Molecular Diagnosis - An Indispensable Tool to Clinch the Diagnosis in Familial Haemophagocytic Lymphohistiocytosis – A Case Report with Review of Literature

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INTRODUCTION

Haemophagocytic Lympho-Histiocytosis (HLH) includes variety of life-endangering conditions caused due to defective immunity and represented as uncontrolled hyperinflammatory response. HLH is mostly instigated by infection. Familial form is due to genetic defects in cytotoxic T cells & natural killer cells. HLH mostly goes unrecognized, and hence has high morbidity and mortality. Prompt diagnosis is critical to ensue curative treatment. Diagnosis of familial Haemophagocytic Lymphohistiocytosis is ellusive due to variable presentation, coexistence with other diseases, & select availability of confirmatory tests. We present a case of a 3-year-old girl child, with organomegaly, fever and pancytopenia diagnosis could not be reached for a long period.

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PRESENTATION OF CASE

Familial Haemophagocytic Lymphohistiocytosis (FHL) is a type of inherited Haemophagocytic Lympho-Histiocytosis (HLH). This rare autosomal recessive disorder is often fatal, with only cure being haematopoietic stem cell transplantation. The cause is mutations in genes like *PRF1, UNC13D, STX 11* which are involved in granule mediated cellular cytotoxicity.[1,2] The primary pathogenesis is hyperactivation of cytotoxic T lymphocytes & macrophages causing hypercytokinemia along with excessive T-cell and macrophage infiltration in multiple organs.

FHL generally affects children in early infancy, with maximum cases diagnosed between one to six months of age. However, late presentation in childhood and even adulthood has also been identified.[3] Common clinical presentation is fever, failure to thrive, cytopenias, organomegaly and presence of haemophagocytosis in bone marrow, liver, or lymph nodes. It may mimic other childhood diseases like storage disease, autoimmune diseases, primary immunodeficiencies, malignancies, and infections and hence prompt diagnosis is challenging. Correct diagnosis of FHL & its distinction from secondary form needs genetic work-up.

We hereby report a case of FHL which presented with non-specific signs & symptoms and is prototype of diagnostic dilemmas faced by both clinicians and pathologists needing molecular work-up to clinch the diagnosis.

3-year-old female child, resident of Madhya Pradesh, born of non-consanguineous marriage, was referred with fever since 6 months and progressive abdominal distension since 3 months. Haemoglobin electrophoresis revealed AA pattern. A prior report of bone marrow done for persistent pancytopenia showed erythroid hyperplasia. No abnormal cells were seen. She had received 3 blood transfusions in last 2 months for anaemia and cytopenia. Family history was not significant.

On admission she was febrile (39° C) with a pulse rate of 114 / min, BP – 110 / 70 mm of Hg, and had pallor & mild icterus. Abdominal examination revealed Hepatomegaly 7 cm, and huge splenomegaly beyond umbilicus. Rest of the systemic examination was normal. No skin, bone lesions or any neurological symptoms noted.

CLINICAL DIAGNOSIS

Persistent Pancytopenia with Organomegaly

DIFFERENTIAL DIAGNOSIS

Storage Disease
Langerhans Cell Histiocytosis
Haemophagocytic Lymphohistiocytosis

PATHOLOGICAL DISCUSSION

Fresh Investigations confirmed persistent pancytopenia with haemoglobin 9.6 gm / dL, TLC 1750 / cumm, and platelet count 31,000 / cumm & normal RBC indices. Differential count showed Neutrophil 13 %, lymphocytes 68 %, Monocytes 19 %. PS revealed anisocytosis of RBC's with presence of both Microcytes & macrocytes along with hypochromasia and polychromasia. There was relative lymphocytosis, with presence of activated lymphocytes. Platelets were depleted, however giant platelets were seen. No haemoparasite was seen. Other biochemical investigations showed AST 64.8 U / L, GGT 130 U / L, Creatinine 0.08 mg / dL, VLDL 91 mg / dL, & raised triglycerides (456.8 mg / dL), with mildly raised serum ferritin (546.1 ng / ml). Serological tests for kala azar and HIV were negative. USG Abdomen showed hepatomegaly with diffuse echotexture and massive splenomegaly without nodularity.

