Case report: Secondary HLH in COVID-19

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Abstract
We present case of a 53 year old lady who is a known case of rheumatoid arthritis and hypertension in Karnataka, South India, who presented with Severe Acute Respiratory Infection (SARI) like symptoms (fever, cough and breathlessness) due to COVID-19. During the course of treatment further investigations revealed evidence of Hemophagocytic Lymphohistiocytosis (HLH). This case emphasizes the need for a thorough workup to identify the etiology of HLH as it may unmask a treatable entity in a COVID-19 patient.

Keywords: Hemophagocytic lymphohistiocytosis, COVID-19, Severe acute respiratory infection

Introduction
It is known that COVID-19 can cause an intense cytokine mediated immune response causing various complications - acute respiratory distress syndrome. However one extreme of such a strong immune response is Hemophagocytic Lymphohistiocytosis (HLH). HLH being a rare condition in itself, is lately diagnosed and it mimics the cytokine storm in COVID-19.

Case report
We present the case of a 53-year-old female, with history of hypertension & rheumatoid arthritis, taking cilnidipine daily, leflunomide daily & methotrexate once in 7 days with no history of cigarette or alcohol consumption and no known drug allergies. The patient comes to seek medical care at A.J. Hospital & Research Centre on 7/10/2020 following 10 days treatment (antibiotics and Remdesivir) at another hospital for RT-PCR proven nasopharangeal swab for SARS-CoV2 RNA virus, which was diagnosed on 1/10/2020. She reported that the reason for seeking medical care was the worsening of fever & dyspnea on 7/10/2020. On general physical examination, patient was extremely tachypneic with a respiratory rate of 38 rpm, when breathing in room air. On lung auscultation, there were bilateral crackles in the lower and middle segments of lungs, Saturation of 77% on 15litres oxygen High concentration mask and temperature 100 ºF. In the background of swab positive RT-PCR 1 week back, the possible differential diagnosis was COVID -19 associated Acute respiratory distress syndrome, possibly in a cytokine storm phase, so a repeat nasopharyngeal swab specimen was collected along with other routine investigations - complete blood cell count, blood chemistry, inflammatory markers (IL-6, D-Dimer, ferritin, LDH, CRP). (See table 1).

The patient was shifted to covid intensive care unit and high flow nasal oxygen (HFNO) was offered, thereby improving her oxygen saturation to 94% and was encouraged to do awake proning as well as Incentive Spirometry. Apart from her antihypertensives and disease modifying anti rheumatic drugs (DMARDs), she was started on - Meropenem, Voriconazole, Oseltamivir, Ivermectin, Low Molecular Weight Heparin- Enoxaparin sodium (60mg subcutaneous bid), Methylprednisolone (120mg/ day), Mucolytics, vitamin C & zinc and 2 doses of tocilizumab (400mg) were initiated on 7/10/2020 in view of elevated inflammatory markers. A baseline 2d echocardiography was done and it revealed normal ventricular functions with no pulmonary artery hypertension and chest computed tomography (CT) reported as multiple patchy ground glass opacities with consolidation and fibrotic bands involving bilateral lungs, predominantly the mid and lower lobes-CORAD 6 with CT severity index 23/25.
Non-Invasive ventilation (NIV) was started on 25/10/2020 due to sudden oxygen desaturation despite on HFNO. However, due to persistent desaturation despite NIV, patient was electively intubated using an Hi-Lo Evac endotracheal tube with a Macintosh laryngoscope blade, sedated with fentanyl and vecuronium; and connected to invasive mechanical Ventilation; central venous catheterisation was done using right jugular vein for venous access, an arterial line was placed using right ulnar access for mean arterial pressure (MAP) monitoring as patient was on inotropic supports (dopamine and norepinephrine); a urinary catheter was added to meropenem for both hemodynamics, and Methylprednisolone (120mg/day) with fentanyl-midazolam/fentanyl-vecuronium infusion were continued.

