

# Extreme Hypertriglyceridemia in an Infant with Hemophagocytic Lymphohistiocytosis and Hydroxycobalamin Deficiency

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## SUMMARY

**Introduction** Hemophagocytic lymphohistiocytosis (HLH) is a severe hyperinflammatory condition characterized by fever, cytopenias, hepatosplenomegaly and hemophagocytosis. HLH may be primary or secondary to infection, autoimmune disease or malignancy. Hypertriglyceridemia is a common abnormality in HLH and one of the HLH-2004 diagnostic criteria.

**Case Outline** We present an infant with severe hypotonia and hypoproteinemic edema who also had extreme hypertriglyceridemia (21 mmol/l) and was diagnosed with HLH based on six of eight HLH-2004 criteria (fever, hepatosplenomegaly, bicytopenia, hypertriglyceridemia with hypofibrinogenemia, sIL-2R > 2400 IU/ml, hemophagocytosis). The presence of IgM antibodies to Epstein-Barr virus and cytomegalovirus indicated a probable infectious trigger. The child was cured by the HLH-2004 protocol for secondary HLH (consisting of dexamethasone and cyclosporine). He was also found to have low serum hydroxycobalamin levels, promptly corrected upon hydroxycobalamin administration.

**Conclusion** The presented case history underlines the need to ascertain the presence or absence of each of the eight HLH-2004 criteria in any patient suspected to suffer from HLH.

**Keywords:** hemophagocytic lymphohistiocytosis; infant; hypertriglyceridemia; hydroxycobalamin deficiency

## INTRODUCTION

Hemophagocytic lymphohistiocytosis (HLH) is a severe hyperinflammatory condition characterized by prolonged fever, cytopenias, hepatosplenomegaly and the phenomenon of hemophagocytosis, exerted by abnormally and excessively activated macrophages that cause a 'cytokine storm' [1]. HLH may be caused by a genetic defect (primary HLH, either a separate disease designated familial HLH or part of certain primary immunodeficiency disorders) or it may be secondary to other conditions, such as infection (particularly that caused by herpesviruses and *Leishmania*) [2, 3], autoimmune disease (where it is often called macrophage activation syndrome) [4] or malignancy (i.e. lymphoma) [5]. As envisaged by analyzing the functions of the genes involved in primary HLH, it appears that the common element in the pathogenesis of all types of HLH is a defect in immune functions that are dependent on exocytosis of vesicles containing cytotoxic granules – the action of natural killer (NK) cells and cytotoxic T-lymphocytes (CTL). The functional impairment of these cells leads to inefficient immune response with the persistence of invading microorganism and consequent inflammatory hyperstimulation, as well as to inefficient termination phase of the immune response, where NK cells and CTL also appear to play a key physiological role. It is less

clear what causes the impairment of cytotoxic functions in secondary HLH, and the extent of underlying genetic predisposition toward the abnormal response that characterizes HLH is difficult to measure against the contribution of exogenous factors. Since the initial clinical course of secondary HLH resembles closely the expected course of infection or other triggering condition, it is important that treating clinician bears in mind the possibility of this disorder. In addition to fever, cytopenias and hepatosplenomegaly, diagnostic criteria for HLH proposed by the Histiocyte Society [6] include hypertriglyceridemia, hyperferritinemia, hypofibrinogenemia, increased concentration of the receptor for interleukin (IL)-2 in the plasma, as well as decreased NK cell functional capacity. Hemophagocytosis (as demonstrable by bone marrow aspirate) may or may not be present, and its absence by no means excludes the diagnosis of HLH, a fact that is often overlooked, delaying the diagnosis. Although hypertriglyceridemia is a diagnostic criterion for HLH, its presence in patients affected with this disorder is far from universal, and its severity, where present, tends to be highly variable [7].

## CASE REPORT

A seven-month-old male infant of body length of 72 cm and body mass of 6,530 g (body mass

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index of 12.6 kg/m<sup>2</sup>, under the 5th percentile: underweight) was admitted at the University Children's Hospital, Belgrade, Serbia, due to extreme hypotonia, high fever and hepatosplenomegaly. His mother indicated that the hypotonia was first apparent at the age of 4.5 months. However, the child had been diagnosed with perinatal asphyxia and a slight hypotonia had been medically documented since birth. Upon examination, the child was revealed to have moderate hepatomegaly (later confirmed by ultrasonography at 100 mm right lobe craniocaudal diameter). The spleen was also slightly enlarged (craniocaudal diameter of 80 mm). Blood analyses showed bicytopenia (red blood cell count  $2.71 \times 10^{12}$  cells/l, hemoglobin 9.9 g/l, white blood cell count  $6.6 \times 10^9$  cells/l, absolute number of granulocytes  $0.6 \times 10^9$  granulocytes/l, platelet count  $527 \times 10^9$  platelets/l) with high mean corpuscular volume (110 fl). There was an extreme hypertriglyceridemia (21 mmol/L) and hypofibrinogenemia (1.1 g/l), while serum ferritin level was normal (147.6 µg/ml). Findings in the cerebrospinal fluid were also normal. The patient was an exclusively breast-fed infant, and his personal and family histories were unremarkable, as was the nutritional history of the mother. The patient is the only child of his non-consanguineous parents, who showed a normal lipid profile. Bone marrow aspiration showed a reduced myeloid precursor count with predominance of erythroid lineage exhibiting megaloblastic appearance. Scarce hemophagocytosis was noted. Bone marrow biopsy confirmed dysplastic/megaloblastic changes of the erythroid lineage.

