Hemophagocytic Lymphohistiocytosis in Imported Pediatric Visceral Leishmaniasis in a Nonendemic Area

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Objectives To describe characteristics of visceral leishmaniasis-associated hemophagocytic lymphohistiocytosis (HLH) with focus on diagnostic clues and pitfalls, including the frequency of central nervous system (CNS) involvement, and to determine the efficacy of liposomal amphotericin B (L-AmB).

Study design We retrospectively analyzed clinical and laboratory features, diagnostic procedures, and treatment of 13 patients with HLH with imported visceral leishmaniasis, reported to the German HLH reference center (1999-2012).

Results The spectrum of presentations was indistinguishable from patients with hereditary HLH or with acquired HLH because of infections with other pathogens. In 8 patients, disease onset occurred before the age of 2 years, coinciding with the typical age of manifestation of primary HLH. Two patients had mild nonspecific CNS findings. Misleading antiviral IgM (n = 6) and autoantibodies (n = 2) led to inaccurate interpretation of the etiology of HLH, sometimes with inappropriate therapeutic consequences. False negative results for *Leishmania* were obtained by initial bone marrow microscopy in 6/13, serology in 1/12, bone marrow culture in 2/5, and polymerase chain reaction of peripheral blood in 1/3 patients, and all bone marrow samples tested were *Leishmania*-positive by polymerase chain reaction (n = 7). L-AmB was administered to 12 patients, 5 of whom had no prior HLH-directed immunosuppressive therapy; sodium stibogluconate was administered to 1 patient. Persistent remission was achieved in 11 cases. Two patients required repeated or prolonged L-AmB therapy.

Conclusions Awareness of diagnostic pitfalls may save patients from unnecessary toxic treatment. CNS involvement is rare. L-AmB shows efficacy in visceral leishmaniasis-associated HLH. *(J Pediatr 2014;165:147-53)*.

Leishmania are flagellated protozoa transmitted by sand flies. *Leishmania infantum*, endemic in the whole Mediterranean, can cause cutaneous, mucocutaneous, or visceral leishmaniasis, also known as kala azar. The latter is characterized by fever, hepatosplenomegaly, pancytopenia, and weight loss and is fatal, if untreated.[1] Hemophagocytic lymphohistiocytosis (HLH) is a rare, potentially lethal clinical syndrome of severe hyperinflammation occurring in a wide spectrum of conditions. Hereditary HLH has to be distinguished from the acquired form that can be caused by infections, malignancies, rheumatologic, or metabolic diseases.[2] Hallmarks of HLH are fever, hepatosplenomegaly, pancytopenia, and a characteristic set of abnormal laboratory test results including ferritin, soluble CD25 (also known as soluble interleukin-2 receptor), triglycerides, and fibrinogen. Hence, there is per se substantial overlap between visceral leishmaniasis and the syndrome of HLH. However, case reports and small series have reported courses of visceral leishmaniasis that not only overlapped with HLH but fulfilled all diagnostic criteria of HLH.[3,4] *Leishmania* parasites have been found to be the most common protozoan trigger of acquired HLH.[5,6] The clinical picture of visceral leishmaniasis with HLH initially can be indistinguishable from HLH of other etiology, potentially leading to misdiagnoses and inappropriate therapies. To further identify diagnostic clues and to delineate potential pitfalls, we analyzed a cohort of pediatric patients, in whom the first diagnosis was HLH and who were later diagnosed with visceral leishmaniasis.

CNS	Central nervous system
CSF	Cerebrospinal fluid
EBV	Epstein-Barr virus
HLH	Hemophagocytic lymphohistiocytosis
IFT	Immunofluorescence test
L-AmB	Liposomal amphotericin B
NK	Natural killer
PCR	Polymerase chain reaction

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The treatment of choice for visceral leishmaniasis in the Western world consists of liposomal amphotericin B (L-AmB). Less costly medications are drugs containing pentavalent antimony, paromomycin, or miltefosine.[7] HLH-directed therapeutic approaches include corticosteroids, cyclosporine A, etoposide, T-lymphocyte directed antibodies, and other biologicals. Hereditary HLH can only be cured by hematopoietic stem cell transplantation.[2] Case reports and small series anecdotally have reported the successful use of L-AmB in visceral leishmaniasis complicated by HLH.[4] To provide additional data on the therapy for visceral leishmaniasis-associated HLH, we documented treatment outcome.

