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Case report

Neonatal Hemophagocytic Lymphohistiocytosis – Case Report

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A B S T R A C T

Hemophagocytic lymphohystiocytosis (HLH) represents a severe hyperinflammatory condition with the cardinal symptoms prolonged fever, hepatosplenomegaly, and cytopenias. The most prominent histopathological feature of HLH is an accumulation of activated T lymphocytes and macrophages predominantly in lymphoid tissues. Although it can occur in all age groups, neonatal-onset HLH is very rare. We report on a case of HLH presenting with anemia and respiratory distress at birth. Several weeks prior to diagnosis the symptoms were attributed to a systemic infection. The child developed typical clinical and laboratory findings, and was diagnosed with HLH according to HLH-2004 guidelines. Chemo-immunotherapy was initiated, but after a temporary control of the disease the patient succumbed to rapidly progressive HLH. Post-mortem, extensive hemophagocytosis was found in multiple organs. No specific genetic defect was identified. HLH is potentially fatal childhood disease. It is important for pediatricians to be able to early identify this disorder and commence the therapy before overwhelming disease activity develops.

Key words: *hemophagocytic lymphohistiocytosis, neonate*

Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a rare disorder characterized by widespread accumulation of lymphocytes and mature macrophages, sometimes with hemophagocytosis, primarily involving the spleen, lymph nodes, bone marrow, liver and cerebrospinal fluid[1]. The hallmark of HLH is an uncontrolled hyperinflammation, caused by unremitting activation of CD8+ T cells and macrophages, and excessive levels of cytokines[2]. The symptoms and signs may vary widely, but the most typical findings are prolonged fever, hepatosplenomegaly and cytopenias[3].

HLH comprises two different conditions: primary or genetic HLH, and secondary or acquired hemophagocytic syndrome (secondary HLH, sHLH). These two forms may be difficult to distinguish from one another[1].

Familial hemophagocytic lymphohistiocytosis (FHL) is a subgroup of genetic HLH inherited in an autosomal recessive manner. The incidence has been estimated to 1.2/1.000.000 children *per* year, i.e. 1:50.000 live-born[4]. Most children develop the disease very early in life with around 70% less than 1 year of age at onset. FHL may also present at birth or even at prenatal investigations. Several genetic defects underlying FHL have been discovered recently and have elucidated the pathophysiology of the disease. Without treatment, FHL is invariably fatal, with a median survival of 2 months after diagnosis[4,5].

We present a two-month-old male infant with very early neonatal presentation. The patient was misdiagnosed with sepsis and accompanying anemia for several weeks prior to diagnosis of HLH.

Case Report

A 4-day-old male neonate was transferred to University Children's Hospital Rijeka from the Division of Neonatology, Department of Obstetrics and Gynecology, for

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further observation. He was born at 34 weeks' gestation, with a birth weight of 1.800 g, length 45 cm, and Apgar scores of 5 and 6 at 1 and 5 minutes respectively. During the first hours of life the child developed respiratory distress accompanied by anemia (hemoglobin 80 g/L) requiring transfusion therapy. On the second day of life the patient became icteric and hypotonic. He was the first child of non-consanguineous healthy parents. Maternal serum screening tests for toxoplasmosis, syphilis, rubella, hepatitis B, Epstein-Barr virus (EBV), cytomegalovirus (CMV), herpes simplex virus (HSV), and human immunodeficiency virus (HIV) were negative. Prenatal ultrasound examinations had shown normal fetal growth and development. The family history was notable for two father's siblings having died in infancy for unknown reasons.

