Case Report

Familial hemophagocytic lymphohistiocytosis type 3: case report

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Abstract

Hemophagocytic lymphohistiocytosis (HLH), including familial hemophagocytic lymphohistiocytosis (FHLH), is a rare and underdiagnosed syndrome of excessive immune activation which can be fatal if not treated. We report the case of a previously healthy 13-month-old girl who presented with prolonged fever, anaemia and hepatosplenomegaly, and then developed pancytopenia and biological signs of extreme inflammation. Genetic tests showed mutations in UNC13D gene, involved in FHLH type 3. Patient was treated with immunochemotherapy and underwent hematopoietic stem cell transplantation (HSCT). Awareness of this disease is crucial to make rapid diagnosis, in order to initiate a prompt treatment and quickly proceed to HSCT.

Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a rare and life-threatening syndrome, caused by excessive generation of inflammatory cytokines. There are two forms of HLH: primary HLH, encompassing several genetic diseases in which HLH is the predominant clinical manifestation, and secondary HLH, which is mainly driven by acquired or environmental mechanisms, such as rheumatic disease, malignancy, infection, or drugs (1). Diagnosis is based on a combination of several nonspecific signs and symptoms of extreme hyperinflammation and on genetic testing for the majority of primary HLH (2). HLH has to be rapidly diagnosed and prompt and aggressive treatment should be initiated, because of rapid progression to pancytopenia and multiorgan failure. The only curative treatment of primary HLH is allogenic hematopoietic stem cell transplantation (HSCT) (3).

Case report

A 13-month-old female patient was admitted in the emergency department because of persistent fever and mild anaemia. She was born from non-consanguineous parents and had no relevant personal or familial medical background.

Initially, she was treated for nasopharyngitis, then with amoxicillin/clavulanate for urinary tract infection, but fever did not resolve despite antibiotics. At day 19 of persistent fever, physical examination showed skin pallor, a slight systolic murmur, and hepatosplenomegaly. A blood analysis was performed, revealing a normocytic regenerative anaemia (Hb 8.0 g/dL, reticulocytes 6%) without any signs of haemolysis. Indeed, levels of haptoglobin and bilirubin were normal, and reticulocytes were decreasing in the following blood tests. Currently, inflammatory markers were very low (CRP 8.8 mg/L (normal < 5 mg/L), ferritin 219 mcg/L (normal < 204 mcg/L)).

Ultrasound examination confirmed hepatosplenomegaly and showed lymphadenopathy in the hepatic hilum. At Day 21 of fever (38,4°C), she developed pancytopenia: anaemia (Hb 7.7 g/dL (normal: 10.5-12 g/dL)), neutropenia (990/mm³ (normal: 1500-8500/mm^3)), and thrombocytopenia (114 000/mm³ (normal > 150 000/mm^3)). Liver function test resulted in elevated alanine aminotransferase of 83 IU/L (normal < 34 IU/L) and aspartate aminotransferase of 89 IU/L (normal < 44 IU/L). Ferritin was slightly increased at 258 mcg/L. Acute leukemia was suspected. The patient was transferred to a tertiary centre for further examination. Bone marrow aspiration showed no signs of malignancy or hemophagocytosis. Bacterial and viral check-up were negative. At Day 22 of fever, she received a bolus of methylprednisolone (1 mg/kg) for transfusion reaction and fever transiently resolved but reappeared six days later.

Table 1: *Diagnostic guidelines for HLH, based on HLH-2004 study; incomplete picture of the disease at admission of our patient; criteria fulfilled at diagnosis of our patient.*

Diagnostic guidelines for HLH, established by HLH-2004	Criteria fulfilled at admission of our patient (3 out of 8)	Criteria fulfilled at diagnosis of our patient (6 out of 8)
Diagnostic of HLH if (1) or (2) is fulfilled:		
(1) **Molecular diagnosis of HLH**		
(2) **5 out of 8 of those following criteria**		
• Fever ≥ 38,5°C	+	+
• Splenomegaly	+	+
• Cytopenia (≥ 2 of 3 lineages): Hb < 9.0 g/dL, platelets < 100 x 10^9/L, neutrophils < 1.0 x 10^9/L	-	+
• Hypertriglyceridemia (fasting triglycerides ≥ 3.0 mmol/L) and/or hypofibrinogenemia ≤ 1.5 g/L	+	+
• Hemophagocytosis	-	-
• Low/absent NK-cell activity	Not tested	Not tested
• Hyperferritinemia ≥ 500 mcg/L	-	+
• sCD25 ≥ 2,400 U/ml (or according to the lab norms)	Not tested	+

