Síndrome Hemofagocítica Associada a Leishmaniose Visceral: Um Caso Clínico

Visceral Leshmaniasis Associated Hemophagocytic Syndrome: A Case Report

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Resumo:

A síndrome hemofagocítica é uma condição rara em que um estado hiperinflamatório grave pode resultar em falência multiorgânica e por em risco a vida. Pode ser genético ou adquirido na sequência de infeções, neoplasias malignas, doenças autoimunes ou imunodeficiências. Os autores descrevem o caso de um jovem de 19 anos de idade que recorreu ao Serviço de Urgência por um *sinus pilonidalis* sacrococcígeo infetado, tendo-se verificado, além de febre persistente, pancitopenia. Após melhoria inicial sob antibioterapia empírica, verificou-se novo agravamento clínico relacionado com pancitopenia grave e esplenomegália maciça. A investigação adicional mostrou elevada probabilidade de síndrome hemofagocítica e, perante elevada suspeição de leishmaniose visceral, apesar da ausência de resultados microbiológicos, foi iniciada terapêutica com anfotericina B. No entanto, o quadro evoluiu com discrasia hemorrágica com necessidade de internamento em Unidade de Cuidados Intensivos e realização de esplenectomia. O estudo anatomopatológico do baço confirmou o diagnóstico de leishmaniose visceral.

Palavras-chave: Anfotericina B/uso terapêutico; Leishmaniose Visceral/diagnóstico; Leishmaniose Visceral/tratamento farmacológico; Síndrome Hemofagocítica/diagnóstico; Síndrome Hemofagocítica/tratamento farmacológico.

Abstract:

Hemophagocytic syndrome is a rare severe systemic hyperinflammatory state which results in multiorgan failure, being potentially life threatening. It can be genetic or acquired in the setting of infections, malignancies, autoimmune disorders and immunodeficiency. The authors describe a case of a 19-year-old male admitted to the Emergency Department due to an infected sacrococcygeal pilonidal sinus with pancytopenia in addition to persistent fever. After an apparent initial improvement, there was a clinical deterioration related to severe pancytopenia and massive splenomegaly. The investigation showed high probability of hemophagocytic syndrome and there was high clinical suspicion of visceral leishmaniasis infection. Despite the absence of microorganism tests results, amphotericin B was empirically started. However, hemorrhagic dyscrasia occurred, requiring hospitalization in the intensive care unit and splenectomy, with full recovery. Anatomopathological study of the spleen confirmed visceral leishmaniasis diagnosis.

Keywords: *Amphotericin B/therapeutic use; Leishmaniasis, Visceral/diagnosis; Leishmaniasis, Visceral/drug therapy; Lymphohistiocytosis, Hemophagocytic/diagnosis; Lymphohistiocytosis, Hemophagocytic/drug therapy.*

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Introduction

Hemophagocytic syndrome (HS), or hemophagocytic lymphohistiocytosis (HLH) is a rare and potentially life-threatening condition, characterized by overstimulation of immune system with uncontrolled hypercytokinemia.[1] It can be genetic (primary), when an underlying genetic disorder causes abnormalities of the cytotoxic function of T and natural killer (NK) cells, or acquired (secondary) in the setting of an associated condition causing immune dysregulation, such as infections, malignancies, autoimmune disorders and iatrogenic or acquired immunodeficiency. The genetic form typically manifests in children, while acquired forms tend to occur in older ages, although it can affect any age bracket.[1-4] The cardinal features include fever, cytopenias, hepatosplenomegaly and hemophagocytosis by morphologically benign macrophages.[2,3] The early institution of therapy is critical to control the hypercytokinemia that otherwise will result in multi-organ failure and death.[2]

Leishmaniasis is a parasitic infection, transmitted mainly by sand fly vectors. Although many infections are asymptomatic, they can present as cutaneous, mucosal or visceral disease. Visceral leishmaniasis (VL), also known as *kala-azar*, reflects replication of parasites throughout reticuloendothelial system. It is usually caused by *Leishmania donovani* – endemic in South Asia and East Africa - and *Leishmania infantum*, which causes VL mainly in the Mediterranean basin.[5] The clinical picture resembles that of HS, with anemia, fever, weight