On admission to our hospital the patient was hypotonic, pale and icteric. Antibiotic treatment (ampicillin and gentamycin) was given for presumed systemic infection. During the third week of life his general condition worsened, with pallor, fever (>38.5 °C) and hepatosplenomegaly. The laboratory findings were as follows: RBC 3.49×10^9/L, hemoglobin 75 g/L, WBC 6.7×10^9/L (neutrophils 42%), platelets 289×10^9/L, reticulocytes 2.5%, LDH 437 IU/L, ferritin 662 mg/L, total protein 4.2 g/dL with albumin 2.7 g/dL, and C-reactive protein 24.3 mg/L. Blood cultures were negative. RBC transfusion, broad spectrum antibiotics, acyclovir and intravenous immunoglobulins were given, and his general condition temporarily improved. During the following weeks, the patient suffered from multiple episodes of fever. Repeated bacterial blood cultures were negative. Serologic tests for HSV, CMV, EBV, parvovirus B19, enteroviruses, adenoviruses, and hepatitis B and C viruses were negative. Other findings included persistent hyperferritinemia (max. 1820 mg/L) and hypofibrinogenemia (min. 0.9 g/L). In the eighth week of life the child's condition deteriorated with high fever, irritability, bulging fontanel, hypertonus, altered consciousness, massive hepatosplenomegaly with abdominal distension, and multiple firm subcutaneous nodules. He had severe anemia and mild thrombocytopenia. Examination of a spinal fluid revealed mononuclear pleocytosis and elevated protein content. Magnetic resonance imaging of the brain was normal. Abdominal ultrasound and computed tomography showed enlarged spleen and enlarged hyperdense liver. At this point the child was referred to the hematologist for a further evaluation. Because of the high suspicion of HLH, bone marrow aspiration was performed. Myelopoiesis shifted towards immaturity and limited erythropoiesis was present, without evidence of hemophagocytosis. A biopsy of a subcutaneous nodule from the lower abdomen demonstrated lobular panniculitis and numerous histiocytes with cytophagocytosis (Figure 1). Triglyceride levels were normal. Flow cytometric immunophenotyping of peripheral blood revealed significantly lower percentage of CD19+ B-lymphocytes (25%). Relative percentage of helper T lymphocytes (CD3+CD4+) was higher (67%), while percentage of cytotoxic T lymphocytes (CD3+ CD8+) was lower (10%). The CD4/CD8 ratio was 6.65 (normal range 0.9–3.4). The percentage of the peripheral blood NK-cells (CD56+) was normal (13%). Serum levels of immunoglobulins were decreased. Testing for soluble

