Case Series

CLINICAL PROFILE OF SECONDARY HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS (HLH) - A CASE SERIES

Ajmal N.M1, Arshad Muneerudeen2, Renny Issac3, Muraly C.P4

1Junior Resident, Department of General Medicine, Govt. Medical College, Thrissur, Kerala, India
2Lecturer, Department of General Medicine, Govt. Medical College, Thrissur, Kerala, India
3Associate Professor, Department of General Medicine, Govt. Medical College, Thrissur, Kerala, India
4Associate Professor, Department of Pulmonary Medicine, Govt. Medical College, Thrissur, Kerala, India

Abstract

Hemophagocytic Lymphohistiocytosis (HLH) is an uncommon, life-threatening hyper inflammatory syndrome, caused by severe cytokinemia, due to an excessively stimulated but ineffective immune process. The presenting features of HLH are non-specific, mimicking many diseases, and therefore its early recognition remains a challenge. It requires a high index of suspicion and detailed analysis of clinical and laboratory findings to arrive at a diagnosis. The objective of this study is to study various causes and clinical profile of a series of Hemophagocytic Lymphohistiocytosis (HLH) cases.

INTRODUCTION

Hemophagocytic lymphohistiocytosis (HLH) is a severe hyper inflammatory syndrome resulting from a dysregulated immune response due to various triggers.[1] It is driven by excessive activation of cytotoxic T-lymphocytes, natural killer T-cells and macrophages with subsequent cytokine storm and multi-organ dysfunction.[2] HLH is usually triggered by infection. Familial forms result from genetic defects in natural killer cells and cytotoxic T-cells, affecting perforin and intracellular vesicles. HLH is often under-diagnosed or missed, which contributes to its high morbidity and mortality. Early recognition is hence crucial. Current available treatment options include immunosuppression, immune modulation, chemotherapy, and biological response modification, followed by hematopoietic stem-cell transplant (bone marrow transplant). A number of recent studies have contributed to the understanding of HLH pathophysiology, leading to alternate treatment regimens; however, much work remains to raise awareness and improve the outcomes.[3] HLH can be rapidly progressive and potentially fatal if left untreated. A high level of suspicion for HLH is required in patients presenting with splenomegaly, an increase in liver enzymes, elevation of inflammatory markers such as serum ferritin and cytopenia. This case series contains presents 8 cases of HLH.

CASE SERIES

Case 1

A 17-year-old girl presented with high-grade fever (>103-degree Fahrenheit) associated with chills, more towards evening, for three weeks duration and swelling over the right side of the neck. She complained of sore throat during the initial few days from the onset of fever. Later she noticed painful movements of her fingers and her right knee. The joint involvement was non-migratory and pain used to improve with activity. She also reported recent weight loss of 4 kg in the past one month. There was history of macular rash during fever spikes over the abdomen and trunk, sparing the face which used to disappear when the fever subsided. On examination she had pallor, bilateral cervical lymphadenopathy and tachycardia. There was no tonsillar enlargement or hepatomegaly. Clinically there was mild splenomegaly. Other system examinations were found to be normal. Later on, she developed oral ulcers and one episode of epistaxis. We considered possible diagnoses of lymphoma, tuberculosis, infectious mononucleosis and Still’s disease. Investigations showed pancytopenia with severe neutropenia and elevated transaminases. Peripheral smear report showed a normocytic normochromic blood picture, leukopenia and thrombocytopenia with reactive lymphocytes. Paul-Bunnell test was negative. FNAC of the lymph node showed reactive changes. ANA-IF and Rheumatoid factor were negative and blood...
and urine cultures were sterile. Abdominal ultrasound and chest x-ray were normal. Viral markers (HIV, HBV, and HCV) and tuberculosis work up were negative. She had continued fever spikes and progressively worsening neutropenia and liver enzyme levels. In view of the deteriorating general condition, we considered the possibility of underlying macrophage activation. She had elevated serum ferritin (1510 ng/L), triglycerides (287 mg/dL), LDH (4808 IU/L) and D-Dimer (0.5).

Bone marrow biopsy reported trilineage hematopoiesis with scattered histiocytes. Lymph node excision biopsy showed lymphocyte depletion with proliferation of histiocytes and hemophagocytosis. This picture was suggestive of Hemophagocytic Lympho Histiocytosis (HLH). At this point we considered the possibility of a viral infection triggering HLH. Serological tests for Her IgM and IgG Viral capsular Antigen for Ebstein Bar Virus were positive. She was diagnosed to have EBV infection causing secondary HLH and was started on Inj. Dexamethasone 8mg iv 12th hourly. After 2 days from starting treatment, her blood counts improved, fever subsided, and transaminase levels decreased. Later on, she was put on oral prednisolone 40mg once daily. During follow up visit after 2 weeks, her hemogram and LFT improved and she was symptomatically better.

