Familial Hemophagocytic Lymphohistiocytosis (FHL): An Illustrative Case and Review of Literature

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Abstract

Familial hemophagocytic lymphohistiocytosis (FHL) is a hyperinflammatory disorder which occurs due to a genetic defect in the cytolytic pathway of natural killer cells and cytotoxic T cells. We present here the case of a female infant who presented with the typical features of high-grade fever, hepatosplenomegaly and pancytopenia, and was diagnosed to have FHL due to a homozygous variant in the *STXBP2* gene.

Keywords: Familial hemophagocytic lymphohistiocytosis, *STXBP2*

Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a hyperinflammatory disorder resulting from prolonged and excessive activation of antigen presenting cells (macrophages and histiocytes) and CD8+ T cells. The two forms of HLH are primary (genetic) and secondary (acquired). Primary HLH occurs due to an underlying genetic defect in the cytolytic pathway of natural killer (NK) cells and cytotoxic T cells. The disease usually presents in infancy and early childhood but the first clinically significant episode can present throughout life, ranging from prenatal presentation to the seventh decade. Secondary HLH may be triggered by an infection [Epstein-Barr virus (EBV), herpes simplex virus (HSV), cytomegalovirus (CMV), adenovirus, dengue, ebola virus etc.], malignancy, or autoimmune diseases (such as systemic onset juvenile idiopathic arthritis). Though HLH is classified as primary and secondary, there is a considerable overlap between the two and often secondary HLH is found to have an underlying genetic defect. The estimated incidence of primary HLH is 0.12/100,000 children per year in a Swedish study and 0.34/100,000 children per year in studies from Japan. The prevalence of all cases of HLH under 18 years of age has been estimated as 1.07/100,000 (Sen et al., 2017).

Patient details

Baby X, a 1-month-25-days-old female child, second born to a non-consanguineous couple, was admitted with history of high-grade intermittent fever, breathlessness and occasional cough for 3 days. She was a born by Caesarean section at term, with a birth weight of 3.06 kg and cried soon after birth. The postnatal period was uneventful. She was partially immunised for age (only birth dose vaccines were taken). There was family history of neonatal death; her elder sibling had expired in the newborn period due to multiorgan dysfunction.

On examination, the infant was irritable and did not have any significant facial dysmorphism except for low anterior hair line and small anterior fontanelle. Weight at admission was 4 kg, length 52 cm and head circumference 35 cm. She had mild respiratory distress but her chest was clear. Cardiovascular system was clinically normal. Abdomen was distended and soft to palpation. She had an enlarged liver which was palpable 3 cm below the right costal margin and firm in consistency. The spleen was palpable 2 cm below the left costal margin. Hernial orifices were normal and she had normal female genitalia. There was no skin rash or pigmentation. Neurological examination was normal.

She was initially managed with oxygen by continuous positive airway pressure, intravenous antibiotics (cefotaxime and amikacin) and other supportive measures. On evaluation she was found to have acute liver failure, cholestatic

jaundice and multiple electrolyte abnormalities (Table 1). She was managed with N-acetyl cysteine infusion and other supportive measures. All hepatotoxic drugs were avoided. Electrolyte abnormalities were corrected with intravenous and oral supplements. But the blood counts and liver function progressively worsened (Table 1). There were no episodes of hypoglycaemia. She developed diarrhoea which was managed with oral rehydration solution, probiotics and zinc supplements. In view of high spiking fever and worsening general condition of the baby, antibiotics were upgraded to meropenem and vancomycin, and acyclovir was added suspecting a viral etiology.

