Case Report

Prolonged fever, splenomegaly and pancytopenia in a 4-year-old child: don't forget Leishmania

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Abstract

We report the case of a four-year-old boy returning from Morocco admitted to our hospital with a two-months history of fever and abdominal distension. He had a splenomegaly and a pancytopenia. Anti-leishmania antibodies were found by indirect immunofluorescence test on patient's blood. The presence of *Leishmania infantum* DNA in bone marrow was confirmed by polymerase chain reaction establishing the diagnosis of visceral leishmaniasis due to *Leishmania infantum*. The child was treated with liposomal amphotericin B. Visceral leishmaniasis should be considered in the differential diagnosis of children with persistent fever, hepatosplenomegaly and pancytopenia with travel history to endemic areas.

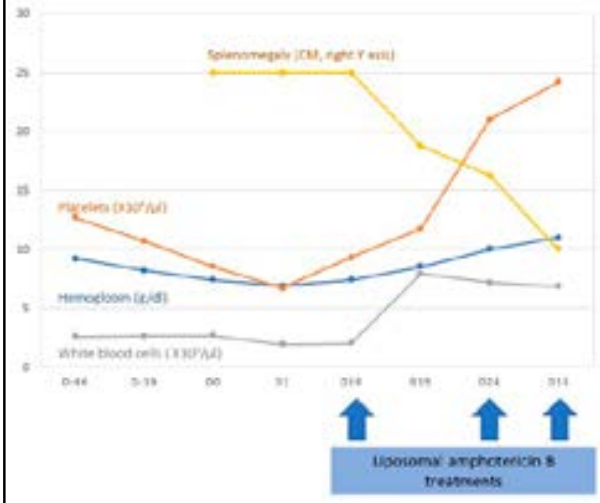
Introduction

Visceral leishmaniasis (VL) also called Kala Azar (Hindi for black fever) is a systemic protozoan infection transmitted by sandflies. It is usually caused by *Leishmania donovani* in Asia and sub-Saharan Africa and *Leishmania infantum* in the Mediterranean region, the Middle East, Central Asia, South America, and Central America (1,2). Another clinical manifestation of leishmaniasis is a cutaneous or mucocutaneous infection caused by different leishmanial species (for example *Leishmania tropica, mexicana, major*) (2).While the infection is often asymptomatic, the most common clinical manifestation of visceral leishmaniasis is fever associated with abdominal pain due to hepatosplenomegaly (3). Disease severity varies with the patient immune status: immunodeficiencies are risk factors for more severe disease with atypical localizations. Systemic treatment is always indicated in symptomatic visceral leishmaniasis. The selection of the appropriate treatment depends on two main factors: the geographical region where the infection is contracted and the immune status of the host. Immunocompromised patients need higher total doses of antimicrobials (2,3). We report the case of a 4-year-old boy who came back from Morocco with fever for two months with painful and distended abdomen.

Case report

During a long stay in Morocco in August 2019, a previously healthy four-year-old boy born in Morocco and living in Belgium since 2018 (with multiple journeys in Morocco between 2018 and August 2019), presented with fever, abdominal pain and abdominal distension. Fever was irregular (with 24 to 48 hours of fever followed by 24 hours of apyrexia). A weight loss was described by the patient's mother at that time but was not measured. The patient sought medical advices in various outpatient clinics in Morocco during this period. A hepatosplenomegaly was clinically observed. A first blood test was done by a general practitioner in Zaio (Morocco) on August 11 and showed a moderate pancytopenia and inflammatory syndrome: hemoglobin: 9,2 g/dl, platelets : 127000/mm³ white blood cells : 2600/mm³, CRP (C-reactive protein) : 4,4 mg/L, ESR (erythrocyte sedimentation rate) : 44mm/h (Figure 1). Hemoglobin electrophoresis was normal and hepatitis A, B and C serologies were negative. Abdominal ultrasound and computerized tomography (CT) confirmed the hepatosplenomegaly. One month later, the parents asked for a second advice in a public hospital in Morocco because of persistent fever and abdominal distention. A second blood test confirmed the pancytopenia (Figure 1). A bone marrow aspiration was normal: rich and polymorphous marrow without signs of malignancy. The patient came back in Belgium in October 2019. He was referred to our tertiary children's hospital to complete the evaluation. At admission (Day 0, two months after the onset of symptoms), he had fever (38.8°C), a massive splenomegaly (10 cm below the costal margin) and hepatomegaly (3 cm below the costal margin), cardiac murmur, pallor and cervical lymph node, with no other clinical manifestations. He had no drug exposure. There was no history of travel besides the stay in Morocco. The blood test confirmed the pancytopenia without lymphoblasts. He had no inflammatory syndrome. Ferritin and triglycerides were not significantly elevated. Immunoglobulin dosages were within the normal range for the age except for an isolated low level of IgA (0,02 g/L- normal value for the age: 0,33-2,35 g/L (4)). Antinuclear antibodies screening (ANA) revealed the presence

