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CASO CLÍNICO / *CLINICAL CASE*

Linfohistiocistose hemofagocítica, infeção por *Leishmania* e *Epstein-Barr:* uma associação incomum

Hemophagocytic lymphohistiocytosis, Leishmania *and* Epstein-Barr *infection: an uncommon association*

/ F. Costa[1] / A. Miranda[1] / A. Figueiredo[1] / P. Correia[1]

[1] Departamento de Pediatria, Hospital Professor Doutor Fernando Fonseca, E.P.E.

Correspondência:
Francisca Costa.
IC19, 2720-276 Amadora, Portugal
Telefone: (+351) 916917366
E-mail: francisca88costa@gmail.com

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/ Resumo

Introdução: O Síndrome Hemofagocítico (SH) caracteriza-se pela ativação imune patológica, com sinais e sintomas de inflamação excessiva.

Relato de caso: Criança de 18 meses, internada por febre persistente com 2 meses de evolução. Negava sintomas associados. Contexto epidemiológico de viagem para quinta no Norte, com contacto com cães. Ao exame objetivo: ar doente, palidez e hepatoesplenomegália. Analiticamente, a pancitopenia com agravamento progressivo associada a hiperferritinemia (828 ng/mL), hipertrigliceridemia (490 mg/dL) e elevação do recetor solúvel da interleucina-2 (11045 u/ml), permitiu o diagnóstico de SH. Da investigação: serologia para EBV IgM EBV-VCA positivo (99U/mL) com IgG EBV-VCA (<20U/mL) e EBNA (<3U/mL) negativos; PCR EBV no sangue periférico negativa; serologia positiva (por imunofluorescência e imunoblot) para *Leishmania* spp no sangue periférico. No mielograma sem hemofagocitose e PCR para *Leishmania* e EBV negativas. Iniciou anfotericina B lipossómica com melhoria.

Conclusão: A serologia, apesar de não ser o *gold-standard* para o diagnóstico, e a revisão da história epidemiológica contribuíram para um desfecho favorável.

Palavras-chave: febre de origem desconhecida; linfohistiocistose hemofagocítica; *Leishmaniose*

/ Abstract

Introduction: *Hemophagocytic lymphohistiocytosis (HLH) is a syndrome of abnormal excessive immune activation, with signs and symptoms of excessive inflammation.*

Case report: *18-months-old girl admitted for persistent fever for the past two months. No other symptoms associated. History of a trip to the north of Portugal with contact with dogs. On examination: ill-appearance, pale and hepatosplenomegaly. Laboratory findings revealed pancytopenia progressively worst, associated with elevated ferritin (828ng/mL), hypertriglyceridemia (490 mg/dL), elevated soluble interleukin-2-receptor (11045 U/mL), which lead to the diagnosis of HLH. Etiologic investigation: IgM VCA-EBV positive (99U/mL), IgG VCA-EBV negative (<20U/mL), IgG EBNA negative (<3U/mL) and PCR for EBV negative; immunofluorescence and immunoblot serologies for* **Leishmania** *spp. were positive. Bone marrow examination did not reveal hemophagocytosis and PCR for* **Leishmania** *and EBV was negative. Treatment was initiated with lipossomic amphotericin with progressive improvement of the clinical condition.*

Discussion: *Although serology for* **Leishmania** *is not the gold standard for diagnosis, it's positivity in this case and the epidemiologic history lead to a favourable outcome.*

Keywords: *fever of unknown origin; hemophagocytic lymphohistiocytosis; leishmaniasis*

/ Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a syndrome of excessive immune activation.[1] It most frequently affects infants aged less than 18 months and usually presents as a febrile disease with multiple organ involvement.[2]

HLH has been linked to a wide variety of diseases, namely infections, malignancies and genetic disorders. Infections, in particular viral infections, are a common trigger both in those with a genetic predisposition and in sporadic cases.[2] The association of HLH and visceral leishmaniasis is well established although its prevalence is unknown. It is thought to be a rare event, but some series have shown higher rates.[1] Some authors argued that *Leishmania* was the second infectious trigger after *Epstein–Barr virus*.[1]