Haemophagocytic lymphohistiocytosis (HLH) is not a disease per se. It is a rare, fatal if untreated, disorder of excessive activation of immune system, marked by fever, cytopenias, hepatosplenomegaly, and hyperferritinaemia. Haemophagocytosis is seen in bone marrow, liver, spleen and lymph nodes. However not necessarily needed for making the diagnosis.[1] The cause of these clinical manifestations is hyperinflammation associated with macrophage activation.[1,4] Scott and Robb-Smith in 1939 mentioned a disorder of accumulating histiocytes with erythro- phagocytosis and labelled it "histiocytic medullary reticulosis" or HMR. Which was later included into malignant histiocytosis.[5] FHL which is the familial form of HLH, was narrated by Farquhar and Claireauxin 1952. They reported two cases of siblings deceased due to HLH.[6,7] Risdall described a that HLH is associated with viral infection and recommended that it should be called virus-associated HLH, thus differentiating it from malignant histiocytosis.[8]

Pathogenesis of HLH is due to activation of MPS. Whenever a healthy person is exposed to any infection, the histiocytes, Natural Killer cells (NK) and cytotoxic T lymphocytes (CTL) get activated releasing inflammatory cytokines like TGF alpha, IL6, IL2 etc. thus killing the infective agent and resulting in culmination of immune response. However, in HLH defective activity of the NK and CTL, results in exaggerated immune response, with defective removal of antigens along with excessive accretion of T cells, histiocytes and macrophages along with raised levels of cytokines. This is called as macrophage activation syndrome.[9]

Universal incidence of FHL is unknown, also incidence of secondary HLH is not available.[10] Sweden is the only country which has studied this disease extensively and reported the incidence of FHL as 1 in 50,000 live births.[1] All together the reported incidence of HLH across ethnicities and races is estimated to be 1 in 100,000. Probably variability of clinical presentations and lack of molecular studies is the cause of under reporting of this condition in resource poor settings.

Signs & symptoms of HLH are the result of increased cytokines secreted by the activated macrophages & histiocytes.[11] (Table 1) The Working Group of the Histiocyte Society (HS) initially in 1987 divided histiocytosis into 3 groups which was redistributed in 2004 into 5 groups.[12] HLH and macrophage activation syndrome belongs to Group "H". (Table 2)[12,13]

HLH could be primary (associated with known genetic mutations) and secondary or reactive (usually due to infection, autoimmunity or malignancy)[12] Genetic HLH can be autosomal recessive or X-linked and is classified into familial disease familial haemophagocytic lymphohistiocytosis (FHL), and HLH with inherited immune deficiencies.[1] Acquired (secondary) HLH is usually secondary to a severe infection, autoimmunity or malignancy. Most common infectious agents associated with secondary HLH are EBV and CMV.

Molecular diagnosis involving mutation studies is considered gold standard for diagnosis and classification of FHL.[14] Diagnosis of HLH is established by clinicoradiological and lab criteria, which are defined by the Histiocyte Society (HLH Study Group) in 1991 and reviewed repeatedly latest in 2009. (Table 3)[15,16] Eight clinical and laboratory criteria are defined, and five of them are essential to make a diagnosis of HLH.[17,18]

Present case had fever, pancytopenia, and organomegaly with raised triglycerides however serum ferritin level was mildly raised. Initially haemophagocytosis was missed on Bone marrow aspirate. It has been reported that haemophagocytosis may not be seen in secondary HLH.[10] Presence of haemophagocytosis clinches the diagnosis in many cases however its absence does not exclude the possibility of HLH.

Diagnosing FHL is difficult due to many confounding signs & symptoms. Furthermore, presence of associated infection, auto-immune disease or malignancy doesn't rule out HLH. Our case also presented with many diagnostic dilemmas.