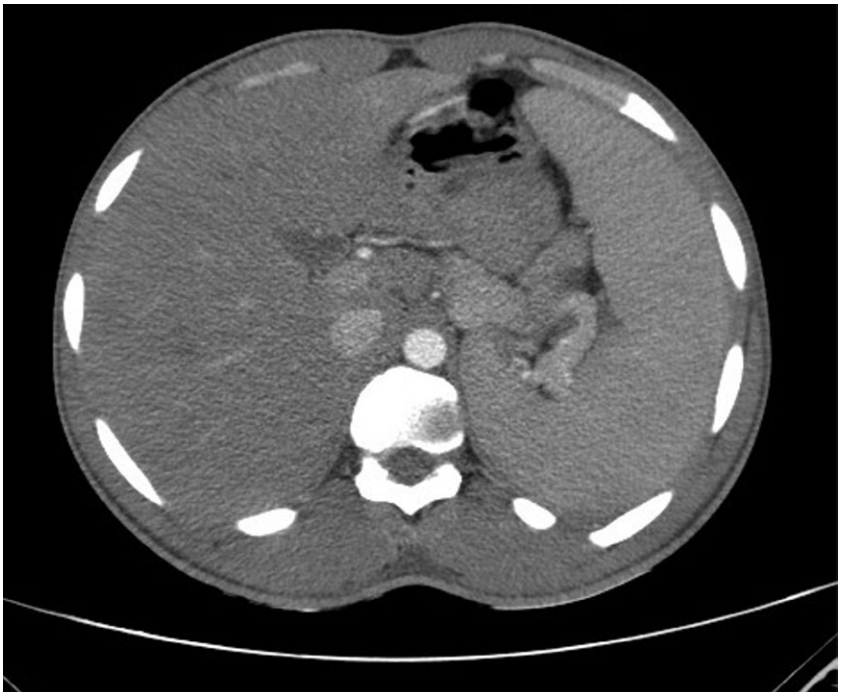

Figure 1: CT scan showing marked splenomegaly (19.6 x 19.5 x 7.6 cm).

loss and hepatosplenomegaly being the most common clinical features.[6] Risk factors include imunossupression and poor domestic sanitary conditions.[5,6] Lipossomal amphotericin B has been recommended as first line therapy.[5] Left untreated, the mortality rate can exceed 95%.[6]

Case Report

A 19-year-old black male from Gambia, living in Portugal for 6 years, was admitted to Emergency Department with lower back pain, suppurative swelling in the sacrococcygeal region for two weeks, fever, chills, profuse sweating, asthenia, adynamia and unquantified weight loss. He had type 1 diabetes mellitus with poor metabolic control. Due to work reasons, he occasionally spent the night in a rural place without proper sanitary conditions. Objectively, he was febrile (39ºC), presenting an infected sacrococcygeal *pilonidal sinus*. The spleen appeared slightly enlarged and an ultrasound confirmed moderate hepatosplenomegaly. The initial laboratorial study noticed the presence of mild pancytopenia (hemoglobin 11.6 g/dL, leukocytes 3900/µL and platelets 127000/µL), hyponatremia (126 mEq/L) and elevation of transaminases (AST 127 U/L; ALT 70 U/L) and LDH (636 U/L) level. So, ceftriaxone and metronidazole were started, and the patient was admitted to the internal medicine ward.

After an initial improvement, with sustained apyrexia and improved anemia, platelet count and transaminases normalization, there was recrudescence of constitutional symptoms, followed by worsening pancytopenia (hemoglobin 9.81 g/dL, leukopenia 1600/µL, with neutrophils 630/µL, and platelets 105000/µL) and increased splenomegaly (19.6 x 19.5 x 7.6 cm) (Fig. 1). High ferritin values (27071 ng/mL), elevated D--dimers (5317 ng/mL), ascending transaminases (up to AST 226 U/L and AST 285U/L), hypertriglyceridemia (263 mg/dL) and low HDL-cholesterol level (5 mg/dL) were also found. Fibrinogen level was within normal range (3.5 g/L). Peripheral blood smear was compatible with increased erythropoiesis and protozoan research was negative. Blood cultures and viral serologies were requested. Febrile neutropenia was assumed and piperacillin/tazobactam was started.

The diagnostic hypothesis of HS was considered, and, in addition to viruses - the most frequent agents - in this clinical context, leishmaniasis was also hypothesized. However, bone marrow aspiration showed no signs of hemophagocytosis or bone marrow infiltration, and direct examination was negative for the presence of *Leishmania*. Myeloculture was not performed due to lack of specific growth medium in our hospital at the time. Still, the Hscore for the diagnosis of reactive HS7 was used with 218 points scored, corresponding to 96% probability of this syndrome even without bone marrow evidence of hemophagocytosis. Other consistent findings included hyponatremia and decreased HDL-cholesterol level.[4,8]

Only Epstein Barr IgM antibodies title was found positive, however, viral load was negative, excluding this infection as the etiology of HS. Serial blood culture tests were all negative and the patient demonstrated worsening hepatosplenomegaly and pancytopenia, despite antibiotic treatment.

On the nineteenth day of hospitalization, a peripheral blood smear revealed blasts. A new bone marrow aspiration was performed, and it showed 14% blasts of non-erythroid series, raising the suspicion of hematological neoplasm. The

Table 1: Microbiological findings for the study of hemophagocytic syndrome trigger.