There is no consensus on diagnostic criteria to HLH.[3] Some authors recommend that it can be based upon the molecular identification of an HLH-associated gene mutation or the identification of at least five of the clinical-laboratory criteria (Table 1 resumes the clinical-laboratory criteria).[2]

/ Case report

An 18-month-old girl, previously healthy, with irrelevant family history, was hospitalized due to relapsing fever for the past two months described as four days of fever, high temperature of 39ºC and 4 hours of apyrexia in the first day followed by 8 hours of apyrexia in the next 3 days, alternating with three days of apyrexia. She had no other symptoms associated, namely rash, abdominal pain, arthralgia, mouth ulcers, nocturnal hyperhidrosis or weight loss. On the physical examination, she appeared ill, pale and had a hepatomegaly and splenomegaly 3 centimeters and 4 centimeters bellow the costal margin, respectively. The epidemiologic history was remarkable for a trip to the north of Portugal 2 months previously to the hospitalization where she stayed in a farm in the countryside and interacted with dogs. The patient was referred to our hospital on the day of admission (end of February of 2016) by her pediatrician who noticed hepatosplenomegaly and a pancytopenia as well as a EBV serologies 1st week of February: IgM antibody against the viral capsid antigen (VCA) 4 U/mL (negative reference value <20 U/mL); IgG VCA antibody 7 U/mL (negative reference value <20 U/mL).

Further investigation, while hospitalized, showed: normochromic normocytic anemia (Hg 8,9g/dL; erythrocytes 3,41x10^12/L; hematocrit 26,1%, VGM 76,5fL; HGM 26,1pg; CHGM 34,1g/dL; RDW 15,9%) with elevated reticulocyte count, negative Coombs test, hemoglobin and protein electrophoresis and glucose-6-phosphate-dehydrogenase all normal. Thoracic radiography was considered normal. Abdominal ultrasound revealed moderate hepatic and splenomegaly structurally

TABLE 1 – HLH CLINICAL-LABORATORY CRITERIA; 5 OF 8 MUST BE PRESENT OR A MUTATION DIAGNOSED	
Fever ≥ 38.5ºC	
Splenomegaly	
Peripheral blood cytopenia (at least two)	Haemoglobin <9 g/dL, platelets <100.000/microL; absolute neutrophil count <1000/microL
Hypertriglyceridemia or	Fasting triglycerides >265 mg/dL
Hypofibrinogenemia	Fibrinogen <150 mg/dL
Hemophagocytosis in bone marrow, spleen, lymph node or liver	
Low or absent NK cell activity	
Hyperferritinemia	Ferritin >500ng/mL
Elevated soluble CD25 (soluble IL-2 receptor alpha)	Soluble IL-2 receptor alpha) two standard deviations above age-adjusted laboratory –specific norms

homogeneous. Transthoracic echocardiogram showed pericardic transudate of 2-3 centimeters. Lumbar puncture was performed showing a normal CSF examination. Interferon gamma release assay (IGRA) was negative. She maintained a pancytopenia (hemoglobin 9,6 g/dL; leucocytes 2600/ mm^3; neutrophils 600/ mm^3; lymphocytes 1800/ mm^3; platelets 63000/uL) associated with elevated ferritin (maximum of 828ng/mL), hypertriglyceridemia (maximum of 490 mg/dL), elevated soluble interleukin-2-receptor (11045 U/mL), elevated d-dimers (maximum 5705 ug/L) and hypoalbuminemia (minimum 2,85g/dL), which lead to the diagnosis of HLH.

The investigation of secondary causes of HLH revealed: positive EBV IgM VCA antibody (99U/mL; negative reference value <20 U/mL), negative EBV IgG VCA antibody (<10U/mL, negative reference value of <20U/mL), negative *Epstein Barr* nuclear antigen 1 (EBNA) (<3U/mL, negative reference value <5U/mL) performed on the 24th February 2016 and negative peripheral blood polymerase chain reaction (PCR) for EBV a week after the previous serologies; positive anti-*Cytomegalovirus* (CMV) IgM antibody (16,6 U/mL), negative anti-CMV IgG antibody and urine PCR for CMV negative; *Human Immunodeficiency Virus, Adenovirus, Toxoplasmosis, Parvovirus, Mycoplasma pneumonia, Bartonella and Herpes simplex* 1 and 2 serologies all negative. Immunofluorescence and immunoblot serologies for *Leishmania* spp. were positive. The urine and blood cultures were negative. The bone marrow was difficult to collect and the sample was small. The direct examination of bone marrow aspiration did not reveal hemophagocytosis, *Leishmania* amastigotes or signs of malignancy. Bone marrow cultures were negative as well as PCR for *Leishmania*, *Mycobacteria tuberculosis* and EBV. The Novy-MacNeal-Nicolle culture for *Leishmania* was not performed.

