

## Suceso de trasplante de médula ósea exitoso en un caso de linfohistiocitosis hemofagocítica familiar tipo 3

### Trasplante de médula ósea exitoso en un caso de linfohistiocitosis hemofagocítica familiar tipo 3

Gabriela Caicedo-Herrera<sup>a</sup>, Estephania Candelo<sup>a</sup>, Manuela Olaya<sup>b</sup>, Paola Pérez<sup>b</sup>, Diego Medina<sup>b</sup>, Harry Pachajoa<sup>a,b</sup>

<sup>a</sup>Centro de Investigaciones en Anomalías Congénitas y enfermedades Raras, Universidad ICESI. Cali, Colombia.

<sup>b</sup>Fundación Clínica Valle De Lili. Cali, Colombia.

Received: Jun 16, 2020; Approved: September 7, 2020

#### What do we know about the subject matter of this study?

Hemophagocytic lymphohistiocytosis (HLH) is a clinical syndrome caused by an inadequate response of the immune system to a trigger, which has a primary (familial) or secondary etiology that is very difficult to differentiate when there is an infection.

#### What does this study contribute to what is already known?

It demonstrated that molecular study is a valuable tool in cases of HLH to determine its etiology, even in the presence of an infection, and how BMT is a curative option for this entity.

#### Abstract

Hemophagocytic lymphohistiocytosis (HLH) is an exaggerated activation of the immune system which can be either primary (familial) or secondary. Familial hemophagocytic lymphohistiocytosis type 3 (FHL-3) is a severe immune disorder, caused by mutations in the *UNC13D* gene, which codes for a protein crucial to the cytotoxic function of lymphocytes. **Objective:** To describe the diagnostic relevance of next-generation sequencing in the approach of a patient with suspected FHL and to demonstrate the effectiveness of bone marrow transplantation as the only curative measure. **Clinical Case:** 4-year-old preschool male, previously healthy, who presented with mononucleosis syndrome and positive IgM for Epstein Barr virus, developing hepatosplenomegaly and progressive clinical deterioration. A lymphoproliferative syndrome was suspected, which was ruled out by bone marrow aspiration, finding evidence of active hemophagocytosis. The patient met the criteria for hemophagocytic syndrome (bone marrow aspiration, pancytopenia, elevated ferritin, and hypertriglyceridemia) and, given the lack of response to first-line management, including antiviral treatment, a possible primary etiology was considered. A molecular study was completed with NGS that was positive for FHL-3. Due to the progressive clinical deterioration, a bone marrow transplantation was performed, presenting successful results after the first year had elapsed. **Conclusion:** NGS is an indispensable tool in the diagnosis of FHL, mainly when the response to standard treatment is not adequate and facilitates the timely implementation of the necessary therapeutic measures.

#### Keywords:

Familial  
Hemophagocytic  
Lymphohistiocytosis;  
*UNC13D*;  
Next-Generation  
Sequencing;  
Bone Marrow  
Transplantation

Correspondence:  
Gabriela Caicedo-Herrera  
mgcaicedo@icesi.edu.co

How to cite this article: Andes pediater. 2021;92(2):268-273. DOI: 10.32641/andespediatr.v92i2.2696

## Introduction

Hemophagocytic lymphohistiocytosis (HLH) is the unrestrained activation of cytotoxic T cells, natural killer (NK) cells, and macrophages<sup>1</sup>. It can be primary (familial) or secondary to different etiologies such as infections, malignancy, rheumatologic disorders, and immunodeficiencies. However, in the absence of family history or molecular testing, it is very difficult to differentiate one from the other<sup>2</sup>.

Familial hemophagocytic lymphohistiocytosis (FHL) is an autosomal recessive disorder that occurs in approximately 1 in 100,000 children<sup>3</sup>. It is caused by mutations in genes involved in the NK cell function and cytotoxic function of TCD8+ lymphocytes (*PRF1*, *UNC13D*, *STX11*, and *STXBP2*)<sup>3-4</sup>. It is characterized by persistent fever, hepatosplenomegaly, elevated ferritin levels, cytopenia, altered NK cell cytotoxicity, and hemophagocytosis<sup>5</sup>. FHL type 3 (OMIM 608898) is the second most frequent genetic form (30-40%). It is caused by mutations in the *UNC13D* gene, which encodes the Munc13-4 protein, which interferes with vesicle maturation during exocytosis and participates in the regulation of cytolytic granule secretion<sup>1</sup>.

The course of FHL is rapidly progressive and fatal in some cases, unless adequate treatment is established. Bone marrow transplant is recommended for all patients with a family history or molecular diagnosis of FHL and patients with severe secondary disease refractory to traditional management, i.e. with persistent clinical symptoms for 8 weeks from the start of management<sup>6-7</sup>.

