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Case Report

Hemophagocytic lymphohistiocytosis and visceral leishmaniasis in children: case report and systematic review of literature

Maria Scalzone, Antonio Ruggiero, Stefano Mastrangelo, Giovanna Trombatore, Vita Ridola, Palma Maurizi, Riccardo Riccardi

Division of Paediatric Oncology, Catholic University of Rome, Rome, Italy

Abstract

Hemophagocytic lymphohistiocytosis is a potentially fatal disorder resulting from excessive activation and non-malignant proliferation of T lymphocytes and macrophages. Neoplasms, autoimmune disorders and systemic infections can cause secondary hemophagocytic syndrome. The association of hemophagocytic syndrome and visceral leishmaniasis is rarely found in childhood. We report a case of an infant affected by hemophagocytic lymphohistiocytosis secondary to visceral leishamniasis and describe all cases of hemophagocytic syndrome associated with visceral leishamniasis in childhood reported in literature, focusing on clinical manifestation, diagnosis and treatment.

Key words: Hemophagocytic lymphohistiocytosis; leishmania; childhood; liposomal amphotericin B.

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Introduction

Hemophagocytic lymphohistiocytosis (HLH), also known as hemophagocytic syndrome, is a clinical-pathological condition characterized by uncontrolled and non-malignant proliferation of macrophages and T lymphocytes with cytokine overproduction. HLH is classified into primary or secondary. Visceral leishamniasis (VL) is a systemic disease affecting the reticoloendothelial system, caused by protozoa of the genus *Leishmania*. HLH secondary to VL is very rare in childhood and the overlap in the clinical characteristics of VL and HLH can be a diagnostic challenge for physicians managing HLH.We describe the case of an infant presenting HLH secondary to VL. Moreover, all cases of hemophagocytic syndrome related to VL in childhood described in literature to date are reviewed.

Case report

An infant of 7,5 months of age was admitted to our Pediatric intensive care unit (PICU) of the Catholic University of Rome with suspected diagnosis of lymphoproliferative syndrome.

Upon admission to our hospital, the child showed poor general condition, pallor, tachypnea and hepatosplenomegaly. Initial hematological investigations revealed severe anemia (hemoglobin 3.3 g/dL) and thrombocytopenia (platelet count 11 × 10^9/L). The direct antiglobulin test was positive. Laboratory testing revealed hypertriglyceridemia (298 mg/dL), hyperferritinemia (4842 ng/mL), hypofibrinogenemia (25 mg/dL), hyoalbuminemia (2.2 g/dL) and elevated levels of lactate dehydrogenase (1200 UI/L). Massive splenomegaly and hepatomegaly were confirmed by abdominal sonography. Bone marrow aspiration (BMA) and biopsy were performed to detect morphological signs of hemophagocytosis.

Because of the presence of erythrophagocytosis to BMA, hyperferritinemia, pancytopenia, hypertriglyceridemia, hypofibrinogenemia and splenomegaly, and HLH diagnosis were performed. Genetic investigation was also performed and found to be negative.

According to the European HLH-2004 protocol, treatment with corticosteroids and etoposide was started. The child's general conditions improved progressively and after ten days the patient was transferred from PICU to our Division.

Treatment with corticosteroids and etoposide (a total of 9 doses) was administered, but elevated ferritinemia and severe hepatosplenomegaly persisted. In addition, the patient received intravenous immunoglobulins (IVIG).

Serological tests for Epstein-Barr Virus (EBV), Cytomegalovirus (CMV), Hepatitis viruses, HIV and *Leishmania* were performed and proved to be

negative. A weak positivity of anti-smooth antibodies and Coombs' positivity were also detected. Serum immunogloblobulin (Ig) G and IgD were found to be elevated.

At 3.5 months after admission, because of deterioration of the overall conditions, a second BMA was performed which revealed some inclusive bodies inside the phagocytic cells resembling *Leishmania* amastigotes. Detection of *Leishmania* by real-time PCR in a bone marrow sample was also performed and found to be positive. Repeated serologic tests for leishmaniasis were negative.

