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Case report

Familial Hemophagocytic Lymphohistiocytosis in a 6-Week-Old Male Infant

Gordana Jakovljević[1], Ika Kardum-Skelin[2,7], Srđan Rogošić[1], Srđana Čulić[3], Jasminka Stepan[1,7], Alenka Gagro[4,7], Ivančica Škarić[5], Lili Mikecin[5], Aleksandra Bonevski[1], Ingeborg Barišić[6,7] and Melita Nakić[1,7]

[1] Department of Hematology and Oncology, Pediatric Clinic, Zagreb Children's Hospital, Zagreb, Croatia
[2] Laboratory for Cytology and Hematology, Department of Medicine, »Merkur« University Hospital, Zagreb, Croatia
[3] Department of Pediatric Hematology and Oncology, Split University Hospital Centre and School of Medicine, Split University, Split, Croatia
[4] Department of Pulmology, Allergology, Immunology and Rheumatology, Pediatric Clinic, Zagreb Children's Hospital, Zagreb, Croatia
[5] Department of Anestesiology, Reanimatology and Intensive Care, Zagreb Children's Hospital, Zagreb, Croatia
[6] Department of Clinical Genetics, Pediatric Clinic, Zagreb Children's Hospital, Zagreb, Croatia
[7] Zagreb University, School of Medicine, Zagreb, Croatia

A B S T R A C T

Familial hemophagocytic lymphohistiocytosis (FLH) is an autosomal recessively inherited multisystem disease. This defect in cellular cytotoxicity is a life threatening condition characterized by fever, rash, splenomegaly, cytopenias and neurologic manifestations. PRF1, UNC13D and STX11 gene defects underlie in about 40–50% of primary cases. Chemoimmunotherapy followed by hematopoietic stem cell transplantation improved disease outcome. We report a case of a 6-week-old boy who presented with a fever, diffuse rash, disseminated intravascular coagulation, hypofibrinogenemia, hypertrigliceridemia, hepatosplenomegaly, leukocytosis with 90% of lymphocytes, granulocytopenia, anemia, trombocytopenia, hyperferritinemia and pathological findings in cerebrospinal fluid. The patient had decreased frequency of NK cells and low NK cell activity in peripheral blood. Bone marrow aspiration analysis showed degenerative changes of histocyte cells, with preserved cytophages (lymphophages and erythrophages) consistent with hematophagocytic syndrome. Given that the molecular diagnosis of the known mutations in genes PRF1 and UNC13D showed a mutation in UNC13D, the diagnosis of familial hemophagocytic lymphohistiocytosis subtype 3 was established. HLH-2004 chemotherapy protocol was performed and partial remission with residual central nervous system disease was achieved. Hematopoietic stem cell transplantation was successfully performed with an unrelated HLA-matched donor. Familiar HLH is generaly a progressive and fatal disease. Early diagnosis with molecular genetic analysis and chemoimmunotherapy followed by hematopoietic stem-cell transplantation is the best approach.

***Key words**: familial hemophagocytic lymphohistiocytosis, UNC13D mutation, HLH-2004*

Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a rare condition, but without exceptions fatal if not treated. It presents itself as overactive macrophages and T-lymphocytes. HLH can be both an inherited and acquired conditon and is thus divided into two main groups: familial (primary) hemophagocytic lymphohistiocytosis (FLH) and secondary hemophagocytic lymphohistiocytosis (SHLH).

SHLH is most commonly linked to an Epstein-Barr virus infection, although infections with other viruses or fungi have been reported. SHLH may also be associated with malignant, genetic or autoimmune diseases[1,2]. Immune diffeciency syndromes such as Chediak-Higashi syndrome 1, Griscelly syndrome 2 and X-linked proliferative syndrome have also been related to HLH[3].

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However, familial form of HLH is an autosomal recessive disorder seen mostly in infancy and childhood and is invariably fatal if untreated. Incidence of FHL has been estimated at 1.2 cases per million, corresponding to 1:50 000 births[4]. Inherited genetic defects result in a cellular immunologic disorder that leads to hyperactivation, proliferation and infiltration of macrophages and T-lyphocytes. The presence of at least five of the eight common clinical findings (prolongued fever, cytopenias, hepatosplenomegaly, hypertriglyceridemia, hypofibrinogenemia, hemophagocytosis, low or absent natural killer (NK) cell activity, hyperferritinemia and high plasma concentrations of soluble CD25) can be sufficient to establish a diagnosis. The diagnosis can also be confirmed by detecting disease-causing mutations in disease-associated genes (PRF1, UNC13D, STX11)[5].