HIV HBV	Negative Imune
HCV	Negative
HAV	Negative
CMV	Past infection
HVS 1/2	Past infection
HZV	Past infection
EBV	IgM positive, IgG negative PCR positive Viral load negative
Syphilis	Negative
Rickettsia conorii	Likely cross reaction
Coxiella brunetti	Negative
Brucella spp.	Likely cross reaction
Plasmodium spp.	Negative
Leishmania spp.	Bone marrow - absent IIFR – positive PCR - negative
Blood cultures	Negatives
Myeloculture	Not permormed

Microbiological findings showing positive IIFR for *Leishmania* spp.

case was discussed with Hematology: bone marrow aspiration and bone biopsy were repeated revealing the presence of CD34 blasts (<5%), with megakaryocytic series showing increased numbers, most of them dysmorphic, although excluding acute leukemia.

Meanwhile, the diagnostic workup was maintained (Table 1), including serologies for *Borrelia, Leptospira, Coxiella burnetti* and *Rickettsia*. Serological techniques of indirect immunofluorescence reaction (IIFR) and polymerase chain reaction (PCR) for the detection of Leishmania were also requested. While waiting for blood sample results, the case was once again discussed with Hematology and, on the twenty--second day, corticosteroid therapy (prednisolone 1 mg/kg/day) was started. It was suspended 4 days later, due to worsening pancytopenia requiring transfusion support.

The clinical team responsible for the patient maintained a high clinical suspicion of leishmaniasis, so therapy with liposomal amphotericin B (4 mg/kg/day) was started - 1 dose per day for 5 days and then 1 dose on days 10, 17, 24, 31 and 38. The ongoing complementary tests showed negative peripheral blood PCR technique, but showed positive immunofluorescence reaction for *Leishmania spp*.

After five days of therapy with amphotericin B, unfortunately, because of major hemorrhagic dyscrasia with severe thrombocytopenia, unresponsive to transfusions, the patient was admitted to the Intensive Care Unit and submitted to splenectomy. Anatomopathological study of the spleen confirmed the diagnosis of VL, with no evidence of hemophagocytosis (Fig. 2).

The patient recovered and was transferred to an intermediate care unit, then to an internal medicine ward and was discharged after completing amphotericin B therapy cycle, with significant clinical and laboratory improvement. The patient-maintained follow-up consultations of Internal Medicine-Diabetes, with no recrudescence of symptoms or skin changes suggestive of post kala-azar dermal leishmaniasis.[6]

Discussion

HS is a potentially life-threatening systemic hyperinflammatory state, with no race or sex predilection. In adults, secondary or reactive forms of HS are probably much more frequent than primary form and can be associated with numerous conditions.[1-3] In 2014, a French study revealed that malignancies were the condition most frequently associated

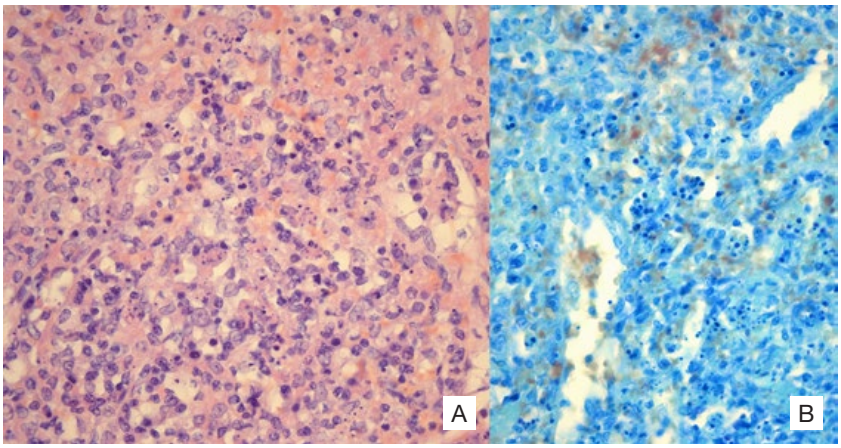

Figure 2: **A.** Spleen parenchyma and sinusoids showing histiocytic infiltrate. **B.** Giemsa staining showing microorganismos compatible with Leishmania in histiocytes.