The child then received intravenous liposomal amphotericin B (AmBisome), 3 mg/kg/day for 5 consecutive days followed by two additional doses given 1 week apart (total dose 21 mg/kg). No side effects related to treatment were detected.

Following administration of AmBisome, the infant's overall condition progressively improved, with a marked reduction of hepatosplenomegaly and the gradual resolution of cytopenia. Inflammatory markers and other laboratory parameters returned to normal limits, except for ferritinemia.

The child was discharged 4 months after admission; results of physical examination were normal except for a mild splenomegaly.

The patient was found to be well, at a follow-up examination performed 4 months after completion of the treatment. Serology for *Leishmania* was still negative. Hematological examination revealed a progressive decrease in ferritinemia and abdominal ultrasound that indicated complete regression of splenomegaly.

Discussion

HLH is a systemic disease characterized by the overwhelming activation of normal T lymphocytes and macrophages leading to clinical and hematologic alterations. This disorder clinically manifests as fever, hepatosplenomegaly and, less frequently, lymphadenopathy, jaundice and rash. Pancytopenia, elevated levels of serum ferritin and triglycerides, coagulopathy with hypofibrinogenemia, and abnormal liver enzymes are commonly present. The diagnosis of HLH is established when five out of eight criteria of HLH are met (Table 1). According to revised diagnostic guidelines for HLH, patients with a molecular diagnosis consistent with HLH do not need to fulfil diagnostic criteria [1].

The laboratory and clinical criteria of HLH were observed in our patient and diagnosis of HLH was established. Primary HLH is a hereditary disorder with autosomal recessive transmission that usually occurs during infancy. Secondary HLH occurs after strong immunological activation induced by malignancies, autoimmune diseases and systemic infections. Viral infections, especially by EBV, represent the most frequent cause of secondary HLH in children [2]. Less frequently, HLH can be secondary to bacterial, fungal and parasitic infections, including *Leishmania.*

VL is a systemic disease caused by parasites of the *Leishmania donovani* complex and transmitted through infected phlebotomine sandflies bites. Around half of VL cases occur in childhood. VL is spread worldwide and it is estimated that there are about 13 million infected people, with about 1.8 million new cases each year. This parasitic disease is endemic in more than 60 countries including North Africa, Central and South America, the Middle East, the Indian subcontinent and Southern Europe. The number of human VL cases in the western Mediterranean basin has increased over the last few decades [3-6].

Table 1. Diagnostic criteria of HLH

The diagnosis of HLH can be established if either 1 or 2 is fulfilled:	
1.	**A molecular diagnosis consistent with HLH**
2.	**Diagnostic criteria for HLH fulfilled (five out of eight criteria)**
	A. Initial diagnostic criteria (to be assessed in all patients with suspected HLH)
	- Fever
	- Splenomegaly
	- Pancytopenia (affecting ≥ 2 of 3 lineages in the peripheral blood with hemoglobin < 10 g/dL, platelets <100 × 10^9/L and neutrophils <1.0 × 10^9/L)
	- Hypertriglyceridemia (triglycerides > 265 mg/dL) and hypofibrinogenemia (fibrinogen ≤1,5 g/dL)
	- Hemophagocytosis in bone marrow or lymph nodes or spleen and no evidence of malignancy
	B. New diagnostic criteria
	- Ferritin ≥500 µg/dL
	- Low or absent NK cell activity (according to local laboratory)
	- Soluble CD25 (soluble interleukin-2 receptor) ≥ 2400 U/mL

HLH: Hemophagocytic lymphohistiocytosis ; NK: Natural killer.

Table 2. Cases of hemophagocytic syndrome related VL in childhood reported in literature to date.