Firstly, haemophagocytosis was not identified in bone marrow initially, it could be appreciated after genetic confirmation highlights the problems associated with its detection. The halo seen around the ingested cells is difficult to find on bone marrow. Also, only erythrophagocytosis is not specific for diagnosis of HLH. Gars et al in their study mentioned that haemophagocytes ingesting granulocytes are the hallmark of HLH, followed by phagocytosis of nucleated red cells and lymphocytes. If granulocytic haemophagocytosis is not seen, then there is only 3 % possibility of HLH. Cases demonstrating at least one granulocytic haemophagocytosis, two or more

haemophagocytes with ingested nucleated red cells, and one haemophagocyte with ingested lymphocytes have confirmed diagnosis of HLH. In our case we could identify haemophagocytes with only nucleated red cell & lymphocyte, Granulocyte haemophagocyte was not identified.[19]

However, Haemophagocytosis is not essential for the diagnosis of HLH and its absence should not result in delay of treatment when other clinical criteria are indicative of this condition.[1,20]

One of the 8 criteria of diagnosis of HLH is hyperferritinaemia > 500 mcg / L. Allen et al have mentioned ferritin level over 10,000 mg / L to be 90 % sensitive and 96 % specific for HLH.[21] In our case ferritin level was only mildly raised, which is very non-specific and can be seen in many other inflammatory disorders. In fact, serum ferritin is an acute-phase reactant and increased values can be seen in various infections, autoimmune and malignant disease.[22]

FHL is a recessive disorder, so family history is usually negative, and these patients can be labelled as secondary HLH.[20] In present case since family history was negative, diagnosis was challenging with patient having signs and symptoms similar to malignancies, infectious and autoimmune diseases,[23]

Increased cytokine levels are hallmark of HLH. Soluble interleukin 2 receptor value > 2,400 units / ml is sensitive and specific for HLH,[21] Various other markers are also available like immunohistochemical stain for CD163. Levels of other cytokines like interferon γ, interleukin 10 & interleukin 6 can be used to differentiate HLH from bacterial or viral infections. CD107 can be used as surrogate marker for decreased or absent NK lymphocyte and cytotoxic T-lymphocyte activity. However, utility of these markers for primary diagnosis is limited as these are not the markers routinely tested for, and they require specialized labs. Thus, a strong index of suspicion with molecular testing is central to diagnosis.

Genetic studies showed that FHL is caused due to mutations in *PRF1*, *UNC13D, STX11,* or *STXBP24* genes. These encode for proteins taking part in granule exocytosis and perforin-dependent target cell apoptosis.[23] Majority of cases of FHL (15 – 50 %) are due to mutations in perforin gene *PRF1* at locus *10q24*. These are defined as FHL2. Maximum cases are reported in African Americans (> 50 %), followed by Turkey (43 %), Japan (42 %), and India (41 %). FHL 3 occurs due to mutation in UNC13D at locus 17q25 and amounts for 15 to 20 % cases of FHL. Defect in STX11 gives rise to FHL 4 which is predominantly seen in Turkish families.[1,2]

Flow cytometry can be employed for perforin staining in cytotoxic lymphocytes, including NK-cells, CD8 + T-cells, and CD56 + T-cells, but detection of Perforin gene mutation can help in diagnosis of only type 2 FHL. Molecular studies may still be needed for detection of other mutations with resultant increase in cost.

The dilemmas in diagnosis arise due to spatial presence of signs and symptoms during evolution of disease. One or more of the features may be absent at a particular time in disease progression and when the clinical and lab criteria are borderline, the molecular diagnosis clinches it.

DISCUSSION OF MANAGEMENT

In view of marked thrombocytopenia patient received steroids for few days. Fever disappeared, Splenomegaly regressed, and platelet count increased to more than one lakh. Meanwhile Liver biopsy was done for storage disease, which showed chronic hepatitis with mild portal fibrosis (ISHAK stage 2 / 6) and mild activity. No storage cells or Langerhans cells were identified. IHC for CD1A was negative.

To rule out storage disease patient's sample was sent for molecular studies which revealed *PRF1* gene mutation, confirming the diagnosis of Familial haemophagocytic lymphohistiocytosis Type II (FHL 2).

