Hemophagocytic Syndrome: A Misleading Complication of Visceral Leishmaniasis in Children—A Series of 12 Cases

Marie-Helene Gagnaire, MD*; Claire Galambrun, MD‡; and Jean Louis Stéphan, MD*

ABSTRACT. Objective. To describe the difficulties in diagnosing visceral leishmaniasis (VL) when revealed by hemophagocytic syndrome (HS) in young children.

Design. Retrospective study of patients identified over a 17-year period in French pediatric units.

Results. This series comprises 12 cases of VL that were either revealed (n = 11) or complicated (on starting treatment with antimony salts [n = 1]) by HS. Clinical manifestations were those of severe VL with sustained high fever and hepatosplenomegaly in children in very poor condition. Biological manifestations always included pancytopenia, marked hypofibrinogenemia and hypertriglyceridemia, hepatic cytolysis, and prominent hemophagocytosis on the bone marrow smear. These features led to transfer to a hematology unit. Ten children were very young (<38 months) at onset (and consequently at infection). Signs of autoimmunity (Coombs’ test-positive erythrocytes, antinuclear factors, and various autoantibodies) were found in 4 cases and were probably secondary to polyclonal B cell activation. Serologic tests for Leishmania were negative at onset in 6 children, and no amastigotes were found on the first marrow smear in 8 of 12 cases despite extensive search. Seven patients had not visited foreign countries. All these factors explain the initial diagnostic confusion. Three cases were initially misdiagnosed as familial erythrophagocytic lymphohistiocytosis or infection-associated HS, and these patients were treated with etoposide (once for 5 months) to control the HS after failure of steroids. The diagnostic delay in these cases was 50, 74, and 134 days. When VL was finally diagnosed, amphotericin B monotherapy was effective in 4 cases. Eight patients were treated with antimony salts; 4 were cured, 3 required adjunctive treatment, and 1 worsened (HS) and was cured with steroids and liposomal amphotericin. Regardless of the type of therapy, all 12 children are presumed cured with a mean follow-up of 7 years (range: 6 months–16 years).

Conclusions. A diagnosis of VL should, therefore, be seriously considered in all young patients with HS exposed to visceralizing Leishmania sp in Southern Europe. Clinicians and cytopathologists must be aware of the association. Early diagnosis of VL will minimize unnecessary hospitalization and potentially harmful investigations and treatments.

ABBREVIATIONS. HS, hemophagocytic syndrome; FEL, familial erythrophagocytic lymphohistiocytosis; VL, visceral leishmaniasis; RHS, reactive hemophagocytic syndrome; HB, hemoglobin; VAHS, virus-associated hemophagocytic syndrome; CRP, C-reactive protein; IgG, immunoglobulin G.

Hemophagocytic syndrome (HS) is a clinicopathologic entity characterized by activation and uncontrolled nonmalignant proliferation of T lymphocytes and macrophages, leading to cytokine overproduction. The latter accounts for the primary biological signs. Patients usually present with an acute febrile illness, hepatosplenomegaly, pancytopenia, marked hypofibrinogenemia, and hypertriglyceridemia. HS in children has been linked to viral, bacterial, fungal, and parasitic infections (the so-called infection-associated HS) and to a broad spectrum of malignancies and genetic disorders, such as Chédiak-Higashi disease, Griscelli disease, XLP syndrome, and familial erythrophagocytic lymphohistiocytosis (FEL).1,2 Infection by the protozoan pathogen Leishmania is a public health problem in most countries bordering the Mediterranean basin. One important reservoir is dogs. Although there are a number of different species, all of which are transmitted by phlebotomine sandflies, there are only 2 primary types of clinical disease. Cutaneous leishmaniasis is extremely common in tropical countries, the Middle East, and many Mediterranean areas. Visceral leishmaniasis (VL) is somewhat less common, affecting tropical areas of the Old World.3 However, VL may be contracted on short visits.4 Particularly in young children, VL revealed by HS (an exceedingly rare event) can cause considerable diagnostic difficulty. The first case of leishmaniasis revealed by a reactive HS (RHS) was reported by Matzner et al5 and concerned a 22-year-old adult. We are aware of only 2 other reported pediatric cases, both in Scandinavia.6,7 We reviewed the medical records of 12 children with RHS associated with VL diagnosed in France during the past 16 years. Clinical and biological features of this misleading presentation of VL are discussed together with treatment.

