Hemophagocytic syndrome and COVID-19

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A B S T R A C T

The case of a woman hospitalized due to COVID-19 is presented. The patient developed a severe macrophage activation syndrome diagnosed by bone marrow biopsy, which did not respond to immunoglobulin therapy.

1. Case presentation

We present the case of a 60-year-old female, with history of diabetes and hypertension, taking losartan, gabapentin, insulin glulisine, and metformin daily. Reports no history of cigarette or alcohol consumption and no known drug allergies. Surgical history: C-section and hysterectomy. (see Table 1, Fig. 1)

The patient sought medical care at the Hospital Samaritano Botafogo on 3/31/2020 presenting dry cough, dyspnea, prostration, fever (not measured), and dizziness. She reported that the reason for seeking medical care was the worsening of dyspnea and the onset of dizziness on 3/31/2020. Physical examination revealed dyspnea and tachypnea when breathing room air. On lung auscultation: bilateral crackles in the lower segments of the lungs bilaterally, blood pressure (BP) 112/57 mmHg, heart rate (HR) 81 bpm, respiratory rate (RR) 18 rpm, Sat O2 97%, and a temperature of 36 °C (96.8 °F). No further relevant information was found. A high concentration oxygen mask was offered, with O2 at 10 L/min, thereby improving O2 saturation up to 94%. The diagnostic impression was a coronavirus infection; a swab specimen collection for coronavirus was ordered, along with a respiratory virus panel; blood cell count; blood chemistry; chest computed tomography (CT) (report: diffuse ground glass infiltrates in over 50% of lung fields, mosaic paving pattern, basilar consolidations, and linear atelectasis in the lower lobes). The patient was admitted to the intensive care unit (ICU). Azithromycin and hydroxychloroquine were initiated on 3/31/2020 while in the ICU.

Non-invasive ventilation (NIV) and dexmedetomidine were started on 1/4/2020 due to oxygen desaturation with the use of a high concentration oxygen mask. However, due to persistent desaturation despite NIV, endotracheal intubation was indicated with a McGrath blade and bougie; the patient was adapted to ventilatory support, sedated with lidocaine, etomidate, and rocuronium; central venous catheterization was performed in the right internal jugular vein (IJV), an arterial line was placed for mean arterial pressure (MAP) monitoring; a urinary catheter and a nasoenteric tube were inserted. The following tests were ordered: chest X-ray, sample collection for culture, and arterial blood gas (ABG). Therapy was initiated with amoxicillin/clavulanic acid, norepinephrine, fentanyl-midazolam, enoxaparin sodium, azithromycin and hydroxychloroquine.

On 4/2/2020, insulin infusion was started and a transthoracic echocardiogram (TTE) was ordered, which showed mild to moderate systolic dysfunction, with signs of congestion in the inferior vena cava. A lung ultrasonography was also carried out, showing consolidation in the lower two-thirds of the right lung, with coalescent B-lines in the right lung apex and lower two-thirds of the left lung. ECG recorded on 4/2/2020: QTc 530 msec; azithromycin was discontinued. Vascular Surgery rotated the femoral access placed the previous day (4/1/2020) due to a slight hematoma at the site of MAP line insertion, slight distal cyanosis in both lower limbs, reduced perfusion; a EV100 kit was placed in the femoral artery without complications, maintaining normal blood flow, backflow, and pedal pulse after procedure completion. The left IJV was cannulated, a new chest X-ray was ordered, along with ABG, lactate, and electrolytes. Therapy with enoxaparin sodium + azithromycin + hydroxychloroquine, dobutamine, fentanyl-midazolam, insulin (infusion pump), and norepinephrine was maintained.

On 4/3/2020, the parameters on EV1000 showed a low cardiac...
On 4/4/2020, the placement of a hemodialysis catheter was indicated (urine output: 400 mL and fluid balance -330 mL). Hemodialysis was started with an UF rate of 100 mL/h. Azithromycin was indicated again for severe COVID-19. Hydrocortisone was stopped due to a lack of blood sugar control without reducing the dose of norepinephrine once it was initiated.

On 4/5/2020, the patient was hemodynamically unstable, with norepinephrine and amiodarone infusion; highest fever value 100.2°C (37.9°C); glucose level 137 to 249, requiring regular insulin 8 IU SC; receiving hydroxychloroquine + azithromycin + piperacillin/tazobactam + teicoplanin; urine output 0 mL, hemodialysis 1000 mL, fluid balance +699 mL. EV1000: SVV: 9, CO: 3.9. On hemodialysis, the patient continued with 100 mL volume reduction. The patient presented supraventricular tachycardia with an HR of 190 bpm; vagal stimulation was unsuccessful, and a 300 mg amiodarone loading infusion was administered in 20 min. and a maintenance infusion was continued at 20 mL/h.

