine		< 0.47 mg/dL
Triglycerides	260 – 430 mg/dL	40 - 150 mg/dL

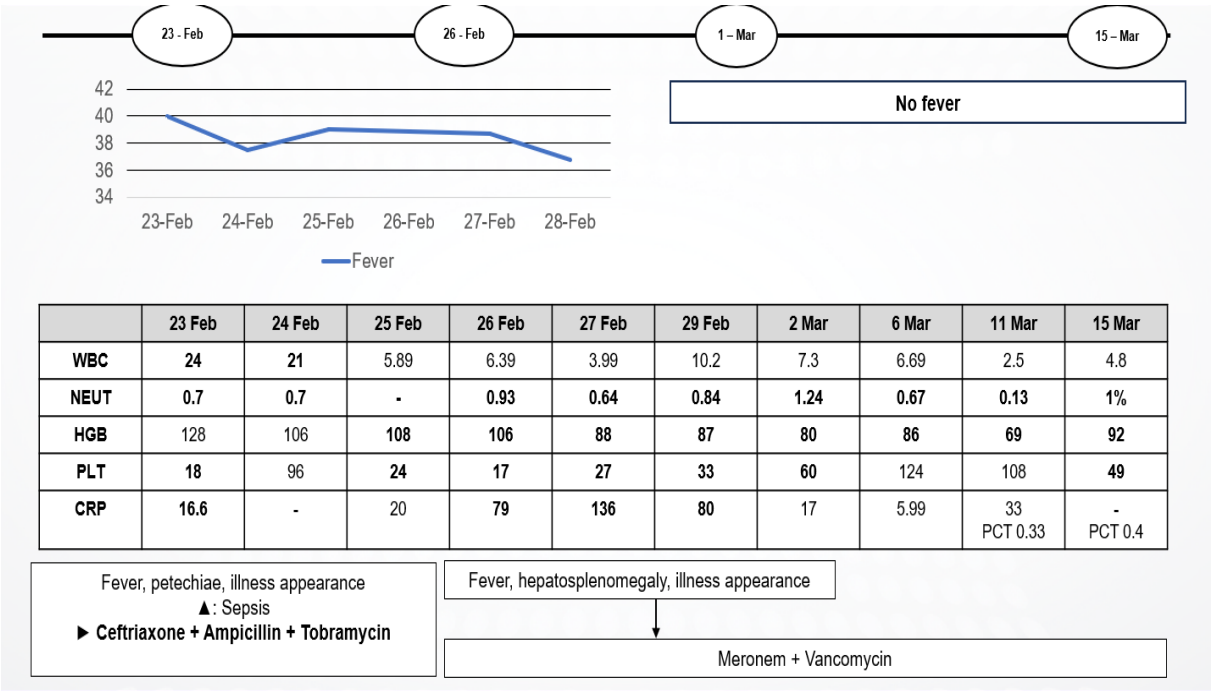
CRP: C-reactive protein, IL-6: Interleukin-6, LDH: Lactate dehydrogenase, PT: Prothrombin time, INR: International Normalized Ratio, aPTT: Activated partial thromboplastin time, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, BUN: Blood urea nitrogen

Viral serology was negative for CMV, EBV, coronavirus, HIV, rubella, measles, adenovirus, rotavirus. Blood culture, stool culture, and nasopharyngeal swab culture were also negative (see Table 3).

Table 3. **Case study. 1.5-month-old male infant with HLH. Virology and bacterial culture results**

	Patient's result
PCR CMV	Negative
IgM CMV	Negative
IgM CMV	Negative
PCR EBV	Negative
VCA-IgG EBV	Negative
VCA-IgM EBV	Negative
HSV 1+2 antibody	Negative
IgM Rubella	Negative
PCR Sars-CoV-2	Negative
HHV6	Negative
Blood culture	Negative
Stool culture	Negative
Urine culture	Negative
Nasopharyngeal swab culture	Negative
PCR 65 pathogen in blood	Klebsiella pneumoniae

PCR: polymerase chain reaction, IgM: Immunoglobulin M, EBV: Epstein-Barr virus, CMV: cytomegalovirus, VCA-IgG: Viral capsid antigen Immunoglobulin G, HHV6: Human Herpesvirus 6



	23 Feb	24 Feb	25 Feb	26 Feb	27 Feb	29 Feb	2 Mar	6 Mar	11 Mar	15 Mar
WBC	24	21	5.89	6.39	3.99	10.2	7.3	6.69	2.5	4.8
NEUT	0.7	0.7	-	0.93	0.64	0.84	1.24	0.67	0.13	1%
HGB	128	106	108	106	88	87	80	86	69	92
PLT	18	96	24	17	27	33	60	124	108	49
CRP	16.6	-	20	79	136	80	17	5.99	33 PCT 0.33	- PCT 0.4

Fig 1. **Clinical and laboratory test progression and treatment in 3 weeks treated with broad-spectrum antibiotic**.