METHODS

Study Design

All pediatric units in France were surveyed for cases of HS in patients with VL. The charts of 12 patients referred to 8 pediatric hematology units between 1983 and 1999 were studied. The history, physical findings, and laboratory results were recorded. The diagnostic criteria for reactive HS included fever, splenomegaly,
obtained by dermal scraping. Which amastigotes were identified in a thin smear lesion on the forehead disappeared after a few days found in 2 cases: one (patient 1) in which a chronic showed slow waves. A likely portal of entry was fluid studies were normal. Electroencephalogram tonia). Computed tomography and cerebrospinal neurologic signs (obtundation, weakness, and hypoalbuminemia (serum level: including axillary and inguinal chains. The enlarged fus e adenopathy was appreciable in 3 patients, in-

Clinical Findings

The median incubation period for full-blown VL in this series was ~6 months. The clinical manifestations were fairly uniform. Persistent fever was found in all 12 cases and was irregular, high (≥39°C), and accompanied by a marked alteration of the general state, pallor, fatigue, severe weight loss, and poor feeding. Failure to thrive (weight: <2 standard deviations) was observed in 6 cases. Splenomegaly, reaching the iliac crest in 7 cases, was always present at initial presentation. The liver was also enlarged (n = 11), often >5 cm below the costal margin. Diffuse adenopathy was appreciable in 3 patients, including axillary and inguinal chains. The enlarged nodes (1–2 cm in diameter) were nontender. Extreme hypoalbuminemia (serum level: <20 g/L) caused edema and ascitis in 3 patients. Two patients had neurologic signs (obtundation, weakness, and hypotonia). Computed tomography and cerebrospinal fluid studies were normal. Electroencephalogram showed slow waves. A likely portal of entry was found in 2 cases: one (patient 1) in which a chronic lesion on the forehead disappeared after a few days of treatment, and also in the other (patient 2) in which amastigotes were identified in a thin smear obtained by dermal scraping.

The other signs are shown in Table 1.

<table>
<thead>
<tr>
<th>TABLE 1. Clinical Findings</th>
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<tbody>
<tr>
<td><strong>Clinical Manifestations</strong></td>
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<tr>
<td>Spleen enlargement (median: 11 cm; 5-26)*</td>
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<tr>
<td>Hepatomegaly (median: 8 cm; 3-17)*</td>
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<tr>
<td>Purpura</td>
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<tr>
<td>Edema or ascites†</td>
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<tr>
<td>Fatigue, pallor, general deterioration</td>
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<tr>
<td>Weight loss</td>
</tr>
<tr>
<td>Loss of appetite</td>
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<tr>
<td>Protracted fever &gt;15 d</td>
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<tr>
<td>Enlarged abdomen</td>
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<tr>
<td>Failure to thrive</td>
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<tr>
<td>Identified portal of entry‡</td>
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<tr>
<td>Neurological deterioration (lethargy, hypotonia)</td>
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<tr>
<td>Lymphadenopathy</td>
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<td>Joint pain</td>
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* Below the costal margin.
† Physical or ultrasound examination.
‡ One case formally documented by dermal scraping.

Results

The study population was composed of 6 boys and 6 girls, whose age at onset ranged from 7 months to 9 years (median: 18.5 months; 10 patients: <38 months old). Six were of French origin and 6 were of other origins (Algeria, 3; Rwanda, 1; and Morocco, 2). Three children lived in the South of France (an endemic area for leishmaniasis) and 9 had stayed in the South of France (4) or in North Africa (5) for a mean of 5.8 months (3 weeks–10 months) before clinical onset.