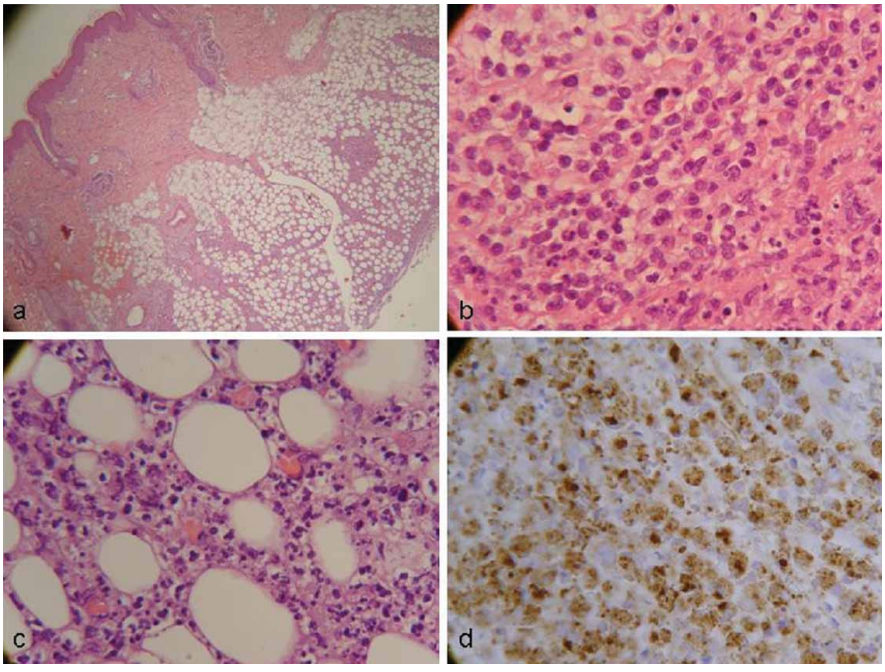

Fig. 1. Cytophagic histiocytic panniculitis: (a) an intense inflammatory cell infiltration present at the junction between the dermis and subcutaneous fat; (b) consisted predominantly of histiocytes with abundant eosinophilic cytoplasm and irregular vesicular nuclei; (c) with some cells which demonstrated erithrophagocytosis; (d) and positive for CD68 immunostaining.

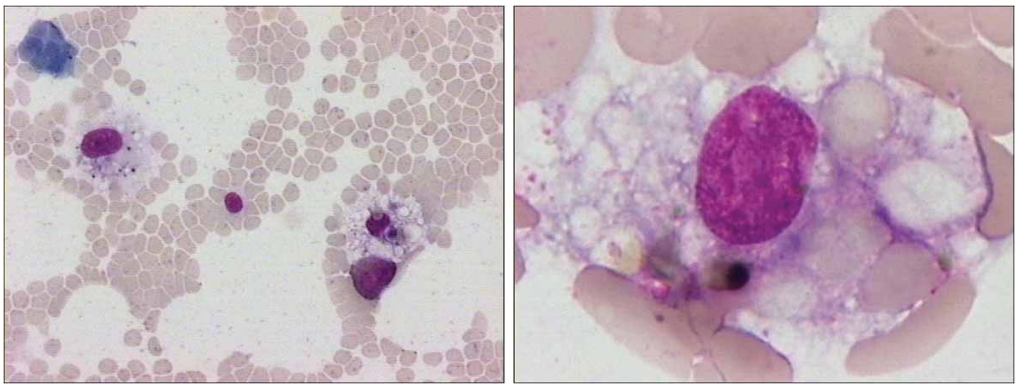

Fig. 2. Bone marrow aspirate showing: (a) marked hypocellularity and (b) erithrophagocytosis.

interleukin-2 receptor, perforin expression, and natural killer (NK)-cell activity was not available.

Altogether, clinical and laboratory parameters fulfilled the criteria for the diagnosis of HLH, and the treatment according HLH-2004 protocol was initiated (dexamethasone, etoposide, cyclosporine A). The child's general condition, neurological disturbances and organomegaly much improved, with a fever and skin nodules resolving within 2 days and 7 days respectively. His laboratory parameters also improved significantly. The patient had no available HLA-identical related donor, and it was decided to carry out the international search for an unrelated donor for hematopoietic stem cell transplantation.

Two weeks after the start of the treatment the patient's condition rapidly deteriorated with high fever, generalized edema, enlarged spleen, severe anemia, thrombocytopenia, and hyperferritinemia (5100 ng/mL). The bone marrow was hypocellular, with normal hematopoietic elements being replaced by macrophages that exhibited erythrophagocytosis (Figure 2).

The clinical condition of a child further deteriorated with circulatory and respiratory insufficiency requiring mechanical ventilation. Chest X-ray showed bilateral interstitial pneumonia and cardiomegaly. Echocardiography confirmed marked enlargement of left ventricle with significantly decreased contractility. Multiple organ dysfunction syndrome (MODS) developed, and the patient died of cardiorespiratory failure and disseminated intravascular coagulation at the age of 3.5 months, four weeks after the initiation of the treatment.

At autopsy, extensive lymphohystiocytic hemophagocytosis was identified in the bone marrow, lungs, liver and spleen. Post-mortem genetic tests for FHL including *PRF1* (FHL2), *UNC13D* (FHL3), and *STX11* (FHL4) were negative. No mutations were found in both parents.