The treatment of this condition involves chemotherapy and immunotherapy in order to achieve clinical stability prior to allogeneic hematopoietic stem cell transplant (HSCT) as the only curative therapy. Here we present a case of a 6-week-old infant with an FHL subtype 3.

Case Report

A six-week-old male infant was admitted to our hospital due to diarrhea with slight traces of blood in the stool and confluent maculo-petechial rash after receiving antibiotic treatment for a respiratory infection. He had a normal prenatal history. Family history has no particularities apart from two great grandmother's children deceased shortly after birth from an unknown cause. The patient is the first child of unrelated parents and was born from mother's first and only pregnancy which was without any significant events until a week before birth when the complications were manifested in terms of hypertension. Birth was at term, with vacuum extraction. Infancy period was without any abnormalities and with regular development.

Upon initial physical examination he was found to be without fever but with hepatomegaly 6 cm below the rib cage and splenomegaly 8 cm below the rib cage. Laboratory findings revealed high leukocyte levels at 25.28×10^9/L (granulocytes 1%, lymphocytes 90%, monocyte 4%, reactive lymphocytes 5%), erythrocytes 3.35×10^{12}/L, hemoglobin 100 g/L, hematocrit 0.286, low thrombocyte levels at 28×10^9/L, alanine aminotransferase 80 U/L, aspartate aminotransferase 63 U/L, gamma glutamyl transpeptidase 404 U/L, lactat dehydrogenase 549 U/L, blood glucose 6.6 mmol/L, ferritin >5000 ng/mL, cholesterol 2.68 mmol/L, triglycerides 4.76 mmol/L, high-density lipoprotein 0.35 mmol/L, low-density lipoprotein 0.17 mmol/L, prothrombin time was not detectable, fibrinogen was also undetectable, thrombin time 34.7 seconds, antithrombin III (ATIII) 76.2%, D-dimer 6.3 mg/L. The following day the boy's coagulation test results worsened, all of which were indicative of the development of disseminated intravascular coagulation. He developed a fever and his general condition also deteriorated so he was treated with intravenous broad spectrum antibiotic along with fresh frozen plasma, thrombocytes, cryoprecipitate and ATIII substitutions. First bone marrow aspirate was taken on the 5th day of the admission and showed no abnormalities. Due to thrombocytopenia we tested for antithrombocytic antibodies in peripheral blood and found binded antithrombocyte antibodies of IgG class. At that time the boy received intravenous immunoglobulin therapy but with no clinical impact. Leukopenia and anemia slowly developed along with thrombocytopenia. Rapid exacerbation together with constantly deteriorating laboratory results (leukocytes 1.7×10^9/L, thrombocytes 27×10^9/L and hemoglobin 80 g/L) on the 9th day of the admission indicated another bone marrow aspiration. Bone marrow aspiration analysis showed mediocellular bone marrow with regeneratory granulopoiesis mainly up to a stage of metamyelocyte and a few mature forms. Plenty of megakaryocytes were found together with abundant erythropoiesis. We observed degenerative changes of histocyte cells, alone and in clusters, with preserved cytophages (lymphophages and erythrophages) consistent with hemophagocytic syndrome (Figure 1). Decreased frequency of NK cells and low NK cell activity (<1%) were detected in peripheral blood. Intracellular perforin expression in peripheral blood mononuclear cells determined by flow cytometry was within the age-dependent reference range. Cytotoxic lymphocyte activity was performed. The cytotoxicity was immediately above the limit set as pathological (<10 LU). NK cell degranulation was deficient.

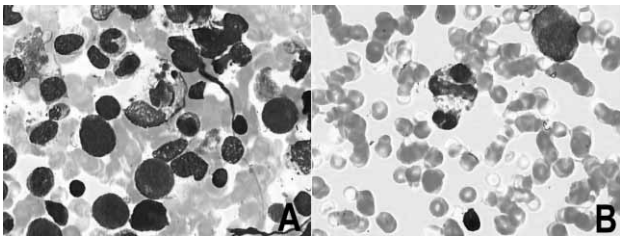

Fig. 1. The bone marrow aspirate smears: a) phagocytosis; b) lympho- and granulophagocytosis in the same macrophage.

EBV PCR results as well as EBVCA IgM serology were negative. PCR and correspondingly RT-PCR analysis results for herpes simplex virus, cytomegalovirus and parvovirus B19 were negative. Serologic testing detected no IgM antibodies for parvovirus B19 or adenovirus. All other infectious, neoplastic or hereditary metabolic diseases have also been ruled out. Genetic testing revealed a single UNC13D mutation which was together with all the other results compliant to the diagnosis of familial hemophagocytic lymphohistiocytosis subtype 3.