References	N. pts	Sex	Age (years)	Country	Hepato/ splenomegaly	Fever	Pancytopenia	Diagnosis delay	Mode of diagnosis: BMA/ serology/other	Therapy	Outcome
Present case (2012)	1	M	7.5 months	Italy	+	+	+	3.5 months	2° BMA positive*/serology negative/ BM PCR positive	Cs +VP16+Ampho B	Alive
Kokkini *et al* (1984)	1	M	3	Greece	+	+	+	U	1° BMA positive/ serology positive	U	Alive
Sukovà *et al* (2002)	1	M	7	Czech Republic	+	+	+	9 weeks	5° BMA positive*/serology positive	Cs + VP16 + IVIG + Ampho B	Alive
Marom *et al* (2001)	1	M	2	Israel	+	+	+	1 month	3° BMA positive*/ serology positive	Cs+Cyc A+IVIG + LAmb	Alive
Michel *et al* (1988)	1	F	1	France	+	+	+	U	BMA positive/serology positive	MA + Pe	Alive
Thabet *et al* (1999)	1	M	1.8	Tunisia	+	+	+	U	BMA negative/serology positive	S SGL	Alive
Sineidi *et al* (2002)	1	F	4	Oman	+	+	+	9 weeks	3° BMA positive*/serology not performed	Cs + VP16 +S SGCL + LAmB	Alive
Agarwal *et al* (2006)	1	M	6	India	+	+	+	2 weeks	BMA positive /serology positive	S SGL	Alive
Bogdan *et al* (2001)	1	M	1.25	Germany	+	+	+	5 months	2°BMA positive*/serology positive	Cs + LAmb	Alive
Sipahi *et al* (2005)	1	M	1.5	Turkey	+	+	+	Died in 12 h	Serology positive + post mortem spleen biopsy positive	None	Died
Billiau *et al* (2005)	1	M	4	Belgium	+	+	+	U	BMA and liver biopsy: positive for HLH/serology not performed	Cs + Cyc A+ IVIG+ Ampho B	Alive
Tunc *et al* (2001)	1	M	4	Turkey	+	+	+	3 months	3° BMA positive*/serology negative	IVIG+S SLG+LAmB	Died
Granert *et al* (1993)	1	M	1	Sweden	+	+	+	U	BMA negative/spleen asp review positive	S SCL	Alive
Ozyurek *et al* (2005)	1	M	4	Turkey	+	+	+	1.25 months	BMA positive/serology positive	LAmB	Alive
Nadrid *et al* (1996)	1	M	2	Tunisia	+	+	+	3 months	2°BMA positive*/2°serology positive*	S SGL	Alive
Bouguila *et al* (2010)	2	M	1.5	Tunisia	+	+	+	U	BMA positive/serology positive	MA+Cs+IVIG	Died
		M	1.8	Tunisia	+	+	+	U	BMA positive/serology positive	MA+ VP16+Cs	Alive
Kocak *et al* (2004)	1	M	1.5	India	+	+	+	1 week	BMA positive	LAmb	Alive
Minen *et al* (2007)	1	F	2.5	Italy	+	+	+	U	BMA positive/serology positive	LAmb	Alive

Table 2 (continued). Cases of hemophagocytic syndrome related VL in childhood reported in literature to date.