Managing HLH is major challenge due to its varied clinical presentation & universal dismal outcome in absence of treatment. Without treatment patients with familial HLH do not survive.[21] The immediate goal of treatment of patients with HLH is suppression of hyperinflammation and immune dysregulation using chemotherapeutic drugs or immunosuppressant like corticosteroids, cyclosporins and immunoglobins. The aim of treatment is to substitute the flawed immune system by haematopoietic Stem cell transplantation[20] Without treatment persistent hyperinflammation may lead to prolonged neutropenia and death by repeated fungal and bacterial infections.[13,15]

FINAL DIAGNOSIS

In view of molecular diagnosis, previous bone marrow aspiration slides were reviewed for evidence of haemophagocytosis. On meticulous search, few histiocytes with haemophagocytosis of lymphocytes and nRBC's could be identified. Thus, the final diagnosis of the present case was given as Familial haemophagocytic lymphohistiocytosis type II due to *PRF1* gene mutation. Steroids were continued and patient is on follow up.

HLH Manifestations Possible Mechanism
Fever Increased IL-1; IL-6, TNF-α Cytopenia action of TNF-α, INF-γ, haemophagocytosis
Increase triglycerides in blood increased levels of TNF-α suppresses lipoprotein lipase causing secondary hypertriglyceridemia
Decreased fibrinogen in blood Macrophages secrete plasminogen activator Which stimulates plasmin causing hyperfibrinolysis
High Serum Ferritin Secreted by activated histiocytes / macrophages
High sIL-2R Secreted by activated T lymphocytes
Organomegaly Infiltration by activated histiocytes / macrophages

Table 1. Clinical Manifestations of HLH & Possible Reasons (Modified from M. Madkaikar)

Histiocytosis of the H Group
Primary HLH: Due to inherited genetic mutations
1. HLH associated with defects in cytotoxic activity of T lymphocyte & NK cells
2. HLH associated with defects in inflammasome activation
Secondary HLH: Mostly Non-Inherited
Infection-associated HLH (Viruses like EBV, CMV, HIV, bacteria, parasites & Fungi) Malignancy-associated HLH (commonly lymphoma)
Malignancy-triggered HLH (Haematological malignancies, Solid tumours)
Macrophage activation syndrome secondary to autoimmune disease

Table 2. Causes of Primary & Secondary HLH[12,13]

Future goals involve raised awareness among clinicians & pathologists regarding this disease, identifying better

biomarkers for diagnosis and early initiation of HLH directed therapy which will help reduce mortality.[11] The diagnosis of HLH can be established if Criterion 1 or 2 is fulfilled.

The Diagnosis of HLH Needs to Fulfil either Criterion 1 or 2	
1.	A molecular diagnosis of HLH or X linked lymphoproliferative syndrome
2.	At least 3 of 4: a) Fever b) Splenomegaly c) Cytopenias (minimum 2 cell lines reduced) d) Hepatitis
3.	At least 1 of 4: a. Haemophagocytosis b. Increased serum Ferritin c. Increased soluble Interleukin 2 Rα (age based) d. Absent or very decreased NK function
4.	Other results supportive of HLH diagnosis: a. Hypertriglyceridemia b. Hypofibrinogenemia c. Hyponatremia

Table 3. Diagnostic Criteria for Haemophagocytic Lymphohistiocytosis (HLH) (Revised in 2009) [18]

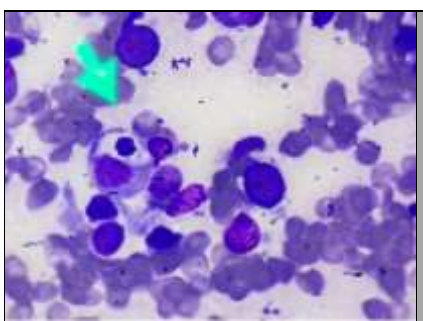

Figure 1. Haemophagocytosis in Bone Marrow

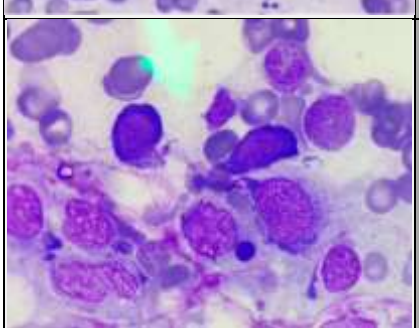

Figure 2. Haemophagocytosis in Bone Marrow

Arrow shows lymphocyte haemophagocytosis, macrophage engulfing lymphocyte (bone marrow) (Oil immersion 100X)

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