Discussion

HLH encompasses a heterogeneous class of rare but potentially fatal disorders characterized by multisystem inflammation, which results from prolonged and intense activation of antigen- presenting cells (macrophages, histiocytes) and CD8+T-cells, and excessive proliferation and ectopic migration of T-cells[2]. It comprises two different conditions: primary or genetic, and secondary or acquired form. Genetic HLH is inherited in an autosomal recessive or X-linked fashion and can be divided into two subgroups: familial hemophagocytic lymphohystiocytosis (FHL) in which the clinical syndrome of HLH is the only manifestation, and HLH associated with inherited immune deficiencies Chediak-Higashi syndrome, Griscelli syndrome, and X-linked lymphoproliferative syndrome which have distinctive clinical features besides the sporadic development of HLH[5,6]. Acquired (secondary) forms of HLH may develop as a result due to strong immunological activation of the immune system, which may be caused by a severe infection and malignancy. Leading triggering agents in infection-associated hemophagocytic syndrome (IAHS) are viruses of the herpes group, especially EBV and CMV. Acquired HLH in association with malignant disease (malignancy-associated hemophagocytic syndrome, MAHS), especially lymphomas, can develop before or during treatment. Macrophage-activation syndrome (MAS) is a special form of HLH which occurs in children and adults with autoimmune diseases[5].

Despite attempts to differentiate primary from secondary HLH, the clinical presentation is highly overlapping[2]. Prolonged fever (>7 days) and hepatosplenomegaly are cardinal findings. Neurological symptoms may dominate the initial clinical course, including irritability, seizures, hypo- and hypertonia, cranial nerve palsies, and altered consciousness. Lymphadenopathy, skin rash, jaundice, edema, and diarrhea are less frequent. In the early days to months of the disease, symptoms may improve spontaneously, followed by exacerbations[2]. Characteristic laboratory findings are cytopenias, especially anemia and thrombocytopenia, coagulopathy with hypofibrinogenemia, and hypertriglyceridemia. Liver dysfunction, hypoalbuminemia, elevated lactate dehidrogenase, and hyponatremia are often present[1,2]. Two highly diagnostic parameters are an increased plasma concentration of the alpha chain of the soluble interleukin-2 receptor (sCD25), and impaired NK cell activity. In patients with FHL, NK cell number is normal, but the activity is per-

TABLE 1
REVISED DIAGNOSTIC GUIDELINES FOR HEMOPHAGOCYTIC LYMPHOHYSTIOCYTOSIS

The diagnosis HLH can be established if one of either 1 or 2 below is fulfilled
(1) A molecular diagnosis consistent with HLH
(2) Diagnostic criteria for HLH fulfilled (five out of the eight criteria below)
(A) Initial diagnostic criteria (to be evaluated in all patients with HLH)
Fever
Splenomegaly
Cytopenias (affecting ≥ 2 of 3 lineages in the peripheral blood):
Hemoglobin <90 g/L (in infants <4 weeks: hemoglobin <100 g/L)
Platelets $<100 \times 10^9$/L
Neutrophils $<1.0 \times 10^9$/L
Hypertriglyceridemia and/or hypofibrinogenemia:
Fasting triglycerides ≥ 3.0 mmol/L (i.e. ≥ 265 mg/dL)
Fibrinogen ≤ 1.5 g/L
Hemophagocytosis in bone marrow or spleen or lymph nodes
No evidence of malignancy
(B) New diagnostic criteria
Low or absent NK-cell activity (according to local laboratory reference)
Ferritin ≥ 500 mg/L
Soluble CD25 (i.e. soluble IL-2 receptor) ≥ 2.400 U/mL

sistently decreased or absent. Patients with acquired HLH may have low NK cell number; NK cell function is decreased with active disease, but usually reverts to normal after treatment[5]. A lumbar puncture is also recommended as part of a diagnostic workup, and more than half of patients will have a moderate pleocytosis and/or increased protein content, even in the absence of neurological symptoms. The caution with lumbar puncture must be taken with regard to a possibly increased intracranial pressure[2]. All patients should have a bone marrow aspiration. However, frank hemophagocytosis may not be observed early in the course of the disease, and serial marrow aspirates may be helpful[7,8].