Table 1: Main lab results of the patient

<table>
<thead>
<tr>
<th>Date</th>
<th>HB (g/dl)</th>
<th>TC (µl)</th>
<th>Neutrophils %</th>
<th>Lymphocytes %</th>
<th>Monocytes %</th>
<th>Eosinophils %</th>
<th>Basophils %</th>
<th>ANC (µl)</th>
<th>ALC (µl)</th>
<th>AMC (µl)</th>
<th>AEC (µl)</th>
<th>ABC/µl</th>
<th>AMC (/µl)</th>
<th>Platelets (µl)</th>
<th>Procalcitonin</th>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>50,000</td>
<td>0.32</td>
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On 28/10/2020, patients right lower limb was noticed to be swollen, and venous doppler study revealed deep vein thrombosis in right lower limb venous system involving common femoral, superficial femoral, popliteal, anterior tibial and posterior tibial veins. As inflammatory markers were rising and 2d echocardiography started showing increase in pulmonary artery hypertension (PASP by TR jet 40mmHg), a CT-Pulmonary angiogram was done to look for any peripheral thrombus, and it was reported as severe pulmonary artery hypertension. Considering this, the dose of Enoxaparin sodium was increased from 60mg to 90mg subcutaneous and then eventually was switched over to newer oral anticoagulant -Dabigatran (150mg bid) and Inhaled nitric oxide (INO) therapy was initiated. Empirically Teicoplanin was added to meropenem for both gram negative and positive coverage in view of worsening hemodynamics, and Methylprednisolone (120mg/day) with fentanyl-midazolam/fentanyl-vecuronium infusion were continued.

On 29/10/2020, patient developed rectal bleeding. Per rectal examination revealed fresh blood not mixed with stools. On sigmoidoscopy, it was found that distal rectum showed multiple ulcers - Stercoral ulcers. Patient was treated with proctolysis enema and was on liquid paraffin for the same following which there was no rectal bleed noticed.

On 31/10/2020, patients laboratory values showed pancytopenia with very severe neutropenia with hemoglobin 7.7g/dl, platelets 65,000/µl, total counts 320/µl, absolute neutrophil count (ANC) 280 (corrected for nRBCs), serum ferritin 5969, Ddimer 400 ng/ml, procalcitonin 0.3, LDH 840 U/l, total bilirubin 2.2mg/dl, direct bilirubin 1.5mg/dl, with a normal creatinine level 0.8 mg/dl, serum calcium 7mg/dl. Peripheral smear showed pancytopenia with no abnormal or atypical cells. Since patient was on DMARDs, vitamin B12 and folate levels were sought, to rule out drug induced causes of pancytopenia. Vitamin B12 levels were > 2000 pg/ml (1:3 Dilution:4781) and RBC folate level were 386 ng/ml. Direct Coombs test (DCT) was asked in view of

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elevated LDH and mild increase in bilirubin levels, which was found to be negative. Anti-nuclear antibody (ANA) immunofluorescence test was done to rule out autoimmune cytopenia and was found to be negative. Viral PCR (CMV, EBV, Adenovirus, Parvovirus) were sent to rule out infectious causes of pancytopenia, which were found to be negative. Repeat Blood cultures (aerobic & anerobic) from both central and peripheral vessels were sent for culture and sensitivity. HLH work up was done where serum ferritin was >2000, serum fibrinogen levels were 140mg/dl, serum triglyceride levels were 226 mmol/l despite patient was on methylprednisolone (120mg/day) from first day of admission. Therapy wise 1 packed cells RBCs was transfused, methylprednisolone (120mg/day) was continued, antibiotics stepped up from meropenem to colistin and fosfomycin therapy in view of severe febrile neutropenia, and antifungals (anidulafungin) were prophylactically given. 1 dose of granulocyte colony stimulating factor (G-CSF) was administered and methotrexate (last dose 25/10/2020) and Leflunomide were withheld.

On 1/11/2020, 12 hours post G-CSF, complete blood count showed an increase in ANC and she was put on G-CSF twice daily regimen. Tissue diagnosis of HLH in the form of bone marrow biopsy was done and it demonstrated macrophage with nuclear remnants of ingested erythrocytes and leukocytes (Fig.1, Fig.2) and the diagnosis of HLH was made. In view of prolonged ventilation, patient was decided to put on veno-venous ECMO and during the procedure 4 packed cell RBCs and 10 units random donor platelets were transfused and heparin infusion was kept to maintain ACT 150-170 and all other anticoagulation was withheld.

On 2/11/2020, patients hemoglobin was 12.4 g/dl, total counts 50/µl, platelets 49,000/µl. Patient had repeated cardiac arrest despite on Venovenous and venoarterial ECMO. In the presence of repeated swab (RT-PCR, CB-NAAT,RAT) positive test, a negative procalcitonin level and positive laboratory and tissue biopsy diagnostic of HLH in the presence of fever, patient was declared dead on 3/11/2020 at 2.05 am secondary to HLH in COVID-19 despite on methylprednisolone (120mg/day).