with HS, followed by infectious diseases, with a prevalence of approximately 60% and 25% respectively.[9] Among the infectious agents, viruses stand out, particularly Epstein-Barr virus.[1,2,4] A literature review found that non-viral infections account for 20% of all the causes and protozoan infection, including *Leishmania spp.*, as an important cause in endemic countries.[10] Typical features include fever, hepatosplenomegaly and cytopenias. Other frequent findings are hypertriglyceridemia, decreased HDL-cholesterol, hypofibrinogenemia, liver dysfunction, elevated levels of ferritin, hyponatremia, low NK cell activity and elevated soluble interleukin-2 receptor levels (sCD25).[8] Histopathologic findings include macrophage and lymphocyte infiltrates, especially in bone marrow, which occasionally exhibit hemophagocytosis.[2] In fact, cytologic or histologic hemophagocytic picture can be often initially absent and it is not a sensitive or specific finding for the diagnosis of HS,[3,11] although some studies found it in up to 70% of secondary HS cases.[9,12] Henter *et al* developed the HLH-2004 criteria in which the presence of five out of eight criteria confirms the diagnosis.[8] However, this score was developed for pediatric population, lacking validation in adults, and requiring NK cell activity and sCD25 assay that are not universally available in hospital centers. The HScore, published by Farder *et al* in 2014 (Table 2), was the first validated score for the diagnosis of reactive HS based on the largest available data set of adult patients. It is considered a highly sensitive and specific diagnostic tool which assesses the probability of reactive HLH through the calculation of a sum score of nine variables.[7]

According to the HLH-2004 protocol, treatment of HS consists primarily in immunosuppression with dexamethasone, etoposide and cyclosporine.[8,12] However, HLH-2004 has been developed specifically for children and the use of intense immunosuppression without specific antimicrobial therapy administration can be associated with severe adverse outcomes in infection associated-HLH.[11,13] The treatment of secondary HLH is dependent on its cause, so it is imperative to search for and treat underlying triggers.[10,14] Therefore, when caused by an acute infection, appropriate antimicrobial therapy becomes the cornerstone of infection associated-HLH management.[1,14]

In this particular case, the presence of pancytopenia, hepatosplenomegaly and an extremely high value of ferritin raised the suspicion of HS, even in the absence of hemophagocytosis in bone marrow biopsy. HScore was applied resulting in 96% probability of reactive HLH, which was corroborated by HLH-2004 criteria (fever, splenomegaly, pancytopenia, hypertriglyceridemia ≥265 mg/dL and ferritin level ≥ 500 ≥g/L).[8] It is worth noting that NK cell activity and sCD25 level measurements are unavailable at our hospital. On the other hand, the results would probably not be available in useful time and would not change our approach, so they were not requested. With respect to the *trigger* condition, despite being negative in direct observation of blood and bone marrow samples and limitations associated with the lack of access to diagnostic means, VL was the most likely hypothesis due to the suggestive clinical picture and the epidemiological context of the patient. Therefore, after the unfavorable evolution with corticosteroid therapy, amphotericin B was empirically started, and the diagnosis of VL was confirmed after an emergent splenectomy. Supportive care and completion of the amphotericin regimen allowed probable cure of the patient.

Table 2: H-Score for Reactive Hemophagocytic Syndrome.

Know underlying immunodepression (HIV, corticosteroid therapy)	18
Maximal temperature (ºC)	
< 38.4	0
38.4 – 39.4	33
≥ 39.5	49
Organomegaly	
None	0
Hepato- or splenomegaly	23
Hepato- and splenomegaly	38
Cytopenias (Hb ≤ 9.2 g/dL; Leukocytes ≤ 5.000/µL; platelets ≤ 110.000/µL)	
1 bloodline	0
2 bloodlines	24
3 bloodlines	34
Ferritin (ng/mL)	
< 2.000	0
2.000-6.000	35
>6.000	59
Triglycerides (mg/dL)	
< 133	0
133-354	44
>354	54
Fibrinogen (g/L)	
> 2.5	0
≤ 2.5	30
AST (UI/L)	
< 30	0
≥ 30	19
Hemophagocytosis features on bone marrow aspirate	35

Adapted from: Fardet L, et al. Development and validation of the HScore, a score for the diagnosis of reactive hemophagocytic syndrome. Arthritis Rheumatol. 2014;66:2613-20.[7]

Conclusion

Because of overlapping features, VL in the context of an HS can be missed.[11] Despite VL-associated HLH being a rare condition, it should be considered in patients from endemic areas and must be excluded before immunosuppression is considered. Importantly, although many cases of infection-related HS should be treated aggressively with standard HLH protocols, in special EBV, HLH triggered by HIV infection,

tuberculosis, malaria, leishmaniasis, among others, can be exceptions.[2,4,5,10] In this case, specific treatment with lipossomal amphotericin B, along with supportive care, reverted Leishmania-associated reactive HS, resulting in cure.[10,11,13,15] ■

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FSS, DB, MLM, HBV – Redacção e aprovação da versão final
ED – Revisão teórica e aprovação da versão final
SM - Revisão e aprovação da versão final
Todos os autores aprovaram a versão final

Contributorship Statement

FSS, DB, MLM, HBV – Drafting and approval of the final version
ED – Theoretical review and approval of the final version
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Responsabilidades Éticas

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