Genetic studies showed that familial form of HLH may result from mutations in different proteins involved in granule exocytosis and perforin-dependent induction of target cell apoptosis, mediated by NK-cells and cytotoxic T lymphocytes. Depending on the geographical and ethnic origin, 15 to 50% of patients with FHL (also referred to as FHL2) have mutations in perforin gene *PRF1* at locus 10q24 that lead to impaired perforin production. Perforin is a soluble cytolytic protein; it is able, in the presence of calcium, to perforate into the membrane of a target cell, where it polymerizes to form a cell death-inducing pore. *UNC13D* mutations, at locus 17q25, account for 15 to 20% of FHL (also referred to as FHL3). Encoding protein Munc13-4 is essential for priming of secretory vesicles and the subsequent release of cytolytic enzymes. Recently, a third gene defect responsible for FHL4 has been identified in Turkish families on chromosome 6q24 with mutations in *STX11*. The encoded protein, t-SNARE sintaxin 11, facilitates fusion in intracellular membrane trafficking events[2,5].

The revised diagnostic criteria for HLH, based on the recommendations of the Histiocyte Society, are summarized in Table 1. In the absence of a family history or specific molecular diagnosis, an assemblage of at least five of the eight diagnostic criteria are needed for a diagnosis of HLH and initiation of therapy[1].

HLH is a challenging condition not only to diagnose but as well as to treat. The immediate aim of the treatment is to suppress life-threatening hyperinflammation, caused by excessive levels of cytokines[5]. Another aim is to kill pathogen-infected antigen-presenting cells, and thus to remove the stimulus for the ongoing activation of T-cells. The need to treat coexisting infections as potential triggers of HLH is obvious, but usually not sufficient to control hyperinflammation[3].

The overall prognosis for patients with HLH has improved dramatically during the last decades as has the biological understanding of the disease. Current international HLH 2004 protocol is designed for all patients with newly diagnosed HLH, with or without evidence of familial or genetic disease, and regardless of suspected or documented infection[1]. The protocol represents systemic chemo-immunotherapy including dexamethasone, cyclosporine A, etoposide upfront, and, in selected patients, intrathecal therapy with methotrexate. Corticosteroids show cytotoxic effect and inhibit expression on cytokines. In pediatric protocols dexamethasone is preferred than prednisolone since it crosses the blood brain barrier

better. Cyclosporine A prevents T-lymphocyte activation. Etoposide is an antineoplastic agent highly effective in monocytic and histiocytic disorders. Intrathecal methotrexate is used only in patients with persistently abnormal cerebrospinal fluid or progressive neurological symptoms[3]. The overall 3-year disease-free survival is 55%[2].

In genetic HLH the ultimate aim must be hematopoietic stem cell transplantation (HSCT) to replace congenitally defective immune system with normal functioning immune effector cells of healthy donors. Whereas FHL was uniformly fatal without HSCT, with this protocol the overall survival rate is 58 to 64%[9].

Although HLH can occur in all age groups, neonatal onset within 4 weeks after birth is rare, accounting for 4% of all HLH cases[10]. Most patients have been published as case presentations or included in childhood HLH studies, and there are very few reported series[11,12]. The frequency of primary and secondary HLH neonatal cases has not been well defined, and is sometimes indistinguishable from one another clinically and histologically[10]. The diagnosis is frequently delayed, made on autopsy or missed completely. The course of neonatal HLH is quite devastating with high mortality[13]. Chemo-immunotherapy results in the control of the disease in some cases; however, remission is rarely sustained. The only reported survivors were those who received conventional therapy followed by HSCT[10,12].

