Hemophagocytosis in peripheral blood culture preceding hemophagocytosis in bone marrow biopsy in secondary hemophagocytic syndrome

Hemofagocitose em cultura de sangue periférico precedendo hemofagocitose em biópsia de medula óssea na síndrome hemofagocítica secundária

Bruno Macedo Pinto¹, Cristina Han Hue Lin², Tainá Mosca¹, Ademir Veras da Silva², Gustavo Nunes Wakim², Wilma Carvalho Neves Forte¹

ABSTRACT

Hemophagocytic syndrome, which is caused by dysregulation of the immune system, is characterized by excessive macrophage activation, resulting in phagocytosis of normal blood cells in the liver, spleen, and bone marrow. It can be primary (genetic) or secondary (acquired). In adults, it is almost always secondary, with infections, neoplasms, and autoimmune diseases as frequent triggers. The main manifestations of this syndrome are prolonged fever and hepatosplenomegaly. Currently, diagnosis is confirmed through finding hemophagocytosis in a bone marrow biopsy. However, it has been reported that bone marrow biopsy results are still normal on the first day the syndrome manifests. Here we report observing hemophagocytosis in cultured peripheral blood cells from a 29-year-old patient prior to finding hemophagocytosis in bone marrow biopsy. The patient had various infections and a poor general condition, which did not improve after treating the infections. The laboratory findings allowed early treatment of hemophagocytic syndrome and the patient improved. We describe our technique in detail so it can be reproduced, and we provide a non-systematic review of the literature on the syndrome.

Keywords: Hemophagocytic lymphohistiocytosis, hyperferritinemia, persistent infection.

RESUMO

A síndrome hemofagocítica é determinada por desregulação do sistema imunológico, caracterizada por ativação excessiva de macrófagos, resultando em fagocitose de células sanguíneas normais no fígado, baço e medula óssea. Pode ser primária (genética) ou secundária (adquirida). Em adultos quase sempre é secundária, tendo infecções, neoplasias e doenças autoimunes como frequentes desencadeadores. Entre as principais manifestações da síndrome estão febre prolongada e hepatoesplenomegalia. O diagnóstico até o momento é confirmado pelo achado de hemofagocitose em biópsia de medula óssea. No entanto, é descrito que a biópsia de medula óssea é normal nos primeiros dias das manifestações da síndrome. O presente relato tem como objetivo mostrar a observação de hemofagocitose em cultura de células de sangue periférico de paciente de 29 anos precedendo a hemofagocitose em biópsia de medula óssea. O paciente apresentava diversas infecções, com grave comprometimento do estado geral e sem melhora com o tratamento das infecções. O achado laboratorial permitiu o tratamento precoce da síndrome hemofagocítica e a melhora da paciente. No presente relato a técnica utilizada está descrita detalhadamente para que possa ser reproduzida, além de ser apresentada uma revisão não sistemática da literatura sobre a síndrome.

Descritores: Linfo-histiocitose hemofagocítica, hiperferritinemia, infecção persistente.
Introduction

Hemophagocytic syndrome or hemophagocytic lymphohistiocytosis (HLH) or macrophage activation syndrome is characterized by increased levels of pro-inflammatory cytokines, with an excessive activation of macrophages that leads to phagocytosis of normal blood cells in the liver, spleen, and bone marrow.

The main symptoms of HLH are persistent fever above 38.5 °C and hepatosplenomegaly, but general (malaise, weakness), hematological (coagulopathy, petechiae, purpura, ecchymosis, epistaxis), liver, splenic, gastrointestinal (hematemesis, ascites, diarrhea, vomiting, abdominal pain, lower gastrointestinal bleeding), skin (erythematous rash, edema, subcutaneous nodules), respiratory (cough, dyspnea, respiratory failure), and genitourinary (nephrotic syndrome, renal failure) manifestations may also be present, as well as central nervous system (altered mental status, seizures, encephalopathy, coma) and psychiatric disorders (mood swings, delirium and psychosis).1-3 HLH should be investigated in cases of febrile cytopenia, as a delayed diagnosis may lead to multiorgan impairment and increased mortality.

