Mediterranean Sun, Sea, Sand and … Leishmaniasis

Two Case Reports

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Abstract

We present two recent cases of visceral leishmaniasis diagnosed in Belgian children, presenting with typical clinical signs of high fever, splenomegaly and pancytopenia.

Although the differential diagnosis of this clinical presentation is extensive, the diagnosis of visceral leishmaniasis should be considered in the event of a travel history to endemic countries in tropical and subtropical regions, as well as in the south of Europe.

Introduction

Leishmaniasis is a vector-borne disease caused by the *Leishmania* parasite.

The disease is transmitted by the bite of an infected female sandfly, a tiny (2-3mm) and silent insect vector. These sandflies thrive in moist climates and are found in tropical and temperate regions of the world.

While this disease was once a standard textbook "tropical" disease, it can also be acquired in subtropical and other warm climates. In Europe, leishmaniasis is endemic in all southern countries bordering the Mediterranean Sea and the Black Sea.

Infection can range from asymptomatic to severe. Most people who become infected with the parasite, do not develop any symptoms. The ratio of asymptomatic individuals to active disease varies depending on the virulence of the parasite species, host characteristics and study design (1).

Asymptomatic infections can become symptomatic years to decades after exposure in people who have become immunocompromised.

Cutaneous (CL), mucosal (ML) and visceral (VL) leishmaniasis are the 3 clinical syndromes caused by an infection with the *Leishmania* parasite.

CL is the most common form and typically presents with painless, ulcerative lesions on exposed skin, leaving scars. ML is a rare and aggressive form of CL affecting the mucosal areas of nose or mouth. CL and ML can cause substantial morbidity, whereas VL can be life threatening and fatal without treatment (2-5).

Leishmaniasis is endemic in almost all continents, except for Australia and Antarctica. CL is endemic primarily in North Africa, the Middle East and South America. VL is found mainly in East Africa, the Indian subcontinent, Central and Southwest Asia, the Middle East, as well as in Brazil and Latin America. It is also endemic in southern Europe.

Climate change seems to be influencing the spread of the disease through changes in the incidence and geographic distribution of sandflies (5, 6).

We describe two cases of VL in children diagnosed after traveling in Europe.

It is important to raise awareness of this disease and to actively search for the parasite in case of a relevant travel history, especially in the presence of typical signs such as pancytopenia, fever, and splenomegaly.