At Day 35, biochemistry showed hyperferritinemia (1365 mcg/L). EBV and CMV serologies and PCR were negative. Flow-cytometry revealed a very elevated level of CD25s (18 295 pg/ml (normal < 1997 pg/ml)). Bone marrow aspiration performed again and still showed no signs of hemophagocytosis, but bone marrow biopsy revealed an increased number of macrophages. Analysis of cerebrospinal fluid (biochemistry, cytology) were negative. Magnetic resonance imaging of the brain showed a mild abnormal hypersignal in the periventricular white matter on T2 and FLAIR sequences. Diagnosis of HLH was made on all those criteria (Table 1).

Genetic tests showed compound heterozygous nonsense variants in the *UNC13D* gene: c.247C>T p.(Arg83*) and c.640C>T p.(Arg214*). These results confirm the diagnosis of familial hemophagocytic lymphohistiocytosis type 3, an autosomal recessive disease.

The patient was treated according to HLH-2004 protocol and received an HSCT, the only curative treatment (2, 3). Treatment was started before the result of genetic analysis was known.

Discussion

HLH is a rare and life-threatening syndrome. It is characterized by an excessive activation of immune system, due to an uncontrolled proliferation of activated cytotoxic T lymphocytes and macrophages. There are two forms of HLH: primary HLH and secondary (or acquired) HLH. Primary HLH often occurs in infancy, in patients with a genetic disease in which HLH is the predominant manifestation. Secondary HLH can be diagnosed at any age, and may be triggered by malignancy, infection, immunosuppression, or auto-immune disorders (2, 4)

Physiopathology of HLH is resumed in Figure 1. A lack of normal cytotoxic function of cytotoxic T lymphocytes (CTL) and natural killer (NK) cells leads to a deregulated antigenic presentation, and then, to an excessive activation of CTL and NK cells. These hyperactivated cells cause an excessive secretion of cytokines. Hypercytokinemia, and particularly INF-g, leads to hyperactivation of macrophages, which themselves produce cytokines, amplifying the phenomenon. Hypercytokinemia stimulates secretion of cytokines by CTL, NK, and macrophages, causing a cytokine storm. Cytokine storm leads to vascular endothelium damage, myelosuppression, and finally fatal bleeding, severe infections, and multiorgan failure (5, 6).

Primary HLH encompasses a group of genetic diseases. This includes familial HLH (FHLH), but also several other genetic diseases in which HLH is a common manifestation. FHLH type 2 to 5 are autosomal recessive diseases, respectively caused by mutations in *PRF1*, *UNC13D, STX11* and *STXBP2* genes. The gene causing FHLH type 1 has not yet been identified. Those genes are involved in the perforin-granzyme pathway. Perforin-granzyme pathway is one of the multiple pathways of NK/CTL-mediated cytotoxicity, inducing apoptosis. The first genetic defect was described in 1999 and involved the gene encoding perforin (7). In 2003, mutations in *UNC13D* gene, on chromosome 17q25, were discovered (8). *UNC13D* gene encodes a protein, Munc13-4, which is involved in the exocytosis of lytic granules, containing perforin and granzyme, in the immunological synapse (Figure 2). Mutations in *UNC13D* gene lead to FHLH type 3 (8). Other genetic diseases are associated with HLH, such as X-linked lymphoproliferative syndrome, Chédiak-Higashi syndrome, and Griscelli syndrome (2).

Analysis of *UNC13D* gene in our patient revealed a compound heterozygosity for c.247C>T p.(Arg83*) and c.640C>T p.(Arg214*) nonsense variants. Those variants were described in 2008 by Rudd & al (9). These patients were homozygous for one of those variants, while our patient was compound heterozygous for both. In our patient, we also detected another variant on *UNC13D* gene: c.2219C>T p.(Thr740Met) variant. This variant is classified as a variant of uncertain significance.