On 4/6/2020, the patient was anuric and without bowel movements. Fluid balance +1480 mL/24 h. Vascular Surgery removed the hemodialysis catheter from the left common femoral vein due to catheter blockade; an 11 F x 20 cm double lumen catheter was inserted, with good flow and backflow in both lumen; no complications occurred, and the catheter was heparinized and ready for use.

On 4/7/2020, a PTT test was ordered to plan heparin infusion. The following actions were taken: Mifafungin was started, with calcium gluconate replacement. Heparin IV was also initiated. Tocilizumab was switched from piperacillin/tazobactam to meropenem, as scheduled. A bedside abdominal ultrasound was carried out; a bone marrow biopsy was also performed, exhibiting macrophage activity secondary to the syndrome. Midazolam was held, and tocilizumab was initiated at a dose of 400 mg IV.

On 4/9/2020, midazolam was combined with fentanyl to optimize sedation. Sodium glycerophosphate was replaced and immunoglobulin was started. Vascular Surgery prepared the vascular access for hemodialysis. The patient presented ventricular tachycardia with an HR of 190 bpm; vagal stimulation was unsuccessful, and a 300 mg amiodarone loading infusion was administered in 20 min. and a maintenance infusion was continued at 20 mL/h. Immunoglobulin was started and planned to end on 4/13.

On 4/10/2020, the patient did not have a fever throughout the day and was hemodynamically dependent on norepinephrine. Hemodialysis of 200 mL/h, urine output 0 mL.

On 4/11/2020, midazolam, fentanyl, amiodarone, regular metamizole, and hemodialysis were stopped. A blanket was used to increase the temperature to 105°F (40°C). Dobutamine was added and norepinephrine was increased to 12 mL/h. Insulin and IV heparin were administered per the local protocol. The patient presented cardiopulmonary (CP) arrest with asystole following the return from hemodialysis without UF; cardiopulmonary resuscitation maneuvers were carried out for 6 minutes. Two vials of atropine 1 mg and 2 vials of epinephrine 1 mg were used; IV heparin was withheld; PTT was non-coagulable. A Vscan examination showed no evidence of cardiac tamponade, pleural effusion, or pneumothorax. CP arrest with resuscitation maneuvers, a percutaneous pacemaker was inserted, 8 vials of atropine and 3 vials of adrenaline, saline 0.9% 1500 mL, norepinephrine at 100 mL/h at the moment of the CP arrest, and 15 mL/h after CP arrest, without sedation, FiO2 100% in CV mode. At 11:30 pm, another CP arrest for 3 minutes; 8 vials of atropine were administered along with 2 vials of epinephrine, sodium gluconate 30 mL, and sodium bicarbonate 20 mL, increasing norepinephrine up to 40 mL/h.

On 4/12/2020, the patient had the transcutaneous pacemaker implanted during CP resuscitation; there are difficulties with capture, even with maximum output. Respiratory minute volume was reduced in the ventilator; noradrenaline 40 mL/h – 2 mcg/kg/min, achieving MAP 62, HR 80, CI 2.5, SVRI 1900, CVP 8 (EV1000), sodium bicarbonate 250

| Table 1 | Main lab results of the patient. |
|---------|---------------------------------|
|         | 31/03 | 02/04 | 05/04 | 07/04 | 08/04 | 10/04 | 11/04 |
| TROPONIN I | pg/mL | 26    | 2000  | –     | –     | 373   | –     | –     |
| C REACTIVE PROTEIN | mg/dL | 172   | 262   | 164   | –     | 97    | 20    | 23    |
| PROCALCITONIN | ng/mL | –     | –     | 2.8   | –     | 5.6   | –     | –     |
| INTERLEUKIN 6 | pg/mL | –     | –     | 1123  | –     | –     | –     | –     |
| LACTIC DEHYDROGENASE | U/L | –     | –     | –     | 6180  | –     | –     | –     |
| FERRITIN | ng/mL | –     | –     | –     | –     | –     | –     | –     |
| HEMOGLOBIN | g/dL | 12.1  | 10.6  | 9.9   | –     | 6.7   | 7.5   | 7.4   |
| HEMATOCRIT | % | 32.7  | 30.2  | 28.8  | –     | 19.4  | 22.3  | 22.6  |
| LEUKOCYTES | x 10³/µL | 8.76  | 9.82  | 24.87 | –     | 47.75 | 35.36 | 49.15 |
| PLATELETS | x 10³/µL | 193   | 210   | 285   | –     | 413   | 206   | 161   |
| GLUCOSE | mg/dL | 249   | 461   | 197   | –     | 116   | 111   | 126   |
| CREATININE | mg/dL | 75    | 46    | 38    | –     | 20    | 22    | 27    |
| TRIGLYCERIDES | mg/dL | 1.1   | 1.2   | 1.6   | –     | 1     | 1     | 1     |
| AST | U/L | 37    | –     | –     | –     | 128   | –     | –     |
| ALT | U/L | 43    | –     | –     | –     | 709   | 824   | 286   | 297   |
| INR | –     | –     | –     | –     | –     | 2     | –     | 1.36  | 84    |
| TTPA | seconds | –     | –     | –     | –     | >160  | –     | 155   | 38.3  |
| FIBRINOGEN | mg/dL | –     | –     | –     | –     | 351   | –     | 199   | 185   |
| GAMA GT | U/L | –     | –     | –     | –     | 79    | –     | –     | 119   |
| VIRAL PANEL | NOT DETECTED | –     | –     | –     | –     | –     | –     | –     |
| PCR COVID 19 | DETECTED | –     | –     | –     | –     | –     | –     | –     |
ml IV at a high rate; warm saline 1500 ml at a high rate. The patient died at 7:00 a.m.