Case reports

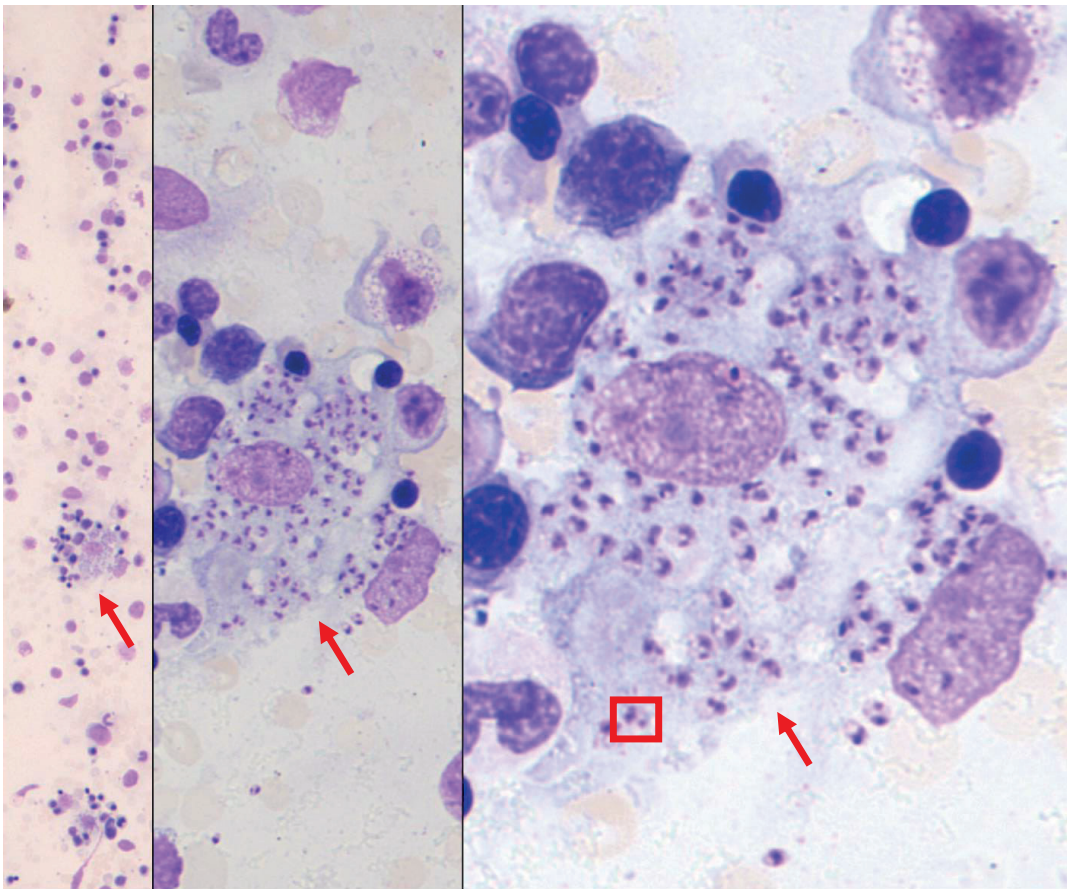
Case 1

A 20-month-old girl is referred to the pediatrician because of a 3-week history of fever and non-bloody diarrhea. Initially she also had a productive cough that resolved after 1 week. Between episodes of fever she is well. The parents noted that she probably lost weight, about 1 kg. Her medical history is uneventful. She was born and lived in Spain until 1 year of age and is vaccinated according to the Spanish schedule. At the time of presentation, she lives with her mother in Belgium, with regular visits to her father in Spain. The parents did not report any contact with domestic or farm animals; no insect or tick bites were noted.

On clinical examination, she is in good general health but appears pale. The clinical and neurological examination is normal except for a splenomegaly of 10 cm below the costal margin.

Initial blood tests show pancytopenia (hemoglobin 6.5 g/dl [N 11.5-13.5], white blood cell count 5430/µl [N 6000-17500] with neutropenia 870/µl [N 1500-8500] and 86000 thrombocytes/µl [N 150000-450000]) and mildly elevated CRP (46,3 mg/l [N <5]), AST (61 U/l [N < 37]) and LDH (548 U/l [N 157-272]). Ferritin is elevated to 1208 µg/l [N 7-140]. Coagulation and renal function tests are normal. Multiple blood cultures were negative, as was a stool bacterial culture. Abdominal echography confirms splenomegaly, without hepatomegaly nor enlarged intra-abdominal lymph nodes. Chest x-ray is unremarkable.

Because of primary suspicion of a hematologic malignancy, the patient is referred to the Pediatric Hematology Department of the Ghent University Hospital for bone marrow examination. This reveals no infiltration by malignant cells, but multiple parasites compatible



Microscopic picture of a May-Grunwald-Giemsa-stained bone marrow aspirate (patient 1). A macrophage (red arrow) with amastigotes is shown at different magnifications (left 10x; middle 50x; right 100x). Amastigotes consist of small, spherical, intracellular inclusions with a prominent nucleus and kinetoplast (red square).

with *Leishmania* amastigotes are seen on direct examination (Figure). Prompt treatment with liposomal amphotericin B is started and given in 7 doses (total dose 21 mg/kg). The girl also receives a red blood cell transfusion for worsening anemia, as the Hb dropped to 5.5 g/dl. The patient defervesces within 24 hours of the first dose of amphotericin B. The diagnosis is later confirmed by PCR performed by the Institute of Tropical Medicine Antwerp (ITM), which reveals the *Leishmania infantum* species.