Laboratory Investigations

All the patients had signs of bone marrow hemophagocytosis (Fig 1) associated with absolute or relative hypofibrinemia in 3 cases (fibrinogen values of 2.5, 1.6, and 2.13 g/L; C-reactive protein (CRP) elevated at 221, 60, and 120 mg/L), hypertriglyceridemia and hypergammaglobulinemia, on which the diagnosis of HS was based. Transaminase activity was high in 10 cases and was 8 times normal on average. Pancytopeny was found in 9 cases, with severe anemia (hemoglobin [Hb]: <7 g/dL) in 10 cases. Two children had severe hypocalcemia (1.43 and 1.79 mmol/L) at onset (Table 2).

Four children had the following autoantibodies at onset: antinuclear (n = 1), positive direct Coombs’ test and antiplatelet antibodies (n = 3), antisMOOTH muscle (n = 2), and rheumatoid factor (n = 1).

Diagnosis (Table 3)

Six of the 12 patients were seropositive for Leishmania, with indirect fluorescence values of 1/160 to 1/1280 at onset. The threshold titer for positivity was 1:80. All but 4 of the patients’ bone marrow aspirates were negative for Leishmania (direct examination) at onset. Eight children who had negative smears at diagnosis had repeat marrow smears, and the parasite was finally identified in 4 of these patients after 1 to 4 months. These last 4 children were also seronegative at onset and only 2 seroconverted after 1 and 2.5 months (Table 3).

The 4 children whose marrow smears remained negative (n = 4) despite repeated testing were seropositive at onset, and their favorable outcome during antiinfective therapy supported the diagnosis of VL. None of the patients had needle biopsy of the spleen.

Culture results were positive in 2 cases. The pathogen (L infantum MON 1) was identified only once by means of an immunoenzymatic method (case 1).

Despite massive infection and positive marrow smears, 3 children remained seronegative. The lymphocyte count, proliferative T-cell responses, and vaccinal antibody assays were normal, and all the children were human immunodeficiency virus-seronegative (data not shown), ruling out an underlying immunodeficiency. One child had Turner’s syndrome.

In one case in which the diagnosis of VL was made

<table>
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<tr>
<th>TABLE 2. Laboratory Findings</th>
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<tr>
<td><strong>Biological Data</strong></td>
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<tr>
<td>Hemoglobin (g/dL)*</td>
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<tr>
<td>Polymorphonuclear lymphocytes (/µL)</td>
</tr>
<tr>
<td>Fibrinogen (g/L)</td>
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<tr>
<td>Platelets (/µL)†</td>
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<tr>
<td>ALT (×N)</td>
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<tr>
<td>Triglyceridemia (mmol/L)</td>
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<tr>
<td>CRP</td>
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<tr>
<td>Serum IgG</td>
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<td>Serum protein</td>
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×N indicates times normal; SEM, standard error of the mean.
* Number of patients with Hb < 7 g/dL at diagnosis: 10/12.
† Number of patients with platelets <50,000 at diagnosis: 6/12.
Number of patients with platelets <100,000 at diagnosis: 10/12.
‡ Figures in parentheses are SEMs.
rapidly, the diagnosis of HS was made retrospectively during marrow slide review 11 years later, based on bone marrow hemophagocytosis and other biological signs.

**Treatment and Outcome**

The mean interval between the first visit to a general practitioner and diagnosis of VL was 49.5 days (range: 13–174 days; median: 34 days). Most (8/12) received ambulatory treatment with antibiotics for a suspected bacterial infection. The mean period between hospitalization and diagnosis was 29.6 days (range: 2–134 days; median: 19 days).

The diagnosis on admission was wrong in 4 cases, all involving very young children (13, 14, 23, and 30 months) and was only corrected after 2, 2.5, and 4 months. The erroneous diagnoses were chronic juvenile myelomonocytic leukemia, FEL (2 cases), and virus-associated HS (VAHS). The diagnostic error led to etoposide therapy in 3 cases and planned bone marrow allografting in 2. These cases are now briefly summarized.

**Case 8 (1992)**

This 13-month-old child of Moroccan origin was admitted to the intensive care unit for gastrointestinal bleeding and fever (40°C). On physical examination, he was chronically ill appearing, febrile, pale, and ecchymotic. His spleen reached the iliac crest.