WBC: White Blood Cell, NEUT: Neutrophil, HGB: Hemoglobin , PLT: Platelet, CRP: C-reactive protein, PCT: Pro-calcitonin

Clinically, the patient showed some improvement, with no fever and unchanged hepatosplenomegaly. However, fever recurred one day after stopping antibiotics. On complete blood count, pancytopenia remained unchanged, with WBC counting nearly within the normal range; however, neutrophil levels consistently fell below 1. Red blood cell and platelet counts showed a gradual decline over time, with some response to transfusions. Inflammatory markers were elevated, but not significantly.

Fig 2. **A 2-month-old male infant showed increasing abdominal distension due to progressive hepatosplenomegaly**

The bone marrow aspirate showed normal cellularity, granulocyte series proliferated and differentiated, immature red blood cell series proliferated, differentiated, normal platelet pattern, no abnormal cells in the marrow with erythrocyte series normal maturation: basophilic erythroblasts 5%, polychromatophilic erythroblasts 40%. The granulocyte series normal maturation. Very rare lymphocytes, rare monocytes, and no atypical cells were observed. The platelet series was represented by normal thrombocytogenic megakaryocytes. The chest X-ray showed no lesions. The abdominal ultrasound confirmed hepatomegaly with a longitudinal diameter of 7.5 cm and splenomegaly with a longitudinal diameter of 9 cm. Cardiac echocardiography showed no defect.

The initial diagnosis was sepsis and the patient was treated with broad-spectrum antibiotics (ceftriaxone 100 mg/kg/day for 3 days, tobramycin 5 mg/kg/day for 3 days, then meropenem x 40 mg/kg/day, vancomycin 60 mg/kg/day, and then fosmicin and colistin for 7 days) and antimycotic therapy (fluconazole 12 mg/kg/day as the loading dose, then 6 mg/kg/day for 7 days). The anemia, thrombocytopenia, and hypofibrinogenemia persisted, and transfusion of blood products became necessary. Despite the intensive treatment, the hepatosplenomegaly and the laboratory abnormalities persisted although the patient had some improvement in clinical condition such as fever and illness appearance. After 2 weeks of intensive treatment with broad-spectrum antibiotics, we found that the child's clinical course was not consistent with isolated sepsis, since the child's response to antibiotic treatment was inconsistent with sepsis. At this point, we considered other differential diagnoses, especially HLH. However, the FHL gene test G4500 package was negative, so we should think of some other diagnosis at that time. The differential diagnosis excluded viral infections (negative serology), bacterial infections (blood culture, pharyngeal exudate, stool culture negative), tuberculosis (X-pert, PCR, T-spot negative, chest X-ray without lesions), malignant or hematology (bone marrow aspiration without atypical cells or image of hemophagocytosis), and metabolic diseases.

With infections, we check for CBC, CRP, pro-calcitonin, urine analysis, lumbar puncture, CMV, EBV, dengue, adenovirus, rhinovirus, influenza type A and B, covid-19, HIV, HBsAg, TORCH, panel bacterial of nasopharyngeal fluids, pertussis, fungal, tuberculosis, PCR 65 bacterial of sepsis, culture (blood, nasopharyngeal fluid, urine, stool), head ultrasound, abdominal ultrasound, chest and abdominal CT scan with no significant except pancytopenia, and PCR 65 bacteria in sepsis positive with *Klebsiella pneumonia* and image of hepatosplenomegaly in ultrasound and abdominal CT scan.

With hematology and malignancy, we check CBC, coagulation, D-dimer, bone aspiration, peripheral blood smear, Coombs test, LDH, IL-6, Ferritin, triglyceride, acid uric, vitamin B12, folate, iron, long bone x-ray, gene test for HLH (G4500 package) and T-cell profiles (i.e, $CD38^{high}$/$HLA\text{-}DR^{+}$) with no significant except pancytopenia, hypofibrinogenemia, hypertriglyeridemia, hyperferritinemia, increase of $CD38^{high}$/$HLA\text{-}DR^{+}$, and G4500 package of HLH gene analysis was negative.

With immunology, we checked C3, C4, anti-ANA, Ds-DNA, Coombs direct and indirect, IgA-G-M, CD3-4-8 and CD19 & CD56 with no significance. With hepatology, we checked AST, ALT, GGT, ALP, total and direct bilirubin, amylase, lipidase and ceruloplasmin with no significant. And finally, one of the important causes is

endocrinology, we check the ABG, NH3, lactate, glucose, AST, ALT, MSMS, cardiac ultrasound, screening for 6 lysosomal storage diseases, lipid panels (HDL, LDL, triglyceride, cholesterol) and karyotypes analysis with no significant results.

In view of the clinical and paraclinical data, with persistent fever, hepatosplenomegaly, pancytopenia, hypofibrinogenemia, a high serum triglyceride level, high serum ferritin level, and increase of $CD38^{high}$/$HLA\text{-}DR^{+}$ marker, we considered the possibility of FHL, as five of the eight diagnostic criteria were present. Although FHL gene test package G4500 negative, we can not rule out the FHL. We checked the whole genome for this patient and final diagnosis was confirmed, as a homozygous UNC13D mutation was detected.