References	N. pts	Sex	Age (years)	Country	Hepato/ splenomegaly	Fever	Pancytopenia	Diagnosis delay	Mode of diagnosis: BMA/ serology/other	Therapy	Outcome
Gagnaire *et al* (2000)	12			France							
				France					BMA positive/ 2° serology positive*		
			1	North							
		F	1	Africa	+	+	+	9 days	BMA positive/serology negative	Cs+LAmb	Alive
		F	3	France	+	+	+	2 days	BMA positive/serology positive	Cs+LAmb	Alive
		M	3	North	+	+	+	1 days	2° BMA positive*/2° serology positive*	MA+Pe+splenectomy	Alive
		M	7 months	Africa	+	+	+	24 days		LAmb	Alive
		M		France	+	+	+	10 days	BMA negative/serology positive	MA+LAmb+Cs	Alive
		M	2.5	North	+	+	+	1.5 months	2°BMA positive*/2°serology positive*	Cs +VP16+Ampho B	Alive
		F	2	Africa	+	+	+	2.5 months		VP16+MA	Alive
		M	1	North	+	+	+	1 week	2°BMA positive*/serology negative	Cs+MA	Alive
		M	1	Africa	+	+	+	4.5 months	BMA negative/serology positive	Cs + VP16+MA	Alive
		F	4	North	+	+	+	1 week	2° BMA positive*/serology negative	MA	Alive
		F	9	Africa	+	+	+	6 days		MA	Alive
		F	1	Africa	+	+	+	2 days	BMA negative/serology positive	MA+Pe	Alive
				France					BMA negative/serology positive		
				France					BMA positive/serology positive		
Sotoca-Fernandez *et al* (2008)	1	F	1.3	Spain	+	+	+	U	BMA positive/serology positive	LAmB	Alive
Tapisiz *et al* (2007)	1	M	2	Turkey	+	+	+	U	BMA positive/serology not performed	LAmB	Alive
Celik *et al* (2007)	1	M	15	Turkey	+	+	+	U	BMA positive/serology positive	MA	Died
Koliou *et al* (2008)	1	F	9 months	Greece	+	+	+	25 days	BMA positive/serology positive/ BM PCR positive	LAmB	Alive
Ay *et al* (2012)	1	M	4.5 months	Turkey	+	+	+	U	BMA negative/serology positive	Os (for positivity for H1N1 virus) + IVIG + LAmB	Alive
Fathalla M (2007)	1	M	10 months	Oman	+	+	+	U	2°BMA positive*/serology not performed	Cs+ VP 16+ LAmB + S SCL	Alive
Prasad e*t al* (2009)	2	M	7	India	+	+	+	U	BMA positive/serology positive BMA positive + trilineage	Ampho B	Alive
		M	10	India	+	+	+	U	myelodysplasia/serology not performed	Ampho B	Alive
Guo X (2011)	4	U	U	China	+	+	+	U	BMA positive/serology positive		U
		U	U	China	+	+	+	U	BMA positive/serology positive	Cs+VP 16 +specific therapy	U
		U	U	China	+	+	+	U	BMA positive/serology positive		U
		U	U	China	+	+	+	U	BMA positive/serology positive		U
Pahwa *et al* (2004)	2	F	6 months	India	+	+	+	U	BMA positive/serology not performed	S SGL	Died
		M	10	India	+	+	+	U	BMA positive/serology not performed	S SGL	Died

VL: visceral leishmaniasis; Pts: Patients; M: Male; F: Female; BMA: Bone marrow aspiration; BM PCR: polymerase chain reaction performed on bone marrow; Ampho B: Amphotericin desoxycholate; LAmB: Liposomal amphotericin; S SGL: Sodium stibogluconate; MA: Meglumine antimoniate; Pe: Pentamide; Cyc A: Cyclosporine; IVIG: Intravenous immunoglobulines; Cs: Corticosteroids; VP16: Etoposide; Os: Oseltamivir; U: Unknown ; *: previous examinations negative

The main manifestations of VL include wasting, prolonged fever, weight loss, splenomegaly and pancytopenia. The broad clinical spectrum of VL and the overlap in the clinical features of VL and HLH may cause diagnostic delay in recognizing forms of HLH secondary to VL [7]. HLH related VL is very rare in childhood, with less than 50 cases reported in literature to date [3–7] (Table 2). In 2 cases, HLH was associated with virus (H1N1 and EBV) and *Leishmania* coinfection [8,9]. Mortality was observed in 6 children affected by HLH associated with VL and it was mostly secondary to infectious and hemorrhagic complications. The median age at onset was 2 years (range 4.5 months-15 years). Males accounted for about 70% cases of HLH secondary to VL. Clinical manifestations is characterized in all patients by fever, hepatosplenomegaly and pancytopenia, associated with high triglyceride levels, low plasma fibrinogen levels and elevated ferritinemia [10–12].

As in our patient, high levels of serum IgG and autoantibodies may be found in patients affected by HLH related to VL. These findings could arise from polyclonal B-lymphocyte activation secondary to this systemic disease [13].