The diagnosis of hemophagocytic syndrome is a real challenge because the signs and symptoms are compatible with other common diseases and are often confused. It is especially important to define whether the condition is of primary or secondary etiology, in the presence of a viral infection that may be the trigger of FHL. This case report aims to present the questions at the time of diagnosis and to clarify when to suspect a primary etiology, in addition to determining the importance and relevance of next-generation sequencing (NGS) in this type of pathology and how bone marrow transplant (BMT) is a curative option for this entity. The objective of this report is to describe the diagnostic relevance of NGS in the approach of a patient with suspected FHL and to demonstrate the effectiveness of BMT as a curative option.

## Clinical Case

A 4-year-old male preschooler, from southwestern Colombia, without paternal consanguinity, but with

forebears from the same locality, with no significant family history (suggesting primary immunodeficiencies or dysregulation syndromes). He was admitted to the emergency department of a local hospital due to 2-week history of fever and cervical adenopathies that was managed as pharyngotonsillitis on an outpatient basis; however, he persisted with fever and the appearance of larger lymphadenopathies. He was hospitalized to start studies and administration of intravenous antibiotics.

During hospitalization, he presented abdominal distension, jaundice, and hepatosplenomegaly associated with pancytopenia. Bone marrow aspiration was performed ruling out neoplastic infiltration, with representation of the three cell lines, and the presence of hemophagocytosis was registered. He was diagnosed with hemophagocytic syndrome due to bone marrow involvement, elevated ferritin levels, hypertriglyceridemia, clinical picture suggestive of infection by Epstein Barr virus (EBV), and positive IgM. He started management with dexamethasone, etoposide, and cyclosporine A according to HLH 2004 protocol, however, the patient presented clinical deterioration (day 15 of hospitalization), with a high risk of ventilatory failure, thrombocytopenia, and severe coagulation alterations, thus he was referred to our institution.

The patient was admitted with generalized jaundice, bilateral cervical lymphadenopathy, hepatosplenomegaly, and marked ascites. Laboratory tests showed panhypogammaglobulinemia, pancytopenia (Hb 8.6 g/dL, platelets 70,000/ $\mu$ L, lymphocytes 2310 cells/ $\mu$ L, and neutrophils 120), elevated ferritin levels (10,637 ng/mL, 4-67 ng/mL NV), hypertriglyceridemia (397.9 mg/dL, 0-200 mg/mL NV), fibrinogen consumption (70 mg/dL), and positive IgM for EBV. Perforin expression by flow cytometry was within normal parameters and NK cytotoxic activity was not measured due to immunosuppressive treatment.

Due to refractoriness to treatment and the patient's age, we considered a possible primary etiology and not secondary to an infection, so the study was complemented with NGS panel molecular testing that included *PRF1*, *UNC13D*, *STX11*, *STXBP2* genes to rule out FHL of primary etiology. It was decided to continue with the management for hemophagocytic syndrome according to the HLH-2004 protocol until completing 8 weeks and valganciclovir was added to the management due to positive PCR tests in cerebrospinal fluid and blood (31,650 copies/mL) for EBV.

The patient completed the HLH-2004 protocol induction with partial remission of the clinical picture and negative viral loads for EBV. Subsequently, he presented progressive clinical deterioration, persistence of elevated ferritin levels, and alteration of liver function, so it was decided to perform a BMT as curative treat-

ment, choosing the Haploidentical 5:10 parent as the donor.

The patient received conditioning regimen with thymoglobulin 5mg/kilo cumulative dose days -9-8-7, cyclophosphamide 14.5mg/kilo/dose days -6 and -5, busulfan 4.8 mg/kg/day -4, fludarabine 30mg/meter/dose days -6 to -2, TLI dose 750 cGy day -1, and rituximab 375mg/meter dose days-8 and -1. On day 84 of hospitalization, he received hematopoietic progenitor cells infusion (source: peripheral blood, donor: HLA 5:10 haploidentical parent, *CD34* cell dose:  $17.8 \times 10^6$ /Kg). He received antimicrobial and antiviral prophylaxis according to institutional guidelines. The post-transplant follow-up did not show reactivation of cytomegalovirus or Epstein Barr. The patient presented platelet and neutrophil engraftment on day +16. The last T cell chimerism was 70% and total chimerism was 60% at 16 months post-transplant. As GVHD prophylaxis he received cyclophosphamide 50 mg/kilo/day +3 and +4, tacrolimus initiated on day +4 to maintain levels between 5-12 ng/dl, methotrexate 7.5 mg/meter/dose days +5, +7, +11, and +15 with calcium folinate rescue. On day +12, he presented marked clinical deterioration associated with hepatomegaly, ascites, and elevated transaminases (ALT 886.1 - AST 1098), thus we suspected hepatic veno-occlusive disease suspending tacrolimus and methotrexate administration and started mycophenolate associated with boluses of methylprednisolone, presenting marked clinical improvement. The patient was discharged on day +29 clinically stable and with hematologic recovery. As a late complication, he presented mild chronic GVHD in the skin and liver treated with steroids and low doses of methotrexate with good evolution evolution, Figure 1.