The homozygous UNC13D mutation was found, the only curative treatment was HCT. After stabilization of the infection, the child was discharged.

III. DISCUSSION

A 1.5-month-old male patient with no significant of past medical history was admitted to the hospital due to high fever and petechiae with prominent problems including fever, unclear infections, hepatosplenomegaly, persistent pancytopenia and inconsistent response with broad-spectrum antibiotics. The diagnosis for this case was really challenging. Clinical and laboratory findings suggest a diagnosis of sepsis. The patient was treated with a combination of broad-spectrum antibiotics and antimycotic, but clinical symptoms worsened, so further assessment was performed to find underlying disease or possible causes, such as other infections (eg, viral, bacterial, fungal), immunology, malignancy, hematology, endocrinology diseases.

After excluding other causes, along with the persistent clinical manifestation with symptoms of high fever and hepatosplenomegaly leading to the diagnosis of HLH, over time the patient satisfied five of the eight criteria (persistent fever, hepatosplenomegaly, pancytopenia, hypertriglyceridemia and hyperferritinemia), but bone marrow aspirate without evidence of hemophagocytosis. According to some authors, hemophagocytosis may not be found in bone marrow aspirate in the early stages of the disease (13).

Hepatic injury or dysfunction in hemophagocytic lymphohistiocytosis (HLH) primarily results from a cytokine storm, which occurs due to impaired natural killer (NK) and cytotoxic T lymphocyte function in genetically susceptible individuals, with triggering factors playing a crucial role. The upregulation of granulocyte-monocyte colony-stimulating factors (GM-CSF) receptors on macrophages, coupled with macrophage proliferation, leads to splenomegaly and hepatomegaly. This inflammation results in elevated transaminases, hepatocyte hemosiderosis, sinusoidal dilatation and congestion, as well as Kupffer cell hyperplasia and hypertrophy, leading to hemosiderosis and hemophagocytosis (14). This patient experienced progressive hepatosplenomegaly; however, liver enzymes remained stable, and biliary markers were nearly within normal limits. Additionally, lymphocyte- or lymphohistiocyte-mediated biliary ductular injury, along with cytokine-mediated impairment of lipoprotein lipase activity (specifically IL-1, IL-6, and TNF-α), causes cholestasis, hyperbilirubinemia, and hypertriglyceridemia (14).

T-cell activation profiles are what we have proposed in this case to help differentiate HLH from early sepsis. In a study of Chaturvedi et all (2021), increased

$CD38^{high}$/HLA-$DR^{+}CD8^{+}$T cells were found to be the optimal marker for detecting active HLH patients (3). In this report, they compared the T-cell phenotype in HLH patients with sepsis patients and found that the immune mechanism in HLH activates T-cells as $CD38^{high}$/HLA-DR^{+}, this population is not present in the peripheral blood samples of patients with sepsis. A study analyzing peripheral blood T cells in 43 HLH children and 19 children with sepsis within 48 hours of symptom onset or before HLH treatment found $CD38^{high}$ or $CD38^{high}$/HLA-DR^{+} populations in HLH patients and absent in most of the sepsis patients. Therefore, although HLH and sepsis have a cytokine storm pathogenesis with overlapping clinical manifestations, T-cell phenotypes can help differentiate and are valuable in diagnosis (3). Another study multiparametric analysis of 6 pediatric HLH patients concluded that flowcytometry on peripheral blood of patients with HLH showed > 7% $CD38^{high}$/HLA-DR^{+} cells (15).

Since the child was 1.5 months old, it was likely that this was a familial form of HLH, and genetic testing revealed the presence of the UNCD13D mutation. This mutation belongs to the FHL-3 subtype, which affects the central nervous system more often than others. UNCD13D mutation is responsible for the synthesis of abnormal MUNC13-4 involved in the perforin-mediated cytolysis. For UNC13D mutation, the curative treatment is HCT but has a high risk of mortality.

Fig 3. **A 7-month-old boy with a history of FHL due to a UNC13D gene mutation, after treatment for two infections, is currently relatively healthy and has no other symptoms**

IV. CONCLUSION

Although HLH is uncommon, this is a severe condition and life-threatening if not detected and treated early. Bone marrow aspiration for hemophagocytic features is useful in diagnosing HLH, but this method is invasive. Flowcytometry is a rapid, non-invasive diagnostic test with high predictive value in differentiating HLH from sepsis based on increased > 7% $CD38^{high}$/HLA-DR^{+} cells.

V. DISCLAIMER

The information presented in this report is for educational purposes only and should not be

considered a substitute for professional medical advice, diagnosis, or treatment. The authors declare that there are no conflicts of interest related to this report. Patient confidentiality has been maintained, and consent for publication has been obtained from the patient's guardians.

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