**Case 2**

60 year old male presented with high grade fever with chills and rigor, abdominal pain, significant weight loss, ankle edema, breathlessness dyspepsia early satiety for 9months. Clinically he was anemic, with hepato splenomegaly. The differential diagnoses of Malaria, blood dyscrasias, parasitic infestations, and myelofibrosis were considered. Investigations showed anemia, elevated serum ferritin, and triglycerides. The ultrasonologic evidence of huge splenomegaly rose suspicion of Myelofibrosis and leishmaniasis. The bone marrow biopsy was inconclusive with no evidence of myelofibrosis. A Liver biopsy was done in view of elevated liver enzymes and alkaline phosphatise. Liver biopsy reported showed LD bodies, RK-39 antigen test was positive and leishmaniasis (Leishmania donovani zymodeme MON-37was confirmed with peripheral blood and bone marrow aspirate DNA sequencing. A repeat bone marrow was done which showed numerous intracellular LD bodies consistent with visceral leishmaniasis. Hemophagocytosis and histiocyte collection was also seen. Patient was managed with Liposomal Amphotericin B 10 mg/kg single dose infusion over 2 hours, multivitamins, Iron supplements, human albumin, a balanced diet, good hydration, Calcium and Vitamin D. IV dexamethasone 6mg 8th hourly was given for 5 days (followed by oral steroids in tapering dose) in view of secondary HLH. Fever subsided in 8 days and the patient gained weight in 2months. The spleen size reduced to 2 cm in 1 month. Counts normalised in 45 days but hypoadbuminemia persisted.

On third month of follow up, patient again started to lose weight, recurrent episodes of fever, pedal oedema, exertional dyspnea, anorexia and early satiety reappeared. He had features of treatment failure as the repeat PCR test for leishmaniasis came out as positive. The proposed treatment options for treatment failure viz. pentavalent antimony, miltefosine and paramomycin were not available.

Hence we administered high dose liposomal Amphotericin B 5mg/kg/day for 5 days. He was also treated with broad spectrum antibiotics and other supportive measures including mechanical ventilation but eventually he succumbed to his illness.

**Case 3**

70 year old lady presented with high grade fever with rigor and chills for 3 days. She had severe anorexia and fatigue. She was pale, icterus with tachycardia and tachypnea who desaturated on room air corrected by nasal oxygen. Fine crackles appreciated over lung bases. A polymorpho nuclear leukocytosis, elevated ESR, Hemolytic jaundice with peripheral smear evidence of hemolysis. LDH was highly elevated and the direct Coomb’s test was negative. Chest X ray showed bilateral consolidations. In view of the atypical symptoms with pneumonia, IgG and IgM Mycoplasma antibody also were done and found to be negative. She had an initial relief from fever for 2 days on Inj. Piperacillin Tazobactam and Azithromycin. However fever reappeared in 2 days with clinical and hematological deterioration. Serum ferritin and triglycerides were markedly elevated. A bone marrow study revealed histiocytic proliferation with hemophagocytosis consistent with HLH. Methyl prednisolone pulse of 1 gm was given for 5 days and she started to show improvement both clinically as well as hematologically. On the fifth day, the patient developed dyspnea with fall in oxygen saturation. A repeat chest X-ray showed diffuse bilateral non-homogenous opacities predominantly in lower zones. She was tested positive for COVID-19. Patient was intubated and mechanically ventilated in view of respiratory failure but eventually she succumbed to her illness.

**Case 4**

15 year old male presented with high grade fever, associated with rigor and chills fever and abdominal pain. It was associated with severe abdominal pain which initially was diffuse and later became localized to right iliac fossa. It was also associated with multiple episodes of vomiting and watery diarrhea. On examination of the abdomen features of acute appendicitis were elicited. Ultrasound and CT abdomen showed thickening and inflammation of appendix, cecum and terminal ileum. Blood investigations showed severe leucopenia with neutropenia and reactive lymphocytosis and a high CRP. Serum amylase and lipase were normal. The peripheral smear which showed pancytopenia with severe neutropenia and thrombocytopenia along with
low reticulocyte count. A provisional diagnosis of neutropenic enterocolitis with sepsis induced bone marrow suppression was made.