The patient continued in outpatient management with immunoglobulin infusions initially every 2 weeks and then every 28 days. At the last visit, five years af-

ter transplantation, complete immune reconstitution (humoral and cellular) was observed. It was decided to continue outpatient multidisciplinary management and quarterly visits with the immunology service, pediatric hematology, and clinical genetics.

The NGS panel reported a homozygous mutation in exon 31 of the *UNC13D* c.3049G > A (p.Glu1017. Lys) gene that confirmed the diagnosis of FHL type 3. The finding was confirmed using the Applied Biosystems 3500 Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA) and Sanger sequencing was used to confirm the variant found in the NGS. Bioinformatic analysis was performed using different tools such as PolyPhen-2, MutationTaster, and SIFT, which classified the variant as “disease-causing” and “harmful”. Additionally, a DANN Score of 0.999 was found. Based on the above, the variant was classified as “probably pathogenic” according to the guidelines of the American College of Medical Genetics and Genomics (PM1, PM2, PM3, PP3)<sup>8,9</sup>.

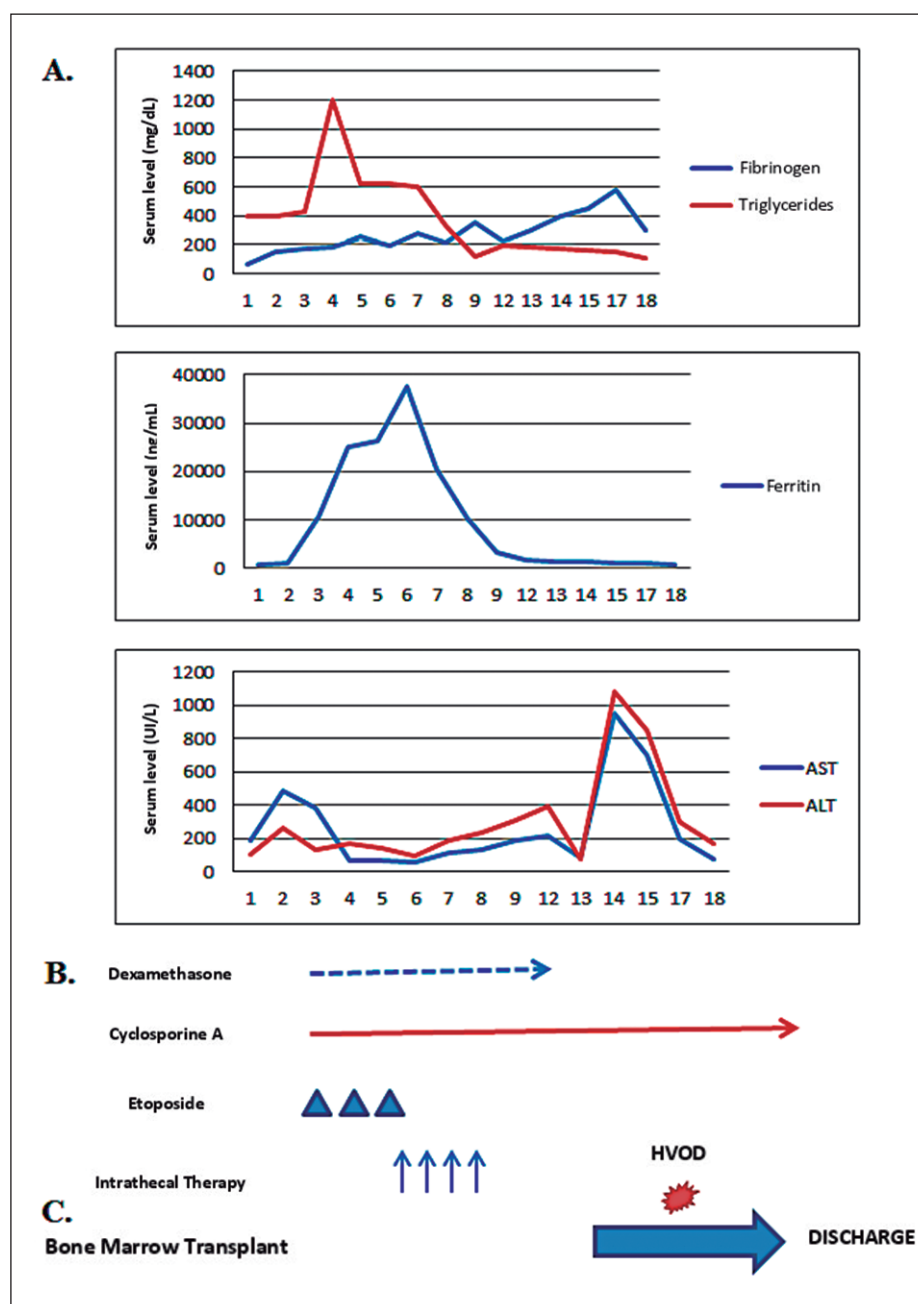
## Discussion

This patient was a previously healthy male preschooler with prolonged febrile illness refractory to treatment, with infectious mononucleosis as the main diagnostic suspicion. However, this pathology is usually self-limited and of benign course<sup>10</sup>, so due to the progressive deterioration, the possibility of malignancy was suspected. After complementary studies, it was found that the patient fulfilled 6 of the 8 criteria for HLH (table 1). In addition, in patients with HLH, symptoms are frequently triggered by viral infections<sup>11</sup>. The association between EBV and the hemophagocytic syndrome has been reported for decades, however, the actual incidence of EBV-HLH is difficult to determine

**Table 1. Diagnostic criteria for hemophagocytic lymphohistiocytosis<sup>5</sup> and findings present in our patient.**

HLH diagnostic criteria	Reported patient
Fever $\geq 7$ days	Present
Splenomegaly	Present
Cytopenias (2 or more lines)	Pancytopenia (Hb 8.6 g/dL, platelets 70.000/ $\mu$ g/L, leukocytes 2310/ $\mu$ g/L)
Hypertriglyceridemia ( $\geq 265$ mg / dL) and / or hypofibrinogenemia ( $\leq 150$ mg / dL)	Maximum triglyceride level (1199 mg / dL) Minimum fibrinogen level (70 mg / dL)
Hemophagocytosis in bone marrow, spleen, or lymphoid node	Present
NK activity absent or decreased	Normal