We commenced treatment according to the hemophagocytic lymphohistiocytosis protocol HLH-2004. It includes etoposide, dexamethasone, cyclosporine A upfront and, in selected patients, intrathecal therapy with methotrexate and corticosteroids. Subsequent hematopoietic stem cell transplantation (HSCT) is recom-

mended for patients with familial disease or molecular diagnosis and patients with severe and persistent or reactivated disease[4]. Already after one week from the beginning of the treatment we observed clinical improvement, organomegaly regression and complete blood cells count (CBC) improvement. The boy reacted well to the induction therapy, but had diarrhea with positivity to Clostridium difficile toxins and perianal ulceration positive to Pseudomonas aeruginosa. Therefore, we conducted a targeted antibiotic treatment, with all supportive therapy recommended by the protocol. He received irradiated and filtered erythrocytes and thrombocytes a few times. After central nervous system (CNS) involvement had been detected [number of cells in CSF 928/3 with 79% lymphocytes (71% CD3 positive), 20% macrophages and 1% plasma cells] (Figure 2), the boy received intrathecal therapy four times. Head MRI showed no lesions. Upon finalization of the induction the boy's general condition improved. Liver and spleen size were within normal parameters, CBC was normal and coagulation tests were satisfying. Since CSF analysis showed persistent signs of CNS involvement (residual cells in CSF 150/3), we introduced intravenous corticosteroids (dexamethasone 10 mg/m^2/day for 7 days and then reduced to 5 mg/m^2/day) and kept those doses for another two weeks. We observed a partial regression in the number of cells in CSF, so dexamethasone pulses were continued with etoposide every second week during the continuation therapy. The entire time the patient was on oral cyclosporin A. He had no HLA-identical related donor and a search for a matching unrelated donor was begun.

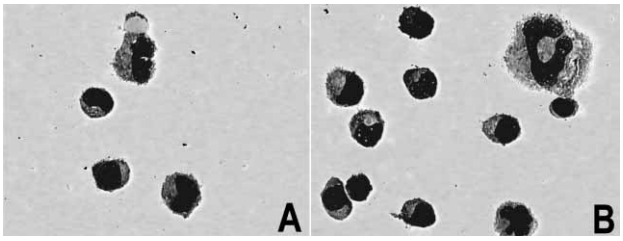

Fig. 2. The cerebrospinal fluid: a) erythrophagocytosis; b) lymphocytes (1), a macrophage (2) and a monocyte (3).

After six months of chemotherapy the boy was referred to another hospital for unrelated umbilical cord blood hematopoietic stem cell transplantation. Reduced intensity conditioning regimen included fludarabine, treosulfan and anti-thymocyte immunoglobulin. In the post transplantation period the patient presented a severe graft-versus-host disease including skin, gut and liver. Immunosuppressive treatment consisting of cyclosporine and prednisolon started at the time of HSCT was continued through more than six-month period after the procedure, during which it was slowly tapered off. Six months following the HSCT the patient was in good condition and with no signs of graft-versus-host disease. Donor and recipient chimerism test showed almost full donor population of transplanted cells. His immunity will take another year or two to function adequately. 18 months following the diagnosis the patient is in remission and shows no signs of illness.

Discussion

HLH is a rare autosomal recessive disease, presented as a severe hyperinflammatory syndrome with intense activation of macrophages and T lymphocytes. The disease usually develops in the first few months of life or rarely in utero, but it is invariably fatal if not treated[6]. So far four genes have been related to the development of HLH, further dividing the disease into four subtypes. FHL subtype 1 was found in four inbred Pakistani families in which locus was identified on chromosome 9q21.3–q22 but the gene is still unknown. FHL subtype 2 is connected to more than 50 mutations of the *PRF1* gene coding for perforin-1 protein, well known as a cytolytic effector. FHL subtype 3 is defined by mutations of the *UNC13D* gene coding for unc-13 homolog D protein involved in cytolytic secretory pathway. FHL subtype 4 is linked to 3 recurrent mutations of *STX11* gene coding for syntaxin-11 known for its role in vesical transport[7]. Depending on the origin, 13–50% of patients with FHLH have mutations in the perforin gene, and 17–30% in UNC13D gene, while STX11 mutations have been found only in patients of Kurdish/Turkish origin[3].