Biochemical blood analyses also demonstrated hypoalbuminemia (14 mg/dl) and hypoproteinemia (31 mg/dl), as well as elevated lactate dehydrogenase (1,270 IU/l), alanine-aminotransferase (350 IU/l) and aspartate-aminotransferase (128 IU/l), while prothrombin and partial thromboplastin times were normal. The patient's lipid profile, apart from elevated triglycerides, showed a normal level of cholesterol (2.6 mmol/l), elevated lipoprotein A (867 mg/dl), reduced apolipoprotein A-I (54.1 mg/dl) and slightly reduced apolipoprotein B (53.9 mg/dl). Metabolic screening of the patient's urine showed increased excretion of all amino acids, positive cyanonitroprusside test (due to cystinuria) and mild proteinuria. Excretion of phenolic acids, organic acids, imidazoles, proline and hydroxyproline were all normal, as well the as anthron test, ferrichloride test, 2,4-DNPH test and toluidin blue test. There was no excretion of glucose or ketones.

Serological assays revealed IgM antibodies specific for Epstein-Barr virus (EBV), as well as both IgM and IgG antibodies specific for cytomegalovirus (CMV), while the antibodies to rubella virus, herpes simplex virus, human immunodeficiency virus and *Toxoplasma* were all absent. Lymphocyte populations, as evaluated by flow cytometry, were all within age-appropriate reference range. The receptor for IL-2 in the serum (sIL-2R) was markedly elevated (3,736 IU/ml). Although NK and cytotoxic T-cell function was not evaluated due to technical limitations, six of the eight HLH-2004 criteria were fulfilled: fever, hepatosplenomegaly, bicytopenia, hypertriglyceridemia with hypofibrinogenemia, and sIL-2R above 2,400 IU/ml. The

diagnosis of HLH was established. The child was subsequently treated according to the HLH-2004 protocol for secondary HLH: dexamethasone daily 10 mg/m<sup>2</sup> for two weeks, then 5 mg/m<sup>2</sup> for two weeks, then 2.5 mg/m<sup>2</sup> for two weeks, then 1.25 mg/m<sup>2</sup> for a week; cyclosporine 6 mg/kg daily divided into two doses, later corrected aiming at plasma level of 200 µg/l; however, no VP16. Shortly after the initiation of treatment, hypertriglyceridemia and neutropenia resolved. After the lipidemia improved, low levels of hydroxycobalamin (<60 pg/ml; reference range >200 pg/ml) were detected in the serum, and 1 mg/kg hydroxycobalamin was given i.m. three times weekly. In a matter of weeks after the institution of hydroxycobalamin treatment, mean corpuscular volume was reduced to 95 fl and signs of megaloblastosis disappeared from the bone marrow. In the course of two months, neurological problems also receded. Upon completion of HLH-2004 treatment, the child has been subjected to follow-up in regular intervals and appears to be in good health. By the time of writing, more than three years have passed without problems. A follow-up measurement of hydroxycobalamin serum level showed that it is now within the normal range (348 pg/ml). Serum hydroxycobalamin level of the mother was also measured and found to be moderately reduced (138 pg/ml).

#### Statement on ethics

Written informed consent for the publication of this case history was obtained from the child's parents in accordance with the Declaration of Helsinki, institutional Ethical Committee guidelines and relevant legal requirements.

#### DISCUSSION

HLH was reported to be the presentation in a case of transcobalamin II deficiency [8]. In the light of this finding, it is possible that very low levels of hydroxycobalamin in our patient could have increased the likelihood of occurrence of secondary HLH upon an appropriate trigger. This trigger was most probably EBV or CMV infection documented by the presence of anti-EBV and anti-CMV IgM antibodies, respectively. Transcobalamin II deficiency in our patient remains a possibility, particularly given the ever-widening clinical spectrum of this disorder of cobalamin metabolism [9]. However, the fact that we found a subclinical deficit of hydroxycobalamin in the mother argues in favor of the explanation that the child simply did not receive a sufficient quantity of hydroxycobalamin from his mother. Rapid resolution of anemia and disappearance of megaloblasts upon hydroxycobalamin treatment are also in accordance with this explanation. Since the mother insisted that her dietary habits were not vegan or vegetarian and did not appear to suffer from any disorder known to be associated with secondary hydroxycobalamin deficiency, the reason(s) for her mild hypohydroxycobalaminemia remain elusive. We had no means to explore potential genetic causes.