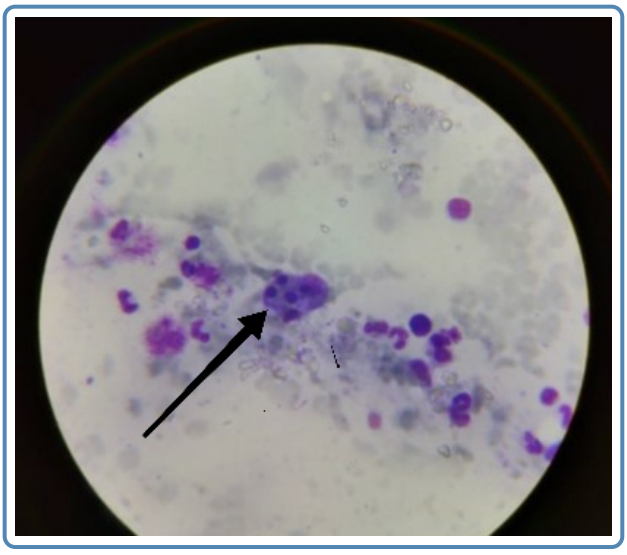

Figure 1 Bone marrow aspiration smear showing hemophagocytosis

Hepatitis B surface antigen (HBsAg), and antibody against hepatitis C virus and hepatitis A virus (anti HCV and anti HAV) were negative. Dengue IgM, Lepto IgM and Scrub IgM serology, test for infectious mononucleosis, and Enterocheck WB test for typhoid were negative. The nasopharyngeal swab viral panel was negative. Real-time reverse transcription polymerase chain reaction (rRT-PCR) test for coronavirus disease (COVID-19) was also negative. Blood PCR for CMV, and HSV 1 and 2 IgM serology were also negative. Serum alpha fetoprotein was 46.88 mg/ml (reference range 0-9.5 mg/ml) and serum lactate dehydrogenase was 971 IU/L (reference range 230-400 IU/L). Urine metabolic screening was negative. Urine examination showed albumin 3+, pus cells 1-3/ high power field, sugar nil, and acetone nil. The other investigations were as follows: urine pH 5.5, serum sodium 113 mEq/L (130-140 mEq/L), serum osmolality 463 mOsm/kg (800-1300 mOsm/kg), and serum chloride 131 mEq/L (80-209 mEq/L).

Serum ferritin was 1473 ng/ml and serum triglycerides were 647 mg/dl. The clinical and biochemical parameters satisfied the criteria of HLH. Hence treatment with intravenous immunoglobulin (IVIG) 2 g/kg and dexamethasone regimen were started. Bone marrow aspirate showed hemophagocytes (macrophage with engulfed erythroblasts) suggestive of HLH (Figure 1). But the child failed to respond to IVIG or steroids. Pancytopenia, liver dysfunction and coagulopathy progressively worsened. Multiple platelet concentrate, fresh frozen plasma and packed red cell transfusions were given. But she developed haemorrhage and shock not responding to inotropes and succumbed to the illness.

The peripheral blood sample was sent for clinical exome sequencing to look for mutations in genes involved in familial HLH. A homozygous missense variant (c.1730G>A; p.Gly577Asp) was identified in the *STXBP2* gene (transcript ID ENST00000441779) in the proband. This variant was previously reported in a patient affected with familial HLH 5 (Pagel et al., 2012). This variant has not been reported in the 1000 genomes database (https://www.internationalgenome.org/1000-genomes-browsers) and has a minor allele frequency of 0.0004% and 0.027% in the gnomAD (https://gnomad.broadinstitute.org/) and internal databases, respectively. The *in silico* predictions of the variant are probably damaging by PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) and damaging by SIFT (https://sift.bii.a-star.edu.sg/), LRT (http://www.genetics.wustl.edu/jflab/lrt_query.html) and MutationTaster2 (http://www.mutationtaster.org/). The reference codon is conserved across mammals. The variant is classified as 'likely pathogenic' as per the American College of Medical Genetics and Genomics/ Association for Molecular Pathology (ACMG/AMP) guidelines (Richards et al., 2015). The child was thus diagnosed to have familial hemophagocytic lymphohistiocytosis type 5 (FHL5) (OMIM #613101). Both parents were heterozygous for this variant. Genetic study of the deceased sibling could not be done due to non-availability of the blood or DNA sample.

Table 1 Table showing the baseline investigations and the progression in the patient