He was treated with IV broad spectrum antibiotics and supportive measures. In view of pancytopenia, we did a bone marrow examination which revealed marked megakaryocytic proliferation and fibrosis with atypical cells and many histiocytes with occasional cells having hemophagocytosis. (To consider hematological malignancy, possibly AML M7 with secondary HLH). S.Ferritin (1695) and triglycerides(288) were elevated. In view of HLH, he was given iv Immunoglobulin at a dose of 1g/kg/day for 2 days and in view of severe neutropenia he was given 3 doses of Granulocyte Macrophage Colony stimulating factor injection. The counts improved and patient became asymptomatic with treatment. A repeat bone marrow biopsy was negative for any hematological malignancy.

In view of suspicion of AML M7, Bone marrow study was repeated which showed trilineage hematopoiesis with areas of stromal edema and fibrosis and occasional megakaryocytes (Flow cytometry: 0.2% myeloblast, 10.9% monocytes, 9.3% mature T lymphoid cells, 2.3% B lymphoid cells, 1.6% NK cells). Since the repeat bone marrow was not suggestive of any hematological malignancy, he was kept under follow up. 1 month later, he had intermittent episodes of fever, bilateral cervical lymphadenopathy. Excision biopsy from the lymph node showed evidence of Non-Hodgkins lymphoma (positive for CD10 and TDT).

**Case 5**

50 year old female, vegetarian, with no known comorbidities presented to surgery OPD with abdominal pain of 1 week duration. It was dull aching type of pain in the right upper quadrant. There was no jaundice. She had a low grade fever which started along with abdominal pain, lasted for 2 days and subsided without any medications. There was no alteration in bowel habits or gastrointestinal bleed. On examination, she was afebrile, had knuckle hyperpigmentation with glossitis and there was tenderness in the epigastrium and right upper quadrant. On investigation, Amylase and Lipase was normal. X-ray abdomen erect showed no evidence of bowel obstruction. USG abdomen showed a normal pancreas and bowel but the gallbladder was minimally distended with a minimal pericholecystic fluid but no calculi. Blood counts showed a bicytopenia (Hb- 8.8g/dL, platelet count-14000/mm\(^3\)). A peripheral smear showed marked anisopoikilocytosis with micro, normo and macrocytes, occasional hypersegmented neutrophils and thrombocytopenia (Platelet count of 15000/mm\(^3\)) suggestive of Vitamin B12 deficiency. She was treated with parenteral methylcobalamine and other supportive measures. She had bleeding from oral cavity while brushing her teeth and on examination we found wet purpura. The repeat platelet counts showed a further fall, which was not fitting with diagnosis of Vitamin B12 deficiency alone. In the background of fever with thrombocytopenia and leucopenia, an IgM dengue was done which came out to be positive. 2 days later, she developed postural hypotension associated with pedal edema and periorbital edema. A working diagnosis of capillary leak syndrome secondary to dengue fever which was supported by the presence of vomiting, with worsening of the abdominal pain and abdominal distension. Chest x-ray showed bilateral pleural effusion. An ultrasound of the abdomen showed acalculous cholecystitis associated with peri cholecystic collection and moderate ascites. Blood counts were repeated to look for hemoconcentration due to capillary leak, but instead showed pancytopenia (Hb- 5.9 g/dL, WBC – 1800/mm\(^3\), platelet count- 8000/mm\(^3\)). Subsequently, a bone marrow study was done which showed normoblastic and megaloblastic maturation in erythroid series and few scattered histiocytes, some showing ingested RBCs suggestive of HLH. Serum Ferritin was 1778 and serum triglyceride was 321, which supported the diagnosis. She was started on Methyl Prednisolone 1g IV. On the 3rd day, she desaturated and a repeat chest x-ray showed bilateral lung infiltrates suggestive of ARDS. She was intubated and mechanically ventilated but she succumbed to her illness.