FHL is a rare disease but with a well defined diagnostic guidelines. The diagnostic criteria based on the recommendations of the Histiocyte Society include at least five of the eight following findings: fever, splenomegaly, cytopenias affecting at least two of three cell lineages in peripheral blood, hypertriglyceridemia and/or hypofibrinogemia, hemophagocytosis in bone marrow, spleen or lymph nodes, low or absent natural killer (NK) cell function activity, hyperferritinemia and high levels of soluble CD25 (soluble IL-2 receptor). Biallelic mutations in PRF1, UNC13D (also known as MUNC13-4) or STX11 genes also confirm the diagnosis of FHL.

The treatment of this disease according to the hemophagocytic lymphohistiocytosis protocols HLH-94 and HLH-2004 includes chemotherapy and immunotherapy followed by HSCT. Survival improved significantly with introduction of HSCT in the treatment protocol and led to three-year survival at approximately 64%[8].

In our case, the patient presented with fever, pancytopenia, hepatosplenomegaly, hypertriglyceridemia, hypofibrinogenemia, hyperferritinemia, low NK-cell activity and hemophagocytosis observed in the bone marrow, thus meeting seven out of eight clinical criteria for the diagnosis of FHL. Additional genetic testing further confirmed the right diagnosis. Our patient was tested negative for PRF1 mutation, but only one mutation of UNC13D was found. Although FHL is an autosomal recessive disease, that kind of finding has been reported and it might be explained by the presence of second presumed mutation in a different HLH-causing gene or within the introns of the UNC13D gene or its promoter region[9]. The distribution of the mutations in Croatian FHLH patients is unknown, as this is the first patient in

which the genetic testing was performed to our knowledge. Further aggravating circumstance was the involvement of central nervous system which has been correlated with a worse outcome[10]. But with slight alteration of the treatment protocol in form of continuing intravenous dexamethasone therapy (10 mg/m^2 / *per* day), we managed to achieve partial remission, sufficient to subdue the patient to an HSCT.

We believe our case states that early diagnosis and rapid and adequate therapy is of great essence in treating FHL. Considering the patient's bad condition and central nervous system involvement as negative predictors, we might conclude that physicians' heightened sense of awareness, timely diagnosis and early beginning of the treatment were important to prevent indubitably fatal outcome and provide a good recovery.

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G. Jakovljević

Department of Hematology and Oncology, Zagreb Children's Hospital, Klaićeva 16, Zagreb, Croatia
e-mail: gordanajakovljevic@yahoo.com

FAMILIJARNA HEMOFAGOCITNA LIMFOHISTIOCITOZA U DJEČAKA STAROG ŠEST TJEDANA

SAŽETAK

Familijarna hemofagocitna limfohistiocitoza (FLH) je autosomalno recesivna nasljedna multisistemska bolest. Ovaj poremećaj stanične citotoksičnosti je životno ugrožavajuće stanje karakterizirano vrućicom, osipom, splenomegalijom, citopenijom i neurološkim manifestacijama. PRF1, UNC13D i STX11 genski defekti nalaze se u podlozi 40–50% slučajeva FLH. Kemoimunoterapija i naknadna transplantacija hematopoetskih matičnih stanica značajno poboljšavaju ishod bolesti. Ovdje smo prikazali slučaj dječaka starog šest tjedana s vrućicom, difuznim osipom, diseminiranom intravaskularnom koagulacijom, hipofibrinogenemijom, hipertrigiceridemijom, hepatosplenomegalijom, leukocitozom s više od 90% limfocita, granulocitopenijom, anemijom, trombocitopenijom, hiperferitinemijom i patološkim nalazom u cerebrospinalnom likvoru. U perifernoj krvi nađen je smanjen postotak i niska aktivnost NK stanica. U analiziranom aspiratu koštane srži otkrivene su degenerativno promijenjene histiocitne stanice i očuvani citofazi (limfofazi i eritrofazi), što je odgovaralo hemofagocitnom sindromu. Uz molekularnu potvrdu mutacije UNC13D gena, postavljena je dijagnoza FHL podtipa 3. Primijenjen je HLH-2004 kemoterapijski protokol i postignuta parcijalna remisija uz prisutnu rezidualnu bolest središnjeg živčanog sustava. Transplantacija hematopoetskih matičnih stanica od nesrodnog HLA-podobnog darivatelja uspješno je izvedena. FHL je progresivna i fatalna bolest čiji se bolji ishod postiže ranim postavljanjem dijagnoze i provođenjem pravovremene kemoimunoterapije s alogenom transplantacijom hematopoetskih matičnih stanica.