Investigation regarding auto-inflammatory syndromes and immunodeficiency was normal: immunoglobulins A 50,5 mg/dL, G 637 mg/dL, M 67,1 mg/dL, D 1,29 mg/dL; complement C4 48,5 mg/dL, complement C3 209 mg/dL, total complement activity CH50 68 U/mL, complement C1q 19,1mg/dL; lymphocyte immunophenotyping – total T lymphocytes 2646 cells/ul (79%), T helper lymphocytes 714 cells/ul (23%), T suppressors lymphocytes 1504 cells/ul (49%), total B lymphocytes 548 cells/ul (15%), Natural Killer (NK) lymphocytes 137 cells/ul (4%), total lymphocytes 3363 cells/ul; negative antinuclear antibody titter; negative anti-double stranded DNA.

Clinical presentation, epidemiologic data and positive serologies for *Leishmania* suggested visceral leishmaniasis. Treatment was initiated on the 16th day of hospitalization with liposomal amphotericin (4 mg/kg) given intravenously for 5 days and then repeated a single dose at the 10th day of treatment. The patient became afebrile three days after initiating treatment and gradually recovered from anemia, pancytopenia and hepatic and splenomegaly. (Table 2 resumes analytical data: before and after treatment.) Follow up of EBV serologies 5 months (11th July 2016) after revealed: negative IgM VCA antibody (<10 U/mL, negative reference value <20U/mL), positive IgG VCA antibody (338 U/mL, positive reference value ≥20 U/mL) and positive EBNA (102 U/mL, positive reference value ≥20 U/mL), confirming co-infection with EBV. The patient remains asymptomatic almost two years after treatment.

/ Discussion

Portugal, as other Mediterranean countries, has a high incidence of visceral leishmaniasis, particularly in the Douro valley region where it accounts for 8,3 cases/100.000 per habitant per year. In the period between 2000 and 2009, 173 new cases of visceral leishmaniasis were diagnosed, 46 of those in children.[3] According with the last national report of compulsory notifiable diseases,

TABLE 2 – LABORATORY DATA OF THE PATIENT BEFORE AND AFTER INITIATING TREATMENT			
	BEFORE	14 DAYS AFTER	4 MONTHS AFTER
Haemoglobin (g/dL)	9,6	11,1	12
Leukocytes/mm^3	2600	6900	6800
Neutrophils/mm^3	600	1700	2700
Platelets/uL	63000	332000	329000
Fibrinogen (g/L)	2,9	-	-
Erythrocyte sedimentation rate (mm/h)	33	-	10
AST (U/L)	70	43	25
ALT (U/L)	46	26	18
Albumin (g/dL)	2,92		4,45
Triglycerides (mg/dL)	490	119	51
Ferritin (ng/mL)	828	73	33
IgG (mg/dL)	637	-	500
IgA (mg/dL)	50,5	-	28,5
IgM (mg/dL)	67,1	-	55,3

AST: aspartate aminotransferase; ALT: alanine aminotransferase; Ig: immunoglobulin

there were 38 cases of visceral leishmaniasis in Portugal between 2012 and 2015 the majority of them between 35 and 54 years of age.[4] Moreover, canine leishmaniasis has increased with a prevalence rate up to 20% found in endemic foci.[3] HLH, on the opposite hand, is a rare disease, difficult to diagnose and therefore hard to estimate the true incidence. A recent review, estimates an incidence of HLH in children of 1 in 100 000, the autosomal recessive forms of familial HLH representing 1 in 50 000 live births.[5]