**Case 6**

38 year old male, mason by profession, smoker and alcoholic with high risk sexual behavior detected to have diabetes 8 years back and had moderate non proliferative diabetic retinopathy, presented with 8 months history of weight loss(20kg in 6 months) and loss of appetite along with recurrent episodes of fever with night sweats for 1 month. It was associated with cough with expectoration but no hemoptysis or breathlessness. He also noticed a painless swelling in his right axilla. On examination, he was emaciated, pale and had icterus with bilateral axillary lymphadenopathy. There was extensive oral candidiasis. The respiratory system examination showed coarse inspiratory crackles on right infraclavicular area. Abdomen examination showed hepatosplenomegaly. Chest X-ray showed right upper zone consolidation. Sputum AFB was negative but CB-NAAT for MTB was detected. HRCT thorax showed cystic bronchiectatic changes in the apical segment of right upper lobe. A lymph node excision biopsy was performed and showed granulomatous lymphadenitis with caseous necrosis suggestive of Tuberculosis. HIV ELISA was positive and HBsAg and Anti-HCV were negative. Blood investigations showed elevated liver enzymes and conjugated hyperbilirubinemia. Ultrasound abdomen showed hepatosplenomegaly and no evidence of any cholelithiasis, which was suggestive of a possible granulomatous hepatitis. Blood counts showed pancytopenia, which was thought to be due to tuberculosis infiltrating the marrow. A bone marrow study showed no evidence of tuberculous infiltration instead, showed the presence of hemophagocytosis with histiocytic proliferation. The ferritin (>40000)
and triglyceride (312) was done subsequently, which favoured HLH. He was initiated on modified anti TB regimen (and HAART was planned after 2 weeks of ATT). In view of HLH with pancytopenia, patient was given high dose steroids (methylprednisolone). The patient went into respiratory failure after a bout of massive hemoptysis and succumbed to his illness despite mechanical ventilation and other supportive measures.

Case 7
14 year old male presented with high grade, intermittent fever with chills and loose stools for 1 week and non-productive cough for 3 days. There was no breathlessness, jaundice, abdominal pain, vomiting, myalgia, neurological deficits or joint pains. He enjoyed some street food while on his visit to north India. Other family members also had similar illness. On examination, the boy had multiple petechial spots over the chest, abdominal wall, thighs and legs which blanched on applying pressure. The routine blood investigations showed pancytopenia with disproportionately low ESR.

A provisional diagnosis of enteric fever was made and the patient was started empirically on IV ceftriaxone with other supportive measures. But fever and pancytopenia were persisting, which prompted us to send serum ferritin, triglycerides, blood culture and a bone marrow biopsy with culture. The serum ferritin was 39800. Suspecting a secondary HLH, patient was referred to hematology for further work up. Investigations showed a high ferritin (>1500) and hypertriglyceridemia. Patient was initiated on IV methyl prednisolone pulse for 5 days. The fever subsided and the pancytopenia improved and he was referred to radiation oncology for further treatment. An echocardiogram showed normal LV function. He was thus initiated on RCHOP-E regimen. The patient went into remission with chemotherapy and is currently under follow up.

Table 1: Cytopenias, Serum Ferritin and Serum triglyceride levels in secondary causes of HLH

<table>
<thead>
<tr>
<th>Cause of HLH</th>
<th>Cytopenias</th>
<th>Ferritin</th>
<th>Triglycerides</th>
<th>Treatment Given</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMN</td>
<td>Hb 7.7  700</td>
<td>40000</td>
<td>1510</td>
<td>187</td>
<td>Dexamethasone</td>
</tr>
<tr>
<td>Leshmaniasis</td>
<td>Hb 6.9  2000</td>
<td>91000</td>
<td>1354</td>
<td>335</td>
<td>Dexamethasone</td>
</tr>
<tr>
<td>Mycoplasma</td>
<td>Hb 7.2  2000</td>
<td>13000</td>
<td>&gt;2000</td>
<td>384</td>
<td>Methyl Prednisolone</td>
</tr>
<tr>
<td>NHL</td>
<td>Hb 8.3  490</td>
<td>29000</td>
<td>1695</td>
<td>283</td>
<td>IV Ig</td>
</tr>
<tr>
<td>Dengue</td>
<td>Hb 5.9  1800</td>
<td>8000</td>
<td>1778</td>
<td>321</td>
<td>Methyl Prednisolone</td>
</tr>
<tr>
<td>HIV+TB</td>
<td>Hb 7.5  560</td>
<td>48000</td>
<td>&gt;40000</td>
<td>312</td>
<td>Not given</td>
</tr>
<tr>
<td>Enteric fever</td>
<td>Hb 12.0  1700</td>
<td>34000</td>
<td>39800</td>
<td>230</td>
<td>Methyl Prednisolone</td>
</tr>
<tr>
<td>NHL</td>
<td>Hb 6.3  2100</td>
<td>22000</td>
<td>&gt;1500</td>
<td>424</td>
<td>Methyl Prednisolone</td>
</tr>
</tbody>
</table>