Our patient presented with severe anemia and respiratory problems already at birth. During the neonatal period he developed typical findings for HLH, but episodes of fever were misinterpreted as systemic infection, although repeated microbiological examinations (bacterial cultures and serologic tests) were negative. All HLH neonatal reports emphasize the difficulties in establishing the diagnosis, as HLH may mimic a number of other diseases most frequent being congenital infection, sepsis, inherited metabolic disorders or hemochromatosis. Misleading in our case might be due to the fact that the patient improved several times in the course of the disease. His transient improvements could be explained by the natural course of the disease in the early phase, unspecific therapy such as transfusions, or at least in part by immunomodulation through immunoglobulins that were given repeatedly because of a suspicion for severe infection. Immunoglobulins down-regulate proinflammatory cytokines, block Fc receptors on macrophages, suppress inducer T and B-cells, and augment suppressor T-cells[4]. They have been mainly used in adults with HLH[14].

Although molecular analysis did not reveal any known genetic defect, very early presentation, serious clinical course, the lack of infectious or immunological triggering, and a family history with reasonable suspicion of siblings that died in early infancy, suggest that the presented case more likely suffered familial HLH. As specific genetic defects account for less than one half of FHL, we can only speculate that reported FHL case is caused by mutations in as yet unidentified genes.

This patient is noteworthy for the presentation in the first days of life. Previous reports from the literature have shown that patients with FHL were usually born healthy but became ill in the first 2 to 6 months of life[10]. In the present case HLH masqueraded as a systemic infection in the following weeks, and too little attention was paid to the severity of other symptoms. The onset of central nervous system involvement heralded progression of the disease, but was overlooked.

We suggest that HLH should be considered in the differential diagnosis of multisystem organ involvement in newborns and particularly in preterm neonates, especially when no infectious or metabolic cause can be found. Ferritin, fibrinogen and triglycerides measurements should be routinely determined in these patients. In the presence of cytopenias and hyperferritinemia, referral to the experienced hematologist is highly recommended. The absence of hemophagocytosis in bone marrow should not be a reason to rule out the diagnosis of HLH, as finding in up to two thirds of initial bone marrow aspirates may be nondiagnostic. If hemophagocytic activity is not proven in bone marrow, material may be obtained from other tissues, or serial marrow aspirates over time may be required to document hemophagocytosis.

Early establishment of the diagnosis of HLH has very important implications for timely commencement of the treatment, before overwhelming disease activity makes irreversible damage and a response to treatment less likely. Because neonatal HLH can be rapidly fatal without specific intervention, it is recommended to start a treatment when a high clinical suspicion exists and results of diagnostic studies are still pending[2]. HSCT should be performed as early as possible, when an acceptable donor is available. Genetic counseling and family planning is of utmost importance. Subsequent pregnancies should be closely monitored and offspring referred for genetic testing.

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NEONATALNA HEMOFAGOCITNA HISTIOCITOZA – PRIKAZ SLUČAJA

S A Ž E T A K

Hemofagocitna limfohistiocitoza (HLH) je ozbiljno stanje prekomjerne upalne reakcije karakterizirano produljenom vrućicom, hepatosplenomegalijom i citopenijom. Najznačajnija patohistološka značajka HLH je akumulacija aktiviranih T limfocita i makrofaga pretežno u limfoidnim organima. Iako se može javiti u svim dobnim grupama, neonatalni početak bolesti je vrlo rijedak. U radu je prikazan slučaj neonatalne HLH s anemijom i respiratornim teškoćama na rođenju. Nekoliko tjedana prije postavljene dijagnoze simptomi su tumačeni sistemskom infekcijom. Dijagnoza HLH je postavljena nakon razvoja tipičnih kliničkih i laboratorijskih znakova prema HLH-2004 smjernicama. Kemoimunoterapijom je postignuta privremena kontrola bolesti, iza koje je uslijedio rapidni progresivni tijek. Postmortalno je nađena opsežna hemofagocitoza u brojnim organima. Nije utvrđen specifični genski defekt. HLH je potencijalno smrtonosna bolest, čije rano prepoznavanje i započinjanje terapije je od presudne važnosti.