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TABLE 3. Evidence of Leishmaniasis at Onset

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<table>
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<tbody>
<tr>
<td>Bone marrow aspirate positive for <em>Leishmania</em></td>
<td>4/12</td>
</tr>
<tr>
<td>Seropositivity (indirect fluorescence)‡</td>
<td>6/12‡</td>
</tr>
<tr>
<td>Culture-positive§</td>
<td>2/3 tested</td>
</tr>
</tbody>
</table>

* Eight of 12 marrow smears were positive after repeated examination.
† Indirect fluorescence was used to detect IgG antibodies to *Leishmania*. The antigen was prepared from promastigotes of the World Health Organization reference strain of *L. infantum*.‡ Titer: ≥1:160 to 1:1280; the serology was positive in 3 cases between 1 and 2.5 months after onset.§ Isoenzyme analysis of cultured promastigotes identified *L. infantum* MON 1 in 1 case.
and the liver was palpable 7 cm below the costal margin. The Hb level was 3.5 g/dL, the platelet count 25 000/μL, and the fibrinogen 45 g/L. Tumoral hepatosplenomegaly, together with signs of hemophagocytosis on the marrow smear and seronegativity for Leishmania, led to a tentative diagnosis of familial lymphohistiocytosis. The child received methylprednisolone intravenously (3 mg/kg/day for 3 weeks), combined with etoposide (230 mg/m²/week for 21 weeks; cumulative etoposide dose: 4.8 g). After a period of clinical improvement (including apyrexia) but persistently low platelet count and hypofibrinogenemia, bone marrow transplantation was envisaged with a genoidentical brother as donor. Etoposide was withdrawn, leading to renewed fever and a deterioration of the overall condition. A fifth marrow smear performed during the pregraft workup revealed Leishmania and hemophagocytosis. The child was then treated with sodium stibogluconate (Pentostam) >6 months after initial presentation. The improvement was judged inadequate and the patient was switched to meglumine antimonystate (Glucantine) then pentamidin isethionate (Pentacarinat), followed by a final course of Glucantine. Recovery was slow, with gradual weight gain, after a disease history of 10 months. Biological abnormalities were corrected after 4.5 months. The pathogen disappeared from the marrow smear 9 months after onset. The serology remained negative 1 year after onset and has not been checked since. The child is considered cured with a follow-up of 9 years.

Case 7 (1992)

The child is cured, with a follow-up of 16 years. The child was then treated with sodium stibogluconate (Pentostam) >6 months after onset but was not subsequently tested. A fourth marrow smear revealed rare amastigotes. The child received etoposide (100 mg/m²; 3 injections in 10 days) and was then transferred to a hematology unit for bone marrow transplantation. During the pregraft workup a new bone marrow smear revealed rare amastigotes. The child received three 10-day courses of antimony meglumine and recovered rapidly. The serology remained negative 3 months after onset but was not subsequently tested. The child is cured, with a follow-up of 16 years.

Case 10 (1983)

This 23-month-old child of Moroccan origin was hospitalized in poor general condition (decreased activity, pallor, and tachycardia), with high fever and pancytopenia (Hb, 6 g/dL; platelets, 7000/μL; neutropenia, 580/μL; immunoglobulin G [IgG], 22 g/L; protein, 83 g/L; calcium, 1.79 mmol/L; fibrinogen, 2.1 g/L; CRP, 16 mg/L; triglycerides, 3.16 mmol/L). She was living in Morocco 7 months before onset. Serologic test results for leishmaniasis were negative, and several smears showed a cell-poor marrow without parasites. No diagnosis was made for 2 months, during which the child’s condition deteriorated slowly despite parenteral nutrition. In particular, the splenomegaly became tumorous and was palpable 17 cm below the costal margin, the fever persisted, and empirical antibiotic therapy was ineffective. Hemophagocytosis was found on a new marrow sample and familial lymphohistiocytosis was suspected. The child received etoposide (100 mg/m²; 3 injections in 10 days) and was then transferred to a hematology unit for bone marrow transplantation. During the pregraft workup a new bone marrow smear revealed rare amastigotes. The child received three 10-day courses of antimony meglumine and recovered rapidly. The serology remained negative 3 months after onset but was not subsequently tested. The child is cured, with a follow-up of 16 years.
The diagnosis of VL was particularly difficult in these cases. VL was considered by the hematologist as a differential diagnosis, but a conclusive diagnosis was never reached. This delay could have been favoring factors.