The diagnostic criteria, proposed by the Histiocyte Society in HLH-2004 study, are listed in Table 1. It is based on a molecular diagnosis consistent with HLH and/or unique combination of nonspecific clinical and biological features: fever, splenomegaly, cytopenia, hypertriglyceridemia and/or hypofibrinogenemia, hemophagocytosis, hyperferritinemia, high rate of soluble CD25, low or absent NK-cell activity (2). Patients may also develop central nervous system (CNS) involvement and liver dysfunction.

In this case, diagnosis was delayed due to several factors. First, fever was attributed to consecutive infections (pharyngitis, urinary tract infection). Secondly, the entire picture of the disease was incomplete when she arrived at the emergency department: initial blood analysis was not typical of HLH, with reticulocytosis and absence of inflammatory markers; and bone marrow was normal (Table 1). NK-cell activity was not assessed, although it is available in Belgium. Finally, CD25s is a nonspecific marker, and it is important to re-evaluate the patient multiple times, to re-consider the diagnosis each time and retest clinical examination and biological test in case of suspicion of HLH, even if the first dosages were normal. Once the diagnosis of HLH is made, in patients with severe systemic symptoms, treatment should be started as soon as possible, whether it is a secondary or primary HLH, except need to treat the cause for secondary HLH. For now, the only curative treatment for primary HLH is allogenic HSCT. The initial therapy of the therapeutic regimen developed by the Histiocyte Society in HLH-2004 study is an immunochemotherapy (2). The aim of this immunochemotherapy is to suppress inflammation and to induce a transient control of the disease before HSCT. This therapy consists of dexamethasone, etoposide and cyclosporine A and, in patients with

Figure 1 : *Physiopathology of HLH. Lack of normal cytotoxic function of CTL and NK cells leads to a deregulated antigenic presentation, and then, to an excessive activation of CTL and NK cells. These hyperactivated cells cause an hypercytokinemia, leading to hyperactivation of macrophages, which themselves produce cytokines, amplifying the phenomenon. Hypercytokinemia stimulates secretion of cytokines by CTL, NK, and macrophages, causing a cytokine storm. It leads to vascular endothelial damage, myelosuppression, and finally fatal bleeding, severe infections, and multiorgan failure.*

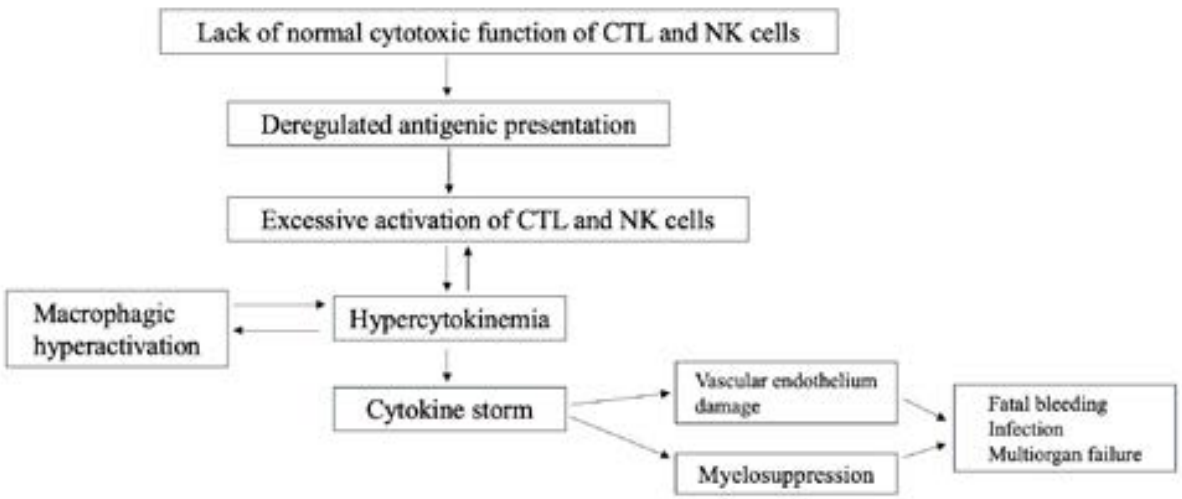
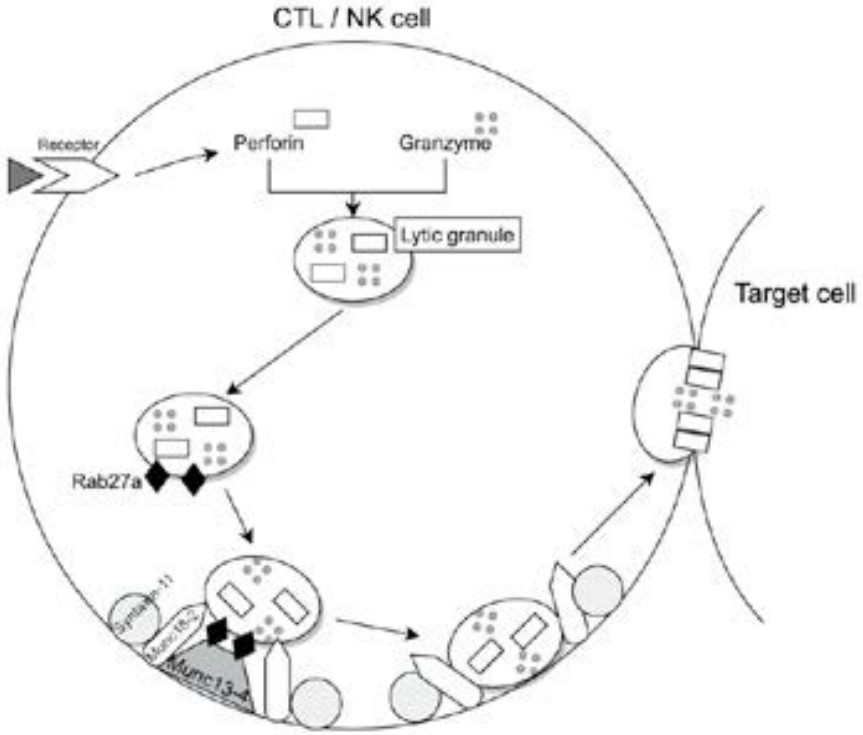