Soluble CD 25 $\geq 2,400$ U/ml	No tested
Ferritin $\geq 500$ ng/mL	Maximum ferritin level (44287 ng/mL)

\*\*HLH: Linfocitosis hemofagocítica. NK: Natural killers



**Figure 1.** Patient Clinical evolution during hospitalization. Schematic representation of some criteria for hemophagocytic lymphohistiocytosis (A) (fibrinogen, triglycerides and ferritin serum level) with their response to the HLH-2004 protocol (B) established up to week 8 of hospitalization. From week 9, the patient presented progressive clinical deterioration and evidence of liver failure (elevated transaminases), so BMT was performed with a haploidentical donor at week 12, as the only curative measure (C). During weeks 14 and 15 he presented hepatomegaly, ascites, and elevated transaminases. Hepatic veno-occlusive disease (HVOD) was suspected, this complication was successfully managed and allowed the patient to be discharged 4 weeks later.

because the findings are similar to other inflammatory entities<sup>12</sup>.

In FHL, 4 genes have been identified as involved *PRF1*, *UNC13D*, *STX11*, and *STXBP2* causing FHL type 2, 3, 4, and 5, respectively<sup>1-2</sup>. The age of presentation of the disease is during the first decade of life, mainly caused by mutations in *PRF1* and *UNC13D*<sup>13</sup>. In this case, the presence of EBV infection was evidenced so we suspected primary etiology due to age, sex, persistent clinical picture despite standard management, and the high probability of parental consanguinity. The patient is a carrier of a homozygous mutation (c.3049G > A)

in the *UNC13D* gene, which encodes the Munc13-41 protein. This is a missense mutation located in exon 31 that causes the substitution of glutamic acid with lysine in a highly conserved region of the C2B domain of the protein and is predicted pathogenic, validated by different bioinformatic predictors. Additionally, in 2011, it was identified in compound heterozygous mutation in a Caucasian patient diagnosed with HLH<sup>14</sup>. In our patient, it was found in homozygosis and it is the first time reported in this state.

Primary and secondary FHL can be rapidly fatal and require early management consisting of progres-

sive immunomodulatory treatment depending on the severity of the disease with a combination of dexamethasone, cyclosporine A, and etoposide, according to the HLH 2004 protocol<sup>6</sup>. Our patient received the proposed scheme, however, the clinical picture persisted and this along with the suspicion of HLH, led to consider BMT.