The diagnosis of VL was made retrospectively by slide review, suggesting that the frequency of the RHS linked to VL may be underestimated. Activated erythrophagocytosis is a conspicuous feature of other common intracellular parasitic diseases in children, such as vivax and falciparum malaria.  

The diagnosis of VL was particularly difficult in these cases. VL was considered by the hematologist as a differential diagnosis, but Leishmania amastigotes were very few in number on the first marrow smear (Table 3), as in the 2 previously published pediatric cases.  

In a recently reported French series of VL, the parasite was not detected in 22% of cases.  

The reason for the parasite scarcity in bone marrow smears of patients with leishmaniasis-associated HS is unclear. Serostatus at diagnosis was noncontributory in one half of the patients who seroconverted either long after their recovery or not at all. This is somewhat surprising because with the exception of patients with acquired immunodeficiency syndrome, anti-Leishmania antibodies are usually present at high titers in patients with VL. US soldiers who served in Operation Desert Storm and developed systemic infection with L tropica (the cause of urban VL in the Middle East) also had low or undetectable antibody titers.  

Spleen needle-aspiration biopsy seems to have a sensitivity as high as 98%, but the risk of hemorrhage in such fragile children with low fibrinogen levels is unacceptable.

FEL is another differential diagnosis in a young child with an intense HS and a negative microbiologic workup. This genetically heterogeneous autosomal recessive disease generally affects very young children, sometimes during the first days of life. In the absence of a relevant family history or parental consanguinity, it is difficult to diagnose this disease, which can be cured only by bone marrow transplantation but is initially managed by cytotoxic and immunosuppressive treatment. Neurologic involvement is nearly always present, with meningeal infiltration by blast-like lymphoid cells and hemophagocytic macrophages, and this should distinguish it from a sporadic HS linked to an infection. The recent description of mutations in the perforin gene in patients with FEL linked to 10q22 should facilitate its diagnosis. Thus, etoposide was wrongly prescribed to 3 patients. This drug, which is cytotoxic for the monocyte-macrophage lineage, can
seem effective in some forms of HS, but it can have catastrophic consequences by increasing the risk of aplasia and aggravating the VL. Moreover, secondary malignancies after epipodophyllotoxin therapy, including myelodysplastic syndromes and acute myelocytic leukemia, have been reported. Bone marrow transplantation was planned in 2 cases, but fortunately the correct diagnosis was made during the pretransplant workup.

Various treatments were prescribed in this retrospective series, which includes a number of old observations. Case 5 is remarkable in that the HS, which was very severe (the patient had required transfusions for a clinical hemorrhagic syndrome), was not present at diagnosis but seems to have been triggered by pentavalent antimonials, because it occurred after 48 hours on treatment. The antimony salts were rapidly withdrawn and the patient recovered on steroids and liposomal amphotericin B. Liposomal amphotericin B (3 mg/kg/day for 5 days, followed by 3 mg/kg administered on an outpatient basis on day 10) was recently shown to be optimal for the treatment of VL in immunocompetent children. Liposomal amphotericin B, which was very effective and well-tolerated in 3 children, seems to us to be particularly suitable for forms associated with a RHS, because lipid-associated amphot B is taken up by macrophages and targets the drug to the site of infection, leading to very high concentrations in the liver and spleen. The efficacy and indications of steroids could not be determined in this small retrospective series. However, intravenous steroid therapy (1 mg/kg/day) should be given when gravity of sociocultural changes.

In the western Mediterranean basin, the number of human VL cases, which used to be relatively low, has increased during the last decade. This is related to the recent increase in the canine population because of 

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