The most common laboratory alterations in HLH are cytopenias (anemia, thrombocytopenia, neutropenia), hyperferritinemia, hypertriglyceridemia, hypofibrinogenemia, hemophagocytosis, decreased T and natural killer (NK) cell function, and elevated levels of the soluble interleukin-2 receptor (sIL-2R or CD25).2,3 Other alterations may include elevated inflammatory markers (C-reactive protein and erythrocyte sedimentation rate); elevated levels of lactate dehydrogenase; cerebrospinal fluid pleocytosis; elevated levels of liver transaminases, total bilirubin, urea, and creatinine; normal or slightly elevated levels of ammonia; hypoalbuminemia; and hyponatremia.2,3

The causes of HLH may be primary or secondary, both with high mortality. Primary HLH is related to genetic mutations in the PRF1, UNC13D, STX11, STXBTP2, SH2D1, XIAP, RAB27A, LYST, AP3B1, NLRC4, MAGTT1, ITK, and CD27 genes and is more common in children up to 18 months of age, but may occur at any age. The incidence of primary HLH ranges from 1:50,000 to 1,000,000 live births, and both sexes are equally affected.3 The etiology of secondary HLH varies greatly according to different places: infectious etiologies are more common in the USA, France, Spain, and South Korea, whereas neoplastic etiologies are more common in Italy, China, Japan, and Taiwan.1 Only a few cases of HLH have been described in South America.

TCD8+ and NK cells normally release perforin (a protein that forms pores on the target cell surface) and promote granzyme activation (proteins that trigger apoptosis).3,4 For this process to occur normally, perforin and granzymes need to be synthesized, transported, packaged in cytolytic granules, and secreted by TCD8+ and NK cells. Mutations in genes transcribing the proteins involved in these 4 processes cause primary hemophagocytic syndrome as a result of inability to clear the antigenic stimulus, which results in a permanently activated immune response and a persistent increase in the pro-inflammatory cytokines tumor necrosis factor α, interferon γ, interleukin (IL)-1β, IL-2, IL-6, IL-12, IL-16, and IL-18. Elevated anti-inflammatory response has also been observed, particularly of IL-10; however, this compensatory response is not enough to suppress excessive macrophage activation.3 The microenvironment of elevated pro-inflammatory cytokines results in persistent macrophage activation with resultant hemophagocytosis, tissue damage, organ failure, and the other manifestations of HLH.3,4

The diagnostic hypothesis of HLH is achieved through the use of the HScore and HLH-2004 scores — both are used in adults and have the same sensitivity and specificity. The HLH-2004 score, which was used in the present case, is derived from studies in children.3,4

HLH treatment aims to control the excessive immune response using potent immunosuppressants, human immunoglobulin, high-dose corticosteroids, and plasmapheresis, in addition to treating the underlying disease and associated multiorgan impairment.

We report the case of a 29-year-old woman in whom hemophagocytosis was first identified in peripheral blood culture and only later in bone marrow biopsy. We also describe in detail the technique used for identifying hemophagocytosis in peripheral blood.

Case report

A 29-year-old woman from Bahia, Brazil, currently living in São Paulo, Brazil, presented to the emergency room with symptoms of shortness of breath, tiredness, and productive cough for 5 weeks, with worsening of dyspnea for 1 day. She worked as a waste collector and had a history of HIV infection (she had abandoned treatment 3 years ago) and chronic kidney disease
Hemophagocytosis preceding bone marrow biopsy in HLH – Pinto BM et al.

Neutrophils

Monocytes

Figure 1
Peripheral blood cell culture showing hemophagocytosis by neutrophils and monocytes. The arrows indicate red blood cells phagocytosed by neutrophils (A and C) and monocytes (B and D) from peripheral blood

Discussion

The patient presented to the emergency room with high and persistent fever and hepatosplenomegaly. No symptoms were suggestive of a specific infection, and the patient responded poorly to medication. She had risk factors for developing secondary hemophagocytic syndrome, including history of HIV infection, previous tuberculosis, and treatment for CMV during the hospitalization.\(^1\,^4\,^5\) Such factors, in association with the patient's clinical condition (high and persistent fever, hepatosplenomegaly, adenomegaly, pancytopenia, and negative cultures), led to the hypothesis of HLH.\(^1\,^2\) This disease, although rare, has a higher incidence in people with HIV and tuberculosis, with a worse prognosis in patients with active infection.\(^5\)

Secondary HLH can be divided into two subgroups: with infectious triggers (viral, bacterial, parasitic, fungal) and noninfectious triggers (neoplasms, autoimmune diseases, medications, and other etiologies). One third of secondary HLH cases has more than 1 cause.\(^1\,^4\) Secondary HLH has an incidence of 1:800,000 individuals and mostly affects adults and women, with a mean age at diagnosis of 50 years.\(^1\)
The patient presented different infectious causes that could trigger HLH, including HIV, tuberculosis, and CMV infection. The most common infectious causes of HLH are HIV and Epstein-Barr virus infection, but also include herpes, CMV, hepatitis A, B, and C, parvovirus B19, influenza, and more recently SARS-CoV-2 infection. Bacterial (Mycobacterium tuberculosis, Staphylococcus aureus, Escherichia coli and Rickettsia spp.), parasitic (Leishmania spp., Plasmodium spp., Toxoplasma spp.), and fungal (Histoplasma spp.) infections have also been described.