Depending on the type of donor and conditioning regimen, allogeneic BMT appears to provide the best curative measure in cases of severe persistent FHL or HLH, with a reported long-term survival of 50-70%<sup>14</sup>. A case of a 20-month-old girl has been reported diagnosed with FHL-3 due to heterozygous mutation in the *UNC13D* gene triggered by EBV infection who was successfully treated with BMT<sup>15</sup>, which is similar to that described in our patient. The patient was discharged at 18 weeks from the onset of symptoms, with complete resolution of the hemophagocytic picture. The last control visit of our patient was 5 years after transplantation, he remained asymptomatic with good quality of life, without requiring immunosuppressants, complete immune reconstitution, and has the complete vaccination schedule for age.

## Conclusions

The molecular study is a valuable tool in cases of HLH to determine its etiology, even in the presence of concomitant infection, if the clinical suspicion is justified, as in our case. Bone marrow transplant is proposed as the only treatment alternative in cases of

HLH, type 3 in this case, and has so far had a successful course.

## Ethical Responsibilities

**Human Beings and animals protection:** Disclosure the authors state that the procedures were followed according to the Declaration of Helsinki and the World Medical Association regarding human experimentation developed for the medical community.

**Data confidentiality:** The authors state that they have followed the protocols of their Center and Local regulations on the publication of patient data.

**Rights to privacy and informed consent:** The authors have obtained the informed consent of the patients and/or subjects referred to in the article. This document is in the possession of the correspondence author.

## Conflicts of Interest

Authors declare no conflict of interest regarding the present study.

## Financial Disclosure

Authors state that no economic support has been associated with the present study.

## References

1. Sieni E, Cetica V, Hackmann Y, et al. Familial hemophagocytic lymphohistiocytosis: when rare diseases shed light on immune system functioning. *Front Immunol*. 2014;5:167.
2. Al-Samkari H, Berliner N. Hemophagocytic Lymphohistiocytosis. *Annu Rev Pathol Mech Dis*. 2018;13(1):27-49.
3. Janka GE, Lehmborg K. Hemophagocytic syndromes-an update. *Blood Rev*. 2014;28:135-42.
4. Gholam C, Grigoriadou S, Gilmour KC, Gaspar HB. Familial haemophagocytic lymphohistiocytosis: advances in the genetic basis, diagnosis and management. *Clin. Exp. Immunol*. 2011;163:271-83.
5. Gupta S, Weitzman S. Primary and secondary hemophagocytic lymphohistiocytosis: clinical features, pathogenesis and therapy. *Expert Rev Clin Immunol*. 2010;6(1):137-54.
6. Henter JI, Horne A, Aricó M, et al. HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer*. 2007;48(2):124-31.
7. Feldmann J, Callebaut I, Raposo G, et al. Munc13-4 is essential for cytolytic granules fusion and is mutated in a form of familial hemophagocytic lymphohistiocytosis (FHL3). *Cell*. 2003;115:461-73.
8. Quang D, Chen Y, Xie X. DANN: a deep learning approach for annotating the pathogenicity of genetic variants. *Bioinformatics*. 2015;31(5):761-3.
9. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17(5):405-24.
10. Luzuriaga K, Sullivan J. Infectious mononucleosis. *N Engl J Med*. 2010;362:1993-2000.
11. Ishii E, Ohga S, Imashuku S, et al. Review of hemophagocytic lymphohistiocytosis (HLH) in children with focus on Japanese experiences. *Crit Rev Oncol Hematol*. 2005;53:209-23.
12. Lehmborg K, Ochi F, Nichols KE, Ishii E. Hemophagocytic Lymphohistiocytosis Associated with Malignancies and with Epstein-Barr Virus. *Histiocytic Disorders*. 2017; 215-31.
13. Trizzino A, zur Stadt U, Ueda I, Histiocyte Society HLH Study Group. Genotype-phenotype study of familial haemophagocytic lymphohistiocytosis due to perforin mutations. *J Med Genet*. 2008;45:15-21.
14. Aricó M, Janka G, Fischer A, et al. Hemophagocytic lymphohistiocytosis. Report of 122 children from the International Registry. FHL study group of the histiocyte society. *Leukemia*. 1996;10:197-203.
15. Olaya M, Alsina L, de Sevilla MF, Catalá A, López-Ramos MG, Martín Mateos MA. Epstein-Barr virus infection triggering a haemophagocytic syndrome. *Allergol Immunopathol (Madr)*. 2014;42:627-9.