Date	DA1	DA3	DA5	DA6	DA7	DA8	DA10	Normal range
Hb (g/dl)	9.5	9.4	9.0	7.2	6.3	6	6.1	9-14
TLC (cells/mm^3)		4,850			3,280	4,800	4,000	5000-19,500
DLC		$P_{34}L_{66}$ ANC 1649			$P_{31}L_{61}$ ANC 992	$P_{12}L_{83}$ ANC 576	$P_{10}L_{90}$ ANC 400	$P_{54-62}L_{25-33}$
Platelet count (cells/mm^3)		65,000			35,000	31,000	10,000	1.5-4 X 10^5
CRP (mg/dl)	1.39				Negative			0-0.6
BU/S Cr (mg/dL)	19/0.5	13/0.4	18/0.4		21/0.5	24/0.4	25/0.3	5-20/ 0.3-0.7
SGOT (IU/L)	368	241	707	1761	395	184	128	<40
SGPT (IU/L)	207	205	378	873	509	419	223	<40
Serum Na (mM/L)	128	147	132	131	136	132	132	135-145
Serum K (mM/L)	2.4	2.5	3.3	2.9	3.8	3.3	5.5	3.5-5
Serum Ca/P (mg%)			4.4/1.8 (corrected Ca- 5)	4.8/2.4	8.5/1.6	9/1.6	8.1/4.3	9-10.6/ 2.5-4.5
Serum Mg (mg%)			1		1.6	1	1.6	1.5-2.5
Serum Bil T/D (mg%)	0.9/0.5	0.8/0.2	0.5			0.5	1.7/0.3	0.2-1/ 0-0.2
Serum ALP (IU/L)	720		472			293	238	210-810
Serum Pr/Alb (gm%)			5.4/3.5			4.3/2.7	4.6/2.4	6.2-7.8/ 3.5-5
PT (sec)/INR		31/ 2.5	31.9/ 2.3		19.7/ 1.42	20.6/ 1.49	27.7/ 2.03	11-15/ 0.8-1.1
APTT (sec)					27.7	28.1	39.5	32

DA – Day of admission; Hb – Hemoglobin; TLC- Total leucocyte count; DLC – Differential leucocyte count; P – Polymorphs; L- Lymphocytes; ANC – Absolute neutrophil count; CRP- C-reactive protein; BU – Blood urea; S Cr – Serum creatinine; SGOT - Serum glutamic-oxaloacetic transaminase; SGPT - Serum glutamic-pyruvic transaminase; Serum Na – Serum sodium; Serum K – Serum potassium; Serum Ca/ P – Serum calcium/ phosphorus; Serum Mg – Serum magnesium; Serum Bil T/D – Serum bilirubin total/ direct; Serum ALP – Serum alkaline phosphatase; Serum Pr/ Alb – Serum total protein/ albumin; PT – Prothrombin time; APPT – Activated partial thromboplastin time

Discussion

Critical to the diagnosis of HLH is the awareness about the disease and high degree of suspicion in children with some of the clinical features. There is considerable overlap between HLH and the symptoms and signs of many other diseases and the combination of clinical and laboratory signs, their severity and the changes over time help in diagnosis. It closely resembles severe systemic sepsis and some consider HLH and severe sepsis to be phenotypes of a spectrum of hyperinflammatory reactions. The cardinal features of HLH are high grade fever, hepatosplenomegaly and cytopenias, with a failure to respond to initial anti-infective treatments. The inflammatory reaction results in elevation of levels of tumour necrosis factor (TNF) α, interleukin 6 (IL-6), interleukin 1 (IL-1) and other interleukins, which causes high fever and infiltration of tissues with activated macrophages and lymphocytes. This leads to multi-organ inflammation and damage.

The HLH-2004 study developed diagnostic criteria for the clinical diagnosis of HLH (Henter et al., 2007). In a child with appropriate clinical presentation, identification of mutation in one of the genes involved or fulfilment of five of the eight criteria, confirms the diagnosis of HLH. Fever and splenomegaly are consistent features in children other than neonates, seen in 90-100% of cases. Hyperferritinemia is a crucial marker of active HLH/macrophage activation syndrome (MAS) and values more than 10,000 µg/L in children were found to be 90% sensitive and 96% specific for HLH. In case of other investigations like blood counts, erythrocyte sedimentation rate (ESR) and transaminases, change in parameters over time rather than absolute values are important for the diagnosis. A paradoxical drop in ESR in spite of active systemic inflammation in the proband is suggestive of HLH. This is due to the fall in fibrinogen level due to fibrinogen consumption and liver dysfunction. A dropping ESR in conjunction with elevated c-reactive protein (CRP) is an important sign of HLH.

While the presence of hemophagocytosis in the bone marrow can help to confirm the diagnosis of HLH, it is frequently absent especially in the early stages of the disease. Moreover, bone marrow aspiration/biopsy is an invasive procedure which may be difficult to do in a sick child. Two other biomarkers, measurement of NK cell function and soluble interleukin 2 receptor α chain (sIL-2Ra, CD25) are available only in specialised immunology or research laboratories.