Even though hypertriglyceridemia is a diagnostic criterion for HLH, the exact mechanism(s) that cause it are not yet fully clarified. It is thought that cytokines that abound in the plasma, above all tumor necrosis factor, inhibit lipoprotein lipase, thereby hampering the uptake of triglycerides from very low density lipoproteins into the adipose tissue [10]. In addition to the role of tumor necrosis factor, IL-18 may also be partly responsible for hypertriglyceridemia in HLH, since it is shown to be specifically present in high concentrations in the plasma of HLH patients [11]. Thus it may be reasonable to assume that the level of triglycerides, which is routinely measurable, reflects the level of cytokines, which cannot be measured but in highly specialized institutions, usually in research settings. Although found to be of uncertain prognostic value so far [7], extreme hypertriglyceridemia, such as that found in our patient, should, at the very least, prompt the physician to include HLH in the differential diagnosis, particularly if other signs of congenital hyperlipidemia syndromes are absent, as was the case here.

The diagnosis of HLH in our patient was established on the basis of six of eight HLH-2004 criteria. However, one of the fulfilled criteria was bicytopenia. Since our patient had a megaloblastic anemia that may be interpreted as a consequence of reduced hydroxycobalamin levels, it is somewhat questionable whether this criterion was legitimately fulfilled, i.e. whether the co-occurrence of leukopenia and anemia still constitutes bicytopenia for diagnostic purposes if there is good reason to believe that their causes are separate and their mechanisms unrelated. In the presented case, this dilemma was compounded by the unusual fact that the child had thrombocytosis rather than thrombocytopenia. There is no specific provision for the above question in the HLH-2004 criteria and, to the

best of our knowledge, no 'official' interpretation. In the absence of the latter, and given that there was little support for any alternative diagnosis, and that, in total, six criteria were fulfilled, we felt compelled to conclude that the diagnosis of HLH was valid. Our confidence in the diagnosis was, to a certain degree, strengthened by the fact that plasma level of sIL-2R was very high, and this parameter showed a considerable promise of high specificity in previous experiences, including our series of children affected by HLH [12].

It is interesting that our patient had a marked hypoalbuminemia and hyperproteinemia. Although this is not a diagnostic criterion for HLH, the severity of hypoalbuminemia was recently reported to be correlated with poor prognosis in adult HLH patients [13]. The most plausible explanation for this correlation is that albumin levels closely reflect the degree of liver damage. It is also worth noting that the presented child did not exhibit hyperferritinemia at any time. Intriguingly, it has recently been shown in Japanese children that hyperferritinemia is associated with poor prognosis in EBV-triggered HLH [14]. The suggested strategy of using ferritin levels as a simple screening test for HLH would clearly not have been useful in establishing the diagnosis in our patient [15], further strengthening the case for the need to ascertain the presence or absence of each of the eight HLH-2004 criteria in any patient suspected to suffer from HLH.

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## Екстремна хипертриглицеридемија код одојчета с хемофагоцитном лимфохистиоцитозом и недостатком хидроксикобаламина

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### КРАТАК САДРЖАЈ

**Увод** Хемофагоцитна лимфохистиоцитоза (ХЛХ) је тешко запаљењско стање које се одликује грозницом, цитопенијама, хепатоспленомегалијом и хемофагоцитозом. ХЛХ може да буде примарна или секундарна услед инфекције, аутоимунских болести или малигнитета. Хипертриглицеридемија је чест поремећај код ХЛХ и један од дијагностичких критеријума ХЛХ-2004.

**Приказ болесника** Представљено је одојче с тешком хипотонијом и хипопротеинским едемима које је имало и екстремну хипертриглицеридемију (21 mmol/l), а дијагноза ХЛХ је постављена на основу шест од осам критеријума ХЛХ-2004 (грозница, хепатоспленомегалија, бицитопени-

ја, хипертриглицеридемија с хипофибриногемиијом, *sIL-2R* > 2400 IU/ml, хемофагоцитоза). Постојање *IgM* антитела на Епстин-Бар вирус и цитомегаловирус указало је на инфекцију као вероватни покретач. Дете је излечено протоколом ХЛХ-2004 за секундарну ХЛХ (дексаметазон и циклоспорин). Такође је утврђен низак ниво хидроксикобаламина у серуму, који се брзо кориговао по давању хидроксикобаламина.

**Закључак** Приказана историја болести наглашава потребу да се постојање или изостанак сваког од осам критеријума ХЛХ-2004 утврди код сваког болесника за којег се посумња да болује од ХЛХ.

**Кључне речи:** хемофагоцитна лимфохистиоцитоза; одојче; хипертриглицеридемија; дефицит хидроксикобаламина

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