Noninfectious causes of HLH must be excluded based on clinical condition or laboratory data, as in the present case. The main noninfectious causes of HLH are cancer (especially hematologic, such as lymphomas, leukemias, solid tumors), autoimmune diseases (systemic lupus erythematosus, Still’s disease, rheumatoid arthritis, vasculitis, and inflammatory bowel disease), medications (methotrexate, nonsteroidal anti-inflammatory drugs, anticonvulsants, immunosuppressants, chemotherapy, gold salts, sulfasalazine), medical procedures (surgeries, biopsies, parenteral nutrition, hemodialysis, liver and kidney transplants), other diseases (diabetes, chronic liver diseases), and pregnancy.

Other causes of systemic immune dysregulation should be ruled out when diagnosing HLH, such as sepsis, systemic inflammatory response syndrome, and multiple organ dysfunction syndrome, which present similar clinical symptoms but often with a faster evolution, unlike in the present case.

The diagnostic hypothesis in this case was based on the HLH-2004 criteria: presence of fever, splenomegaly, cytopenias, hypofibrinogenemia, hypertriglyceridemia, and ferritin levels > 500 µg/L, with a score of 96% chance of having HLH. HLH should be considered when 5 of the 8 clinical criteria of the HLH-2004 score are present: (1) fever > 38.5 °C; (2) splenomegaly; (3) cytopenias affecting at least 2 lineages in the peripheral blood: hemoglobin < 9 g/L, or platelets < 100,000 cells/mm3, or leukocytes < 3,000 cells/mm3, or neutrophils < 1,000 cells/mm3; (4) hypertriglyceridemia (> 265 mg/dL) and/or hypofibrinogenemia (< 150 mg/dL); (5) hemophagocytosis in bone marrow, spleen, liver, or lymph nodes; (6) low or absent NK cell activity; (7) hyperferritinemia (> 500 ng/mL); and (8) soluble CD25 or sIL-2R > 2400 U/mL.

The diagnosis is confirmed by the presence of hemophagocytosis in bone marrow biopsy. However, biopsy results tend to be normal in the first days of symptom onset, as happened in the present case, and the absence of hemophagocytosis does not exclude HLH.

Peripheral blood cell culture showed the presence of fragments of blood cells within mononuclear phagocytes and neutrophils, which led to the diagnosis of HLH before the appearance of hemophagocytosis in the bone marrow and despite the unavailability of tests such as NK cell activity and IL-2R dosage.

Phagocytes were cultured at 37 °C, in an atmosphere of 5% CO2, and isolated from peripheral blood by spontaneous sedimentation. The spontaneous sedimentation technique guarantees a cell suspension of approximately 77% neutrophils. The cell suspension also contained, in order of quantity, red blood cells, eosinophils, monocytes, and lymphocytes. This technique allows direct contact between the different cells, allowing red blood cells to be phagocytosed by other blood cells, as occurs in hemophagocytic syndrome – this led to the diagnosis of HLH in the case presented in this report.

Published studies show that bone marrow biopsy is typically normal in the first days, and early treatment is critical for patient survival. HLH is conventionally treated with etoposide, rituximab, ruxolitinib, or emapalumab, and hematopoietic stem cell transplantation may be conducted in patients refractory to treatment. Because first-line therapies were unavailable and the patient’s condition was worsening, pulse methylprednisolone therapy was administered. Although not a first-line therapy, the use of corticosteroids and the treatment of underlying infections allowed the control of clinical and laboratory manifestations. After treating the hyperinflammatory state, the fever completely resolved, the dyspnea improved, the patient no longer required oxygen therapy, and laboratory tests were normalized. After 4 weeks, there was complete regression of HLH, with improvement of the patient’s general condition, which allowed her to be discharged from hospital for outpatient follow-up.

Conclusion

In this case report, peripheral blood culture identified hemophagocytic syndrome through neutrophilic and mononuclear phagocytes, preceding the presence of hemophagocytosis in the bone marrow. Performing peripheral blood culture may help in cases where a diagnosis of hemophagocytic syndrome is suspected but bone marrow biopsy is normal in the first days.
References


No conflicts of interest declared concerning the publication of this article.

Corresponding author:
Wilma Carvalho Neves Forte
E-mail: wilmanevesforte@yahoo.com.br