Figure 2 : *Perforin-granzyme pathway, one of the multiple pathways od NK/CTL mediated cytotoxicity. The activation signal leads to the formation of the lytic granule, containing perforin and granzyme. Rab27a, Syntaxin-11, Munc18-2 and Munc13-4 are involved in docking, priming and fusion of the lytic granule to the target cell. Polymerization of perforin induces formation of pores in the target cell, through which granzymes are released inducing cell apoptosis. Mutations in genes encoding perforin, Rab27a, Syntaxin-11, Munc18-2 or Munc13-4 are responsible for familial hemophagocytic lymphohistiocytosis. Mutations in UNC13D gene, encoding Munc13-4, leads to familial hemophagocytic lymphohistiocytosis type 3.*



CNS involvement, intrathecal injection of methotrexate and hydrocortisone. This immunochemotherapy has a lot of adverse effects. Etoposide is myelosuppressive and could induce secondary long-term cancers; cyclosporine A may induce hypertensive encephalopathy; and dexamethasone may lead to cardiac hypertension (10). In 2018, the Histiocyte Society published updated treatment recommendations, for the use of etoposide-based protocols and HSCT. Awaiting new alternative emerging drugs, the authors propose adapted doses of etoposide, based on patient's clinical and biological parameters (3).

Primary HLH is fatal if not treated. After diagnosis, median survival of untreated patients with active disease is about 2 months (2, 10). Mortality during treatment is high because of reactivation of disease or treatment-related morbidities. Patients can develop opportunistic infections, spontaneous bleeding, cardiac dysfunction, or multiorgan failure (10). In HLH-study, mortality before HSCT was 19%. 51% of patients underwent HSCT. The 5-year patient survival was 61% (11).

In the future, immunotherapy could have a place in the treatment of primary HLH. In the last years, several studies were conducted, with immunoactive agents as emapalumab, a human anti-interferon-gamma antibody, anti-JAK, alemtuzumab and ATG (1).

Conclusions

HLH is a rare and underrecognized syndrome. It is important to know diagnostic criteria, because issue of this disease can be fatal if not treated. Several factors may lead to a delayed diagnosis, such as the lack of knowledge about this disease, an incomplete picture at the beginning, or the time to obtain some specific tests results. It is important to think about this diagnosis in patient with prolonged fever, pancytopenia and hepatosplenomegaly, and to continuously re-consider diagnosis even if criteria are uncompleted at the first evaluation. Once the diagnosis is made, treatment has to be rapidly started. For now, the only curative treatment for primary HLH is HSCT.

Conflict of interest

The authors declare that there is no conflict of interest.

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