HLH can be either primary due to an autosomal recessive monogenic disorder or secondary to infections, malignancy or autoimmune disorders. Genetic forms of HLH can be further classified into familial HLH and lymphoproliferative syndromes. Familial HLH can be subdivided into conditions without skin hypopigmentation and with skin hypopigmentation (Table 2) (Al-Herz et al., 2014). Genetic testing by DNA analysis of the genes involved will help to confirm the diagnosis. However, most of the patients have severe systemic symptoms at diagnosis, and timely appropriate treatment for HLH is needed before genetic testing to distinguish primary from secondary HLH.

HLH is characterized by multisystem inflammation due to prolonged and excessive activation of antigen-presenting cells (macrophages and histiocytes) and CD8+ T cells, and excessive proliferation and ectopic migration of T cells. NK cells modulate the initial responses of antigen-presenting cells to incoming pathogens like viruses (likely through cytokine signalling) and thus attenuate the subsequent activation of antigen-specific T cells. The perforin/granzymes, Fas/Fas ligand, membrane-bound TNF-α, membrane-bound lymphotoxin, and TNF-related apoptosis-inducing ligand (TRAIL), are the various mechanisms implicated in NK/cytotoxic T lymphocyte (CTL)-mediated cytotoxicity (Madkaikar et al., 2016; Filipovich & Chandrakasan, 2015). Among these, the perforin/granzyme and Fas/Fas ligand interactions are the two most important mechanisms. Various proteins involved in this pathway are lysosomal trafficking regulator (LYST) protein, adaptor related protein complex 3 subunit beta 1 (AP3B1), syntaxin 11, Rab27a, Munc13-4, and Munc18-2 (Figure 2). All genetic forms of HLH are due to variations in one of the genes coding for these proteins (Table 2).

The initial treatment options of HLH consist of combinations of proapoptotic chemotherapy and immunosuppressive drugs targeting the hyperactivated T cells [such as steroids, cyclosporine A, antithymocyte globulins, 2-chlorodeoxyadenosine, and Alemtuzumab (Campath-1H)] and macrophages/histiocytes [etoposide, steroids, and high-dose intravenous immunoglobulin (IVIG)] (Madkaikar et al., 2016; Ishii, 2016). Recently, Alemtuzumab (Campath-1H), a monoclonal antibody to CD52, was found to have a significant response against refractory

Table 2 Classification of genetic forms of HLH (Al-Herz et al., 2014).

Disease name	Gene	Protein	Function
Familial HLH without skin hypopigmentation			
FHL1	Unknown	-	-
FHL2	*PRF1* (first gene reported, in 1999)	Perforin	Pore formation
FHL3	*UNC13D* (second gene reported, in 2003)	Munc 13-4	Vesicle priming
FHL4	*STX11*	Syntaxin 11	Vesicle fusion
FHL5	*STXBP2* (reported in 2009)	Munc 18-2	Vesicle fusion
Familial HLH with skin hypopigmentation			
Griscelli syndrome type 2	*RAB27A*	Rab27a	Vesicle docking
Chediak-Higashi syndrome	*LYST*	Lyst	Vesicle trafficking
Hermansky-Pudlak syndrome type 2	*AP3B1*	AP3B1	Vesicle trafficking
Lymphoproliferative disorders			
XLP1	*SH2D1A*	SAP	Signalling in T, NK and NK-T cells
XLP2	*XIAP*	XIAP	Signalling pathways via NF-κB
ITK deficiency	*ITK*	ITK	Signalling in T cells
CD27 deficiency	*CD27*	CD27	Lymphocyte co-stimulatory molecule
XMEN syndrome	*MAGT1*	Magnesium transporter 1	T cell activation via T cell receptor

FHL - familial hemophagocytic lymphohistiocytosis; HLH - hemophagocytic lymphohistiocytosis; ITK - interleukin-2-inducible T cell kinase; NF-κB - nuclear factor kappa-light-chain-enhancer of activated B cells; NK - natural killer; SAP- signalling lymphocyte activation molecule (SLAM)-associated protein; XIAP - X-linked inhibitor of apoptosis protein; XLP- X-linked lymphoproliferative syndrome; XMEN- X-linked immunodeficiency with magnesium defect, Epstein-Barr virus infection and neoplasia.

HLH, but CMV reactivation and adenoviremia were frequent complications of this therapy. Currently, definitive treatment and potential cure of FHL is only achieved by hematopoietic cell transplantation (HCT). Supportive care including prophylaxis for pneumocystis jirovecii, and other fungal or opportunistic infections by empiric broad-spectrum antibiotics or antifungal therapy also plays a major role in successful treatment. Granulocyte-colony stimulating factor (G-CSF) can also be used to increase neutrophil counts in myelosuppression.