Clinical features of visceral leishmaniasis and HLH are very similar and occasionally it is hard to distinguish one from another. A recent systematic review studied the association between leishmaniasis and HLH reporting 56 cases till 2007, predominantly in children.[6] Fever (100%), splenomegaly (98%), hepatomegaly (80%) and pancytopenia (82%) were described as overlapping findings of HLH and visceral leishmaniasis.[6] Other laboratory findings can be misleading too. Diagnostic criteria of HLH include bicytopenia, hypertriglyceridemia and/or hypofibrinogenemia, low or absent NK levels, increased ferritin and soluble interleukin-2-receptor.[2] A prospective study from 2013 reports similar laboratory abnormalities in patients with visceral leishmaniasis. Nevertheless, cytokine analysis revealed undetectable levels of soluble interleukin-2-receptor and interferon-gama in most patients, only 2 out of 27 subjects had detectable levels of soluble interleukin-2-receptor, which was related with the presence of severe disease.[7] Definitive diagnosis of visceral leishmaniasis can be difficult. *Bode et al*, in a retrospective study, reported false negative results for identification of *Leishmania* in 6/13 subjects in the initial bone marrow aspirate, 1/12 in serology, 2/5 in bone marrow culture and 1/3 PCR results of peripheral blood. Unlike our patient, all bone marrow PCR tested positive for *Leishmania*.[8] Negative results for PCR in bone marrow in our patient could be explained by the small sample. Similar findings were obtained in a series of 12 cases of HLH and visceral leishmaniasis. Remarkably in this study, 8 patients that initially did not have the parasite at direct examination of the bone marrow smear, it was then repeated and there was a positive identification in 4 of them after 1 to 4 months.[9] More bone marrow smears could have been useful in the case presented.

Although, the gold standard for diagnosing visceral leishmaniasis includes identification of the parasite in splenic or bone marrow aspirate or positive PCR for *Leishmania*, immunodiagnostic tests can be useful instruments of diagnosis allied with suggestive clinical features in the appropriate epidemiological context.

Interpretation of EBV serologies suggests that the patient was EBV naïve when the symptoms first started and then had an acute primary infection at the time of the HLH diagnosis.[10] EBV is a well-known infectious trigger of HLH and may have had its role in this case.[1] As far as we know, there are four cases of visceral leishmaniasis and EBV co-infection associated with HLH described

in literature. Three of the four cases are in children (9-month-old, 19-month-old and 22-month-old), two of them with a compatible serology of acute EBV infection and positive viral load and the third one with only a compatible serology of acute EBV infection. The forth case was a 27-year-old with a high EBV viral load of 13,559 copies/mL in serum and *Leishman-Donovan* bodies identified in bone marrow.[11-14] *Koliou et al* suggested that the transient immunosuppression characteristic of the acute phase of EBV infection may interfere with the T-helper 1 immune response for the clearing of phagocytosed *Leishmania* parasites and therefore increase the susceptibility to *Leishmania.*[11]

Initial presentation of the case reported did not suggest primary HLH, therefore the authors considered a secondary cause for HLH. Clinical improvement after liposomal amphotericin B was in favor of leishmaniasis as the trigger of HLH. The treatment of HLH triggered by EBV contemplates addition of rituximab to the HLH treatment.[15] In our case we decided for not treating EBV infection since viral load was not detected. Of the four cases mentioned above only one received treatment with steroids and etoposide additionally to liposomal amphotericin B and none received rituximab. As in our case, all the three patients were successfully treated with liposomal amphotericin B, with reduction of EBV viral load, without initiating HLH treatment.

In the presented case, the treatment option was based in the underlying cause of HLH. Other treatment options should be considered in cases of clinical deterioration despite anti-infective guided therapy. *Bode et al*, highlights that although immunosuppressive therapy might have a good clinical response for a period of time, none of the patients in his series achieved long-term remission without visceral leishmaniasis directed treatment.[8]

In summary, we highlight this case for the diagnostic challenge: atypical pattern of fever associated with the absence of hypergamaglobulinemia and amastigotes in the bone marrow smear. The travel history and positive serology for *Leishmania* were decisive contributors to the diagnosis. Co-infection with EBV is infrequent and possibly led to an increased susceptibility to *Leishmania,* which presented in an unusual manner.

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