The blood count and splenomegaly gradually recovered, with normal WBC, Hb and thrombocytes and no splenomegaly at consultation 2 months after hospitalization. She remains without signs of relapse during the 3 year follow-up period.

Case 2

A 9-month-old Caucasian girl presents with a 4-week history of daily fevers, initially associated with a mild cough. She had received antibiotic treatment with amoxicillin 1,5 weeks before, following a presumptive diagnosis of post-viral bacterial bronchitis, after which she defervesced for two days. There is no history of rash, articular complaints or B symptoms. She has an incomplete vaccination status (polio vaccine only) due to parental vaccine hesitancy. She has traveled to Spain (Valencia region) twice in the past year.

On clinical examination, she is pale and has a marked splenomegaly (6 cm below the costal margin).

The blood sample shows marked anemia (Hb 6.3 g/dl), thrombocytopenia (41000/µl) and leukopenia 3180/µl with neutropenia of 394/µl. Inflammatory markers are elevated with a CRP of 92 mg/l and a high IgG level of 16 g/l [N 3.02-10.37]. Serology is negative for EBV, parvovirus, *Toxoplasma*, rubella, CMV, HIV, hepatitis B and C, mumps, measles, and varicella. Blood and urine cultures are negative. A nasal swab PCR reveals the presence of adenovirus and rhinovirus. The tuberculin skin test and IGRA test are negative.

Chest x-ray shows mild peribronchial attenuation without mediastinal enlargement. Ultrasound confirms splenomegaly of 10 cm without hepatomegaly or enlarged lymph nodes.

After case discussion with the pediatric hemato-oncologists, she is referred to the Pediatric Hematology Department of the Ghent University Hospital for bone marrow aspiration to rule out hematologic malignancy, which is negative, after which she is transferred to the regional hospital for further treatment and follow-up.

However, despite broad-spectrum antibiotics, she continues to have high fever. She appears clinically well, with only persistent splenomegaly. Because of the suspicion of hemophagocytic lymphohistiocytosis (HLH) with worsening cytopenia (Hb 6,9 g/dl, WBC 1560/µl, thrombocytes 31000/µl), increasing ferritin to a maximum of 1624 µg/L and a high triglyceride level of 704 mg/dl [NL ≤ 150], she is again referred to the tertiary center.

With the persistent cytopenia and clinical clue of HLH, the initial bone marrow is reexamined thoroughly and a sparse *Leishmania* amastigote is detected on direct examination, corroborating the diagnosis of VL.

Treatment with liposomal amphotericin B IV is started and continued with a total dose of 21 mg/kg, given in 7 doses. She becomes afebrile within 24 hours. The diagnosis is confirmed by a positive *Leishmania* PCR at the ITM (Antwerp), revealing the *Leishmania infantum* species.

At consultation 6 weeks after the start of treatment, she shows no more splenomegaly and all cell lines have recovered, and she remains well after a 3 months of follow-up.

Discussion

Visceral leishmaniasis results from the dissemination of *Leishmania* parasites throughout the reticuloendothelial system and is usually caused by the species *L. donovani* and *L. infantum*. It has an incubation period ranging from weeks to 6 months. As shown in both cases, the disease typically presents insidiously, which explains the latency of diagnosis in many cases. Clinical manifestations include fever, weight loss, hepatosplenomegaly (usually a more prominent splenomegaly), and pancytopenia due to bone marrow suppression, hemolysis, and splenic sequestration. Blood tests also show a high total protein and low albumin levels, with hypergammaglobulinemia. VL is almost always fatal if untreated and requires prompt evaluation and treatment. The

term kala-azar, which means black fever in Hindi, is often reserved for severe cases of VL, although the terms kala-azar and VL are sometimes used interchangeably. VL is an opportunistic infection in persons with HIV or cell-mediated immunosuppression. HIV infection increases the risk of VL infection, and VL progression of HIV disease, with a high disease burden in parts of Eastern Africa. HIV co-infected patients may present with atypical clinical manifestations (2).