The proband described above had all the clinical features to suspect HLH and molecular testing confirmed the diagnosis. Since there is 25% recurrence risk in each future pregnancy, prenatal diagnosis can be offered in the next pregnancy. A high index of suspicion and proper diagnostic workup including molecular testing will help in confirmation of the diagnosis and appropriate genetic counseling.

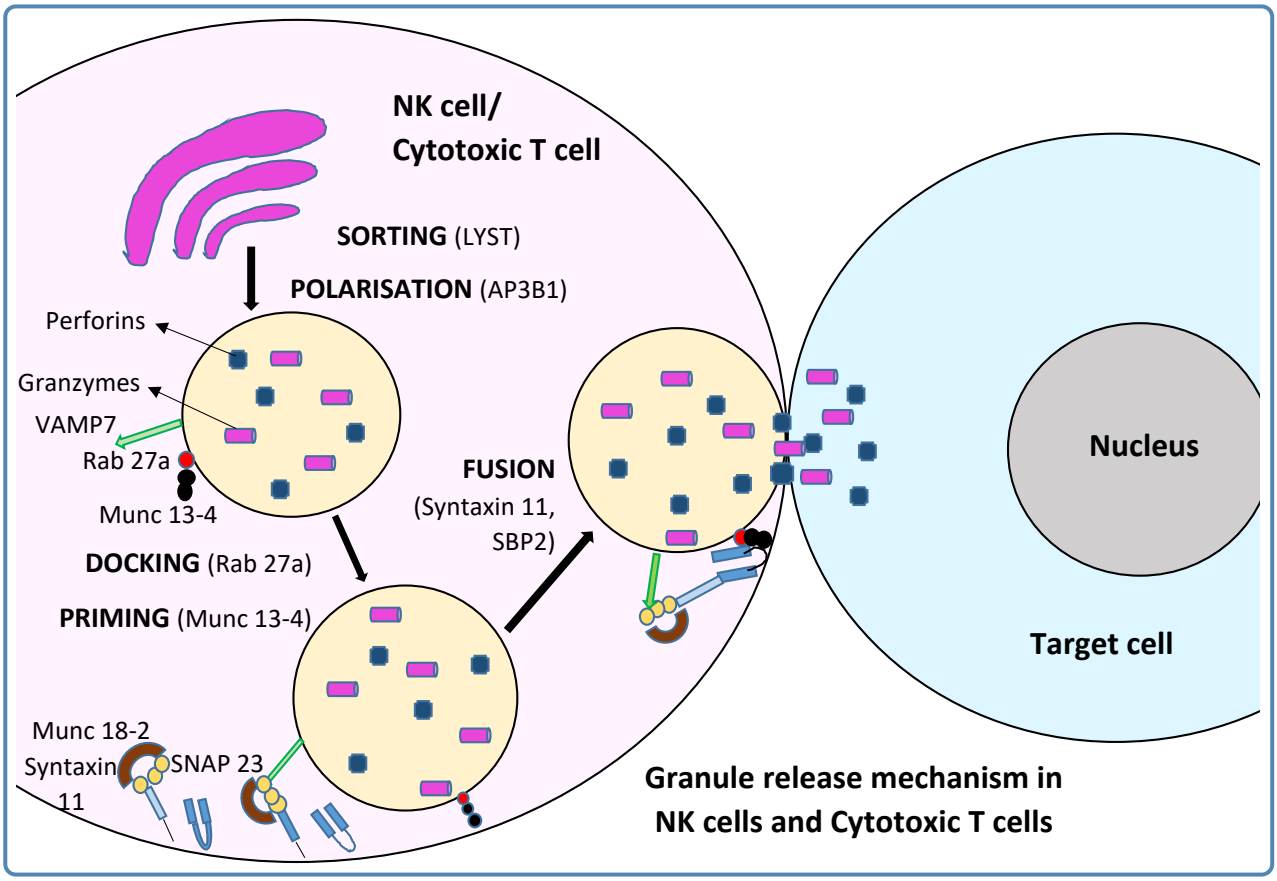

Figure 2 Granule release mechanism in natural killer (NK) cells and cytotoxic T cells showing various molecules and their functions.

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