Discussion

Hemophagocytic Lymphohistiocytosis (HLH), is an inherited severe life-threatening inflammatory disorder, seen most commonly in infants of 18 months of age, and is also seen in patients of all ages. HLH is classified as familial or primary HLH. Secondary HLH occurs in the settings of infections or underlying rheumatologic disorders and also has been seen in some lymphoid malignancies. Secondary HLH is similar to primary HLH, but is characterized by lack of genetic defects, and is triggered by events that disrupt immune balance. HLH is further subclassified into infection-related, malignancy related and associated forms of inflammation / autoimmunity. Secondary HLH is also seen in immunodeficiency following chemotherapy, immunosuppression, biology therapy and organ or stem cell transplantation, insults causing imbalance to the immune system [1, 2, 3]. Abnormal cases are also described in birth disorders such as lysinuric protein incompatibility or biotinidase deficiency. Secondary HLH in metabolic patients is usually atypical, deficient in fever or incorporates non-HLH factors such as vomiting or acidosis [2].

Around 60% of infectious cases are accompanied with primary infection or reactivation of latently present herpesviruses [3]. EBV infects B cells and nasopharyngeal epithelial cells, but in HLH it often infects CTLs and NK cells [4]. Other viruses that are known to trigger HLH are - Cytomegalovirus, herpes simplex virus (HSV), human herpesvirus- 6 and - 8, varicella zoster, parvovirus B19 and adenoviruses. HLH is less frequently seen in RNA viruses (influenza and enteroviruses). Patients suffering human immunodeficiency virus (HIV) infection are prone to develop HLH upon opportunistic infections [3, 5, 6].

Secondary HLH also occurs in autoimmune and autoinflammatory disorders like systemic lupus erythematosus, adult-onset Still's disease, Kawasaki disease, rheumatoid arthritis, dermatomyositis, sarcoidosis, systemic sclerosis, polyarteritis nodosa, inflammatory bowel disease and systemic juvenile idiopathic arthritis. Complicated rheumatological conditions with HLH is termed as...
‘macrophage activation syndrome’ (MAS) [3, 7, 8]. In secondary HLH, the NK cell dysfunction is acquired. It is a temporary phenomenon that often normalizes with disease remission and reverses upon IL2 stimulation in vitro [9, 10, 11, 12]. The cytokine storm is considered to impair NK cell cytotoxicity. Persistent high levels of NK cell- stimulating cytokines (IL12 or IL18), might over activate NK cells, leading to apoptosis [13, 14]. The latent- membrane- protein- 1 seen in EBV is capable of prohibiting SAP expression in T cells, thus creating a setting of XLP1- associated HLH [15]. H5N1 influenza's haemagglutinin protein reduces perforin expression in CTLs, reducing their cytotoxicity, allowing the tenacity of stimulatory H5 antigen- presenting dendritic cells [16]. H1N1 influenza is known to infect and replicate in human NK cells and induce apoptosis, further decreasing NK cell numbers and cytotoxic function [17]. Most viruses conceal anti- apoptotic proteins that delay apoptosis of infected cells, which was recently linked to prolonged cytotoxic synapse duration and cytokine hypersecretion, leading to HLH pathogenesis [18]. EBV and HSV- 1 can provide cells resistant to CTL- induced apoptosis [19]. It is ideal that all patients must have a bone marrow biopsy to evaluate the cause of cytopenias and detect hemophagocytosis and must also be cultured, examined for infectious organisms and looked for secondary or triggering causes and possibilities of malignancy. Bone marrow cellularity may be high, low, or normal in HLH [9]. Hemophagocytosis although can be a marker of excessive macrophage activation, but alone is not enough for the diagnosis of HLH. Some patients show hemophagocytosis in the later course of the disease, when they are clinically improving [20].

Conclusion
Secondary HLH has also been found in patients with severe acute respiratory syndrome and middle east respiratory syndrome (MERS). It is likely that it develops in patients suffering from COVID-19 too, as all three viruses trigger a similar cytokine mediated storm. It is probably existing in more patients than we know with COVID-19 and likely contributes to prognosis and its mortality and needs to be thoroughly evaluated for the same as its a treatable entity.

References