Some cases of VL are associated with hemophagocytic lymphohistiocytosis (HLH), as in case 2. HLH is a life-threatening systemic inflammatory disorder caused by excess immune activation triggered by certain infections. The clinical syndrome includes pancytopenia, fever, splenomegaly, hypertriglyceridemia, and elevated ferritin with bone marrow evidence of hemophagocytosis. Most patients with HLH secondary to VL respond to antileishmanial therapy alone, adjunctive therapy being needed sometimes in case of delayed diagnosis (7).

The diagnosis of VL should be suspected in any patient presenting with compatible signs and symptoms (fever and splenomegaly), blood results (pancytopenia), especially with a travel history to an endemic region.

Diagnosis can be made by microscopic visualization of the characteristic amastigotes in smears or tissues, molecular detection of parasite DNA, parasite isolation by in vitro culture and serologic testing. For optimal guidance in the laboratory diagnostic approach, early consultation with expert advisors and reference laboratories is recommended.

Parasite detection and identification remains the gold standard.

Bone marrow aspiration is the preferred specimen for diagnosis of VL by microscopy, with a high specificity and 50-80% sensitivity. The sensitivity of microscopy on spleen tissue is higher (>90%) but spleen aspiration for diagnosis is discouraged because of the high procedural risk of life-threatening bleeding. PCR on bone marrow smears or tissue samples is the method of choice as it has high sensitivity and specificity and allows for species identification, which is important for treatment guidance. Parasite culture can take weeks to become positive and is used only for specific indications such as drug resistance evaluation and for research purposes.

Serologic testing can provide supportive evidence for the diagnosis and is recommended in persons suspected of having VL in whom bone marrow biopsy or aspiration cannot be performed, or when microscopy and PCR are negative. The sensitivity and specificity of serologic tests depend on the assay and antigens used, as well as host factors. Antibody levels can be lower or even undetectable in persons with HIV or other cellular immunodeficiencies (2, 8-10).

Treatment of VL varies by region and species identification due to variable drug susceptibility and can be challenging. Prompt treatment of VL with liposomal amphotericin B is now recommended as the first-line treatment due to the widespread high-level resistance to previously used antimonial drugs. Total dose and dosing schedule vary according to the clinical presentation, the *Leishmania* species and host factors (immunocompromised or not) (10).

Control of leishmaniasis is based on vector control (reduction of sandfly bites), and prompt diagnosis and treatment of the disease to reduce the infection reservoir. Sandflies are nocturnal and can be found both indoors and outdoors. Bites may go unnoticed because of the small and silent vector. These small sandflies can even pass through the holes of an ordinary mosquito net, so fine-mesh or insecticide-impregnated nets are useful. Travelers can protect themselves from bites by avoiding outdoor activities from dusk to dawn, by minimizing uncovered skin and applying DEET (diethyl-m-toluamide) containing insect repellent to exposed skin (11, 12).

Conclusion

Leishmaniasis is endemic in large parts of the world and can be imported even from a nearby travel destination such as Spain, southern France, Greece, or Italy. Consider the diagnosis of visceral leishmaniasis in patients with a clinical syndrome of fever, splenomegaly, pancytopenia and a relevant travel history (even in the distant past). The gold standard for diagnosis is the detection of the parasite in affected tissue, with bone marrow being the preferred diagnostic sample. In case of suspicion expert advice should be sought for guidance on diagnosis and therapy.

Sandfly bites may go unnoticed because they are very small. The risk of infection can be reduced by vector control, insecticide-treated bed nets, covering the skin (especially from dusk until dawn) and DEET.

The authors have no conflicts of interest to declare with regard to the topic discussed in this manuscript.

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