Figure 1: Lymph node biopsy showing histiocytes and hemophagocytosis (H&E stain)

Figure 2: Bone marrow showing hemophagocytosis consistent with HLH

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DISCUSSION

Hemophagocytic lymphohistiocytosis (HLH) covers a wide range of related diseases viz. HLH, autosomal recessive familial HLH (FHL), familial erythrophagocytic lymphohistiocytosis, viral-associated hemophagocytic syndrome,[4] and autoimmune-associated macrophage activation syndrome (MAS). These disorders are characterized by severe cytopenias due to uncontrolled hemophagocytosis. Other clinical symptoms and laboratory abnormalities result from disordered immune regulation and cytokine storm. The term primary HLH refers to a genetic abnormality causing the disorder,[4-6] whereas secondary HLH means that the disorder is secondary to underlying conditions such as infection, autoimmune/rheumatologic,[3] malignant, or metabolic conditions.

EBV and human immunodeficiency virus (HIV) were the most frequent infections causing HLH, while lymphoma was the most commonly associated malignancy.[6-10] MAS is seen in a variety of different rheumatic diseases in adults and children, but is most frequently reported in systemic juvenile idiopathic arthritis (sJIA) and its adult equivalent, adult-onset Still’s disease.[11] In addition, a diverse range of drugs have been found to cause MAS, including biological therapies.[12,13]

The diagnosis of FHL or secondary HLH is based on a number of clinical signs and laboratory findings. Due to the relatively nonspecific nature of the clinical signs and symptoms, and significant overlap with other illnesses, diagnosis is challenging and often delayed. The diagnosis of HLH is established by the modified 2009 HLH diagnostic criteria and H-score.[14,15] Prior to the use of modern treatment options, death due to HLH was almost inevitable.[16] Briefly, treatment of HLH involves immune-suppressive and modulatory agents, biological response modifiers, treatment of the inciting illness if secondary, and subsequent stem-cell transplantation.[3]

EBV is the cause of heterophile antibody positive infectious mononucleosis (IMN), presenting as fever, sore throat, lymphadenopathy and atypical lymphocytosis and is also associated with some tumors including nasopharyngeal carcinoma and lymphomas.[17,18] Kala-azar (KA) also known as visceral leishmaniasis is a parasitic disease,[19,20] caused by L. donovani. HLH is rare in VL and can complicate the diagnosis of leishmaniasis. Bone marrow study is diagnostic in 78% of cases but is

Table 2: Site of hemophagocytosis, the treatment given and mortality among secondary causes of HLH

<table>
<thead>
<tr>
<th>Cause of HLH</th>
<th>Site of Hemophagocytosis</th>
<th>Treatment given for HLH</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMN</td>
<td>Lymph node</td>
<td>Dexamethasone</td>
<td>No</td>
</tr>
<tr>
<td>Leishmaniasis</td>
<td>Bone Marrow</td>
<td>Dexamethasone</td>
<td>Yes</td>
</tr>
<tr>
<td>Mycoplasma</td>
<td>Bone Marrow</td>
<td>Methylprednisolone</td>
<td>Yes</td>
</tr>
<tr>
<td>NHL</td>
<td>Bone Marrow</td>
<td>IVIG</td>
<td>No</td>
</tr>
<tr>
<td>Dengue</td>
<td>Bone Marrow</td>
<td>Methylprednisolone</td>
<td>Yes</td>
</tr>
<tr>
<td>HIV+TB</td>
<td>Bone Marrow</td>
<td>Not given</td>
<td>Yes</td>
</tr>
<tr>
<td>Enteric fever</td>
<td>Bone Marrow</td>
<td>Methylprednisolone</td>
<td>No</td>
</tr>
<tr>
<td>NHL</td>
<td>Bone Marrow</td>
<td>Methylprednisolone</td>
<td>No</td>
</tr>
</tbody>
</table>
favourable clinical outcome and one patient expired before receiving any immunosuppressive therapy.

**CONCLUSION**

This case series highlights the fact that secondary HLH can occur in a multitude of clinical settings. The features of HLH might take time to evolve and initial bone marrow biopsies may not be suggestive, as was seen in case 2. A high index of suspicion is necessary to have a quick and accurate diagnosis and timely intervention; otherwise the disease can be fatal. Timely detection of this very fatal complication and prompt intervention is life-saving and we hope our case series would guide physicians in the future for early detection of this rare.

**REFERENCES**