At onset, as in our case, the first BMA often does not detect the presence of amastigote forms in the reticuloendothelial system because of the pauci-microbial nature of the disorder. In the case of strong clinical suspicion of HLH associated with VL, repeated BMAs are required.

Serology for *Leishmania* proves negative at diagnosis in about 20% of children with HLH secondary to VL who either seroconverted long after their recovery or remained antibody- negative. In our patient, serology for *Leishmania* was negative at onset and was still negative at 4 months after completion of treatment. The initial negativity of BMA and anti-*Leishmania* antibodies may contribute to delaying diagnosis and treatment. The median diagnosis delay was about 1 month in cases in which the latency time from onset to diagnosis was reported.

Spleen needle-aspiration biopsy has a sensitivity of approximately 98% but is associated with high risk of hemorrhage.

The use of PCR performed on peripheral blood and bone marrow samples can be helpful in the diagnosis of VL. PCR assay appears to have a higher sensitivity and specificity than the conventional diagnostic techniques. The use of PCR has been reported to be more effective than parasite culture or microscopy, mainly in samples with low parasite loads. This test can also provide species identification, which may be significant in the clinical management of VL considering the link between some *Leishmania* species and disease severity. Furthermore, this molecular diagnostic method may be helpful in monitoring disease progression and outcome of antileishmanial treatment by allowing host tissue quantification of parasites to be assessed. However, the use of molecular approaches proves more expensive than conventional diagnostic techniques and requires technological expertise; moreover, currently, there is no standardization of PCR-based protocols [14-15].

Identifying the type of HLH is essential in establishing the most effective treatment. In primary HLH, the treatment includes cytotoxic therapy and bone marrow transplantation. Specific therapy to treat the underlying disease is curative for patients with secondary HLH [16].

The pentavalent antimonial drugs, given intravenously or intramuscularly for 3-4 weeks, are the most commonly used drugs to treat VL but high toxicity and treatment failures have been observed [17]. Liposomal amphotericin B represents the most effective and safest drug. Furthermore, liposomal amphotericin B demands a shorter period of administration than antimonial drugs. Conventional amphotericin B deoxycholate is also curative but can lead to many side effects such as renal toxicity. Pentamidine was previously considered the second line drug in cases of pentavalent antimonial drug resistance, but certain toxicities (diabetes mellitus, gastrointestinal and cardiac side effects) and inferior cure rate to amphotericin B have been reported. Therapeutic response is evaluated clinically in terms of decrease in spleen size, resolution of fever and weight gain [18].

Corticosteroids can be associated with specific therapy, inhibiting cytokine expression and suppressing excessive immune response [19]. The role of intravenous immunoglobulin (IVIG) in the treatment of HLH associated with VL is unclear but if used early offers some chance of success. IVIG can act with multiple mechanisms, including neutralization of cytokines, alteration of cytokine synthesis, T cell and Fas/fas-ligand pathway modulation, down-regulation of C3 activation and deviation of complement deposition [20].

Our patient initially received treatment with corticosteroids and etoposide, according to the European HLH-2004 protocol, as well as IVIG, but no therapeutic response was observed.

Later, specific therapy with liposomal amphotericin B (3 mg/kg/day for 5 consecutive days, and 2 additional doses at day 14 and 21) was performed, leading to clinical response after the first few doses. No adverse events were observed.

Conclusion

HLH associated with VL is very rare but potentially life threatening in childhood. Due to their clinical similarities and the negativity of BMA and anti-*Leishmania* antibodies during early disease course, the diagnosis can be challenging. In case of clinical suspicion of HLH secondary to VL, especially in endemic areas, repeated BMAs are required. Considering the high sensitivity and sensibility of PCR analysis, this test should be performed when the diagnosis of VL is suspected. Liposomal amphotericin B is the most effective and safest drug. Specific treatment yields complete recovery.

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Corresponding author

Antonio Ruggiero, MD
Division of Paediatric Oncology
Catholic University
Largo A. Gemelli 8
00168 Rome - Italy
Phone: ++39-06-3058203
Fax: ++39-06-3052751
Email: ruggiero@rm.unicatt

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