2. Discussion

Hemophagocytic lymphohistiocytosis (HLH) is an aggressive and life-threatening syndrome of excessive immune activation. It most frequently affects infants from birth to 18 months of age, but the disease is also observed in children and adults of all ages.

The pathophysiology of HLH is related to the failure to control the immune response that results in a hyperinflammatory state and consequently to tissue destruction [1]. The dysregulation of the immune system is characterized by a persistent activation of Cytotoxic T lymphocytes (CTLs), Natural Killers cells (NK), and Macrophages.

After encountering a virally infected cell or tumor cell, CD8⁺ CTLs and NK promote cytolytic destruction by releasing proteins involved in triggering the apoptosis of the target cells. The consequence is the elimination of the immune-activating stimulus - the virally infected or tumor cell, for example. This physiologic downregulation is critical for the control of the immune response [2].

HLH is characterized by the inability to clear the antigenic stimulus, resulting in the persistence and amplification of the immune response. Proinflammatory cytokines released by the activated immune cells result in a high level of macrophage activation, which result in hemophagocytosis, tissue damage, organ failure, and the other inflammatory manifestations of the syndrome [2].

HLH can occur as a familial or sporadic disorder. Genetic mutations that disrupt the ability of CD8⁺ CTLs and NK to release the proteins involved in cytolytic destruction predisposes the familial HLH. Sporadic disorder may be caused by diseases that affect the activity of CTLs and NK, like lymphomas and virus infection; or by a disease that increases the activation of macrophages, like macrophage activation syndrome (MAS); or by conditions that increase immune activation, like autoimmune disorders and alloimmune hematopoietic stem cell transplantation (HSCT) [2].

A variety of events that disrupt immune homeostasis can trigger HLH, such as infection, which is a common trigger both in those with a genetic predisposition and in sporadic cases. HLH is often associated with viral infections, including Epstein-Barr virus (EBV), cytomegalovirus (CMV), parvovirus, herpes simplex virus, varicella-zoster virus, measles virus, human herpes virus 8, H1N1 influenza virus, parvovirus, and HIV, alone or in combination. An HLH-like syndrome has been reported in association with SARS-CoV-2 (the novel coronavirus that causes COVID-19) [3,4]. The development of HLH shortly after the initiation of antiretroviral therapy (ART) for the treatment of HIV infection has also been reported [5]. The underlying mechanism associated to infection is related to the toll-like receptors (TLR). TLR are non-antigen-specific receptors on the surface of NK cells that are activated by components of bacteria, fungi, viruses, or mycoplasma. TLR excessive activation by infection can trigger HLH [6].

Patients with HLH have dramatically elevated levels of numerous proinflammatory cytokines, particularly interferon gamma (IFN-γ). High levels of IFN-γ, results in macrophage activation and the subsequent increased production of other proinflammatory cytokines. The persistent activation of macrophages, NK cells, and CTLs in patients with HLH leads to excessive cytokine production by all those cells, a phenomenon named cytokine storm, and is thought to be responsible for multiorgan failure and the high mortality of this syndrome [1,7,8]. Cytokines found at extremely high levels in the plasma of patients with HLH include, besides (IFN-γ), tumor necrosis factor alpha (TNFα); interleukins (IL) such as IL-6, IL-10, and IL-12; and the soluble IL-2 receptor (CD25) [9–11].