Methods

Patients with any form of HLH in Germany are reported to the national clinical reference center in Hamburg, where clinical information is gathered, and the immunologic reference center in Freiburg, where immunological assays are performed. Through a retrospective survey of our database, we identified all patients who had been diagnosed with visceral leishmaniasis-associated HLH since 1999. We collected information on clinical findings, travel history, HLH-diagnostic criteria and related variables, procedures performed for the diagnosis of leishmaniasis (microscopy, polymerase chain reaction [PCR], culture of bone marrow or peripheral blood, and serology), treatment, and outcome. Methods of serologic tests for visceral leishmaniasis were chosen according to center preference, mostly indirect immunofluorescence test (IFT) or enzyme immune assay with commercial or custom-made assays. Testing was done at first suspicion of visceral leishmaniasis. Tests of perforin, signaling lymphocytic activation molecule-associated protein and x-linked inhibitor of apoptosis expression, and natural killer (NK) CD107 degranulation were performed as described previously.[8]

For inclusion, the diagnosis of visceral leishmaniasis had to be based on unequivocal serology, PCR, bone marrow cytology, or a culture positive for *Leishmania*. The diagnosis of HLH was established according to the HLH-2004 criteria,[9] of which at least 5 of 8 had to be fulfilled: (1) fever; (2) splenomegaly; (3) cytopenia of at least 2 cell lines (hemoglobin <90 g/L, platelets <100 000/mm^3, neutrophils <1000/mm^3); (4) hypofibrinogenemia (≤1.5 g/L) or hypertriglyceridemia (≥265 mg/dL); (5) hyperferritinemia (≤150 mg/dL); (6) increased level of soluble CD25 ≥2400 U/mL; (7) visualization of hemophagocytosis; and (8) decreased or absent NK-cell cytotoxicity. Informed consent was obtained from the parents. The study was approved by the ethics committee of the Hamburg Chamber of Physicians.

Results

Fifteen patients with visceral leishmaniasis for whom the initial diagnosis was HLH were identified in the HLH database containing 710 patients (mainly but not exclusively children) with suspected HLH. The resulting prevalence was 2.1%. One patient previously reported,[10] and 1 patient fulfilling only 4 out of 8 HLH criteria were excluded. Age at onset was 0.5-8.2 years, with 8 patients presenting before the age of 2 years. Presumed geographic areas of infection were Spain (n = 7), the Eastern Mediterranean area including Italy, Greece, and the Balkans (n = 5), and Armenia (n = 1). Details of the clinical presentation and laboratory variables are listed in **Table I**. None of the patients was positive for the HIV.

Diagnostic Pitfalls and Inappropriate Therapeutic Consequences

False Negative Results of Bone Marrow Cytology. Bone marrow microscopy was initially negative for *Leishmania* parasites in 6 of 13 patients (46%), even when re-analyzed retrospectively after the diagnosis of visceral leishmaniasis. In 3 of them, amastigotes could be identified in later bone marrow aspirates. One toddler (P12) of this group was treated with the full induction regimen HLH-2004, achieved remission but relapsed. Because at that time most genetic defects associated with HLH were not yet known, the reactivation was considered indicative of a hereditary form of HLH and hematopoietic stem cell transplantation was performed. Five months after presentation and 40 days after transplant, amastigotes were found in the bone marrow.

False Negative Results of Serological Tests and PCR of Peripheral Blood. In P11, initial *Leishmania* serology by IFT was negative. HLH unresponsive to corticosteroids alone prompted HLH-2004 therapy including 2 doses of etoposide that was stopped after receipt of the positive bone marrow PCR results. In P6, PCR from the peripheral blood was negative and visceral leishmaniasis was only proven by positive PCR in the bone marrow.

Unrecognized Travel History. One infant girl (P8) had only been in an endemic area (Spain) in utero. Vertical transmission resulting in congenital visceral leishmaniasis must be assumed, as the mother, who remained clinically asymptomatic, was serologically positive. Diagnosis of visceral leishmaniasis was delayed for more than 3 weeks.

Misleading Serologic Findings of Other Potential Triggers of HLH. Five out of 13 patients (38%) had positive IgM for Epstein-Barr virus (EBV) (n = 3), cytomegalovirus (n = 1, concomitant with EBV), human herpes virus 6 (n = 1), or measles (n = 1). Nonspecific serologic test results because of cross-reactions are likely because no rash was noted in the latter 2 and virus PCRs for 3 patients with serologic test results (×2 EBV, ×1 cytomegalovirus) remained negative. Virus-associated HLH was considered in these patients. In P9, the assumption of EBV-driven HLH because of positive EBV-IgM resulted in the administration of the entire induction regimen of the HLH-2004 protocol including etoposide with intermittent intensification because