One interesting feature of the disease is the hemophagocytosis, which refers to the engulfment (literally “eating”) of host blood cells by macrophages. Hemophagocytosis is characterized by the presence of red blood cells, platelets, or white blood cells (or fragments of these cells) within the cytoplasm of macrophages, and it can be observed in the biopsies of immune tissues (lymph nodes, spleen, liver) or bone marrow aspirates/biopsies.

Prompt treatment is critical, but the greatest barrier to a successful outcome is often a delay in diagnosis due to the rarity of this syndrome, variable clinical presentation, and lack of specificity of the clinical and laboratory findings.

HLH initially presents as a febrile illness associated with multiple organ involvement. Thus, the initial signs and symptoms of HLH can mimic common infections, fever of unknown origin, hepatitis, or encephalitis. With few exceptions, the clinical features are similar regardless of whether an underlying genetic defect has been identified.

The HLH-2004 study, which included 369 patients, reported the following clinical findings and, therefore, were considered as diagnostic criteria [12]:

- Fever ≥38.5 °C (95%);
- Splenomegaly (89%);
- Liver enlargement (91%);
- Splenic enlargement (88%);
- Febrile illness or infection (90%);
- Leukocytosis (75%);
- Lymphadenopathy (76%);
- Hematocrit < 30% (60%);
- Anemia (80%);
- Elevated alkaline phosphatase (73%);
- Elevated LDH (64%);
- Elevated serum ferritin (90%);
- Elevated CRP (77%);
- Elevated reticulocyte count (67%);
- Hypofibrinogenemia (69%);
- Thrombocytopenia (98%);
- Macrophage (hemophagocytosis) in bone marrow aspirates/biopsies.

Fig. 1. Bone marrow aspirate sample examination with hematoxylin and eosin stain. An increased number of activated macrophages with prominent hemophagocytosis of hematopoietic elements is shown in the bone marrow (A), which is displayed in greater detail in panel B.
● Peripheral blood cytopenia, with at least two of the following: hemoglobin <9 g/dL (for infants <4 weeks, hemoglobin <10 g/dL); platelets <100,000/microl; absolute neutrophil count <1000/microl (92%);
● Hypertriglyceridemia (fasting triglycerides >265 mg/dL) and/or hypofibrinogenemia (fibrinogen <150 mg/dL) (90%);
● Hemophagocytosis in bone marrow, spleen, lymph node, or liver (82%);
● Low or absent NK cell activity (71%);
● Ferritin >500 ng/mL (94%)
● Elevated soluble CD25 (soluble IL-2 receptor alpha [sIL-2R]) two standard deviations above age-adjusted laboratory-specific norms (97%).

Some authors prefer to consider a ferritin >3000 ng/mL as more indicative of HLH [13].

Diagnosis of HLH is established when 5 of those eight criteria are fulfilled. Of the 182 patients with sufficient clinical data to judge the HLH-2004 diagnostic criteria for HLH, only 77 (29%) fulfilled at least five criteria.

A Delphi analysis (a method for finding consensus using iterative anonymous questionnaires) from an expert panel determined the following clinical features to be important in adults [14]: underlying predisposing disease; fever; organomegaly; cytopenias; elevated ferritin; elevated LDH; hemophagocytosis on the bone marrow aspirate. Those emerging diagnosis criteria, however, present with several differences from those used in pediatric patients.

It is the consensus that all patients should have a bone marrow aspirate and biopsy to evaluate the cause of cytopenias and/or detect hemophagocytosis. Bone marrow specimens should also be cultured, and examined for infectious organisms and evidence of malignancy, and secondary or triggering causes. Bone marrow cellularity can be high, low, or normal in HLH [15].

Although it can be a marker of excessive macrophage activation and supports the diagnosis of HLH, hemophagocytosis alone is neither pathognomonic of, nor required for, the diagnosis of HLH. Some patients may only show hemophagocytosis later in the disease course, even as they are clinically improving [15]. Hemophagocytosis on bone marrow examination is reported in 25–100% of cases of HLH [16]. A review of 78 hemophagocytosis detected on bone marrow aspirates revealed that approximately half of them were associated with HLH; however, the phagocytosis of nucleated or multiple nucleated cells was strongly correlated with a diagnosis of HLH [17]. A review of adult patients exhibiting hemophagocytosis in bone marrow aspirates revealed that 64% had lymphoma, especially T/NK and B cell lymphoma.

Infiltration of the bone marrow by activated macrophages is consistent with HLH. The macrophages in HLH do not have the cellular atypia associated with malignant histiocytes, and they are clearly different from the CD1a-staining Langerhans cells of Langerhans cell histiocytosis (formerly called histiocytosis-X). It is helpful to stain the bone marrow for the hemoglobin-haptoglobin scavenger receptor CD163 to highlight the macrophages (both hemophagocytosing and not).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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