Figure 1. Clinical and biological evolution during treatment



D0 stands for day of admission in our tertiary hospital. Treatment with intravenous liposomal amphotericin B (Ambisome®) was started on day 10. He received a total dose of 21 mg/kg: 3 mg/kg/day for 5 days (between D10-D15) and then 2 more doses on day 14 (D24) and day 21 (D31). Fever resolved before the treatment at day four after admission, spleen size decreased after 2 weeks of treatment together with normalization of complete blood count

of a low titer (1/80), bacterial and viral serologies performed at admission Cytomegalovirus (CMV), Epstein Barr Virus (EBV), Human immunodeficiency virus (HIV), Parvovirus, *Bartonella*, *Borellia*, *Brucella*) were all negative. A bone marrow aspiration was performed on day 1 of hospitalization to rule out acute leukemia or hemophagocytic lymphohistiocytosis. It revealed a hypocellular marrow without evidence of malignancy or hemophagocytosis. No parasites were observed but a *Leishmania species* serology by indirect immunofluorescence test (IFA) and enzyme linked immunosorbent assays (ELISA) revealed a high anti-leishmanial antibody titer (1/2560). A second bone marrow aspiration was performed and *Leishmania infantum* DNA was detected by polymerase chain reaction (PCR), which confirmed the diagnosis of visceral leishmaniasis due to *Leishmania infantum.* The child was treated with intravenous liposomal amphotericin B. He received a total dose of 21 mg/kg: 3 mg/kg/day on day 1 to 5, day 14 and day 21. Fever had already resolved 6 days before treatment, spleen size gradually decreased after treatment and complete blood count normalized within 2 weeks (figure 1). We did not observe any renal or metabolic toxicity due to liposomal amphotericin B. We are not aware of any relapse but the patient interrupted his follow up after 3 months.

Discussion

Visceral leishmaniasis is a vector borne disease. *Leishmania infantum* is usually found in the Mediterranean Basin, the Middle East, Central Asia, South and Central America (1). In endemic regions the infection can be asymptomatic. The seroprevalence ranges from 7% to 63% in endemic areas. The global prevalence in 2017 was between 50 000 and 90 000 new cases (2). The incubation period for Visceral Leishmaniasis ranges from 2 to 6 months and sometimes up to several years (2).

The disease results from dissemination of the parasite through the reticuloendothelial system. The triad including prolonged fever, hepatosplenomegaly and pancytopenia after a stay in an endemic region must raise high suspicion for visceral leishmaniasis. The most frequently involved organs are bone marrow, spleen and liver. The clinical presentation depends of the involved organs. History of prolonged fever, weight loss and abdominal discomfort due to splenomegaly are the most commonly reported symptoms. Hepatomegaly is less often observed. In rare cases patients have lymphadenopathies (2,3). Pancytopenia is usually present reflecting bone marrow suppression and splenic sequestration (5). Immunocompromised patients, particularly HIV co infected patients usually present a more severe disease sometimes with atypical localizations such as the intestinal or respiratory tract (1). Morbidity and mortality depend on the involved organs. The most common complications are bacterial coinfections or sepsis due to leucopenia, and hemorrhage due to thrombocytopenia and/or hepatic dysfunction. Without treatment the mortality rate of symptomatic visceral leishmaniasis is high (1,3,5).

Multiple diagnostic approaches exist (Table 1). The gold standard for the diagnosis is visualization of the amastigote in affected tissues either by microscopy, histopathology or in vitro culture (1,3,5). The specificity and sensitivity of direct examination depend of the tissues and is well described in different studies: Van Griensven et al. described a sensitivity above 90% in the spleen, 52-85% in bone marrow and 52-58% in lymph nodes (3). Sundar et al. and Costa et al. observed a sensitivity in the bone marrow of about 60-85% (6,7). Parasites can sometimes be retrieved from blood samples in HIV co infected patients because of a higher parasitemia. Splenic aspiration has the highest sensitivity (93- 99 %) but a high risk of life-threatening complications (8). Bone marrow aspiration or biopsy is usually preferred (9). Culture allows greater sensitivity but because it is time consuming and expensive this method is rather used in research labs than in clinical practice (5,9).

Parasite DNA can also be detected by polymerase chain reaction (PCR) in bone marrow and peripheral blood. Molecular testing is the most sensitive method to confirm the diagnosis. As recently reviewed, sensitivity and specificity ranges between 82,6 and 100% and 92 and 100% respectively (9-11). It also allows species identification and diagnosis of the infection in asymptomatic individuals or in patients with atypical clinical presentations such as HIV co-infected patients (2,3,5,11).

Table 1 Advantages and disadvantages of different diagnostic methods in visceral leishmaniasis (3,5-11)

	Advantages	Disadvantages
Cultures / direct examination	• Visualization of the parasite in the tissue → allows definitive diagnosis, high specificity	• sensitivity is relatively low and varies with sampled tissue • Do not allow for species identification • Culture can take up to several weeks • Invasive • Culture is expensive and require specific expertise
Serologic Testing	• Rapid result • Less invasive • Limited cost	• No difference between current from previous infection • Do not assess the response to treatment • Variable sensitivity and specificity (cross reactivity with other parasitic infections) • Lower sensitivity in immunocompromised patient • Do not allow for an early diagnosis (delay between the infection and the immune response)
Molecular Testing by PCR	• Detection of the infection even in asymptomatic immunocompromised patients • Rapid result (<24h) • Do not need viable parasites • Allows species identification • Assess the response to treatment • High sensitivity and specificity	• Access to the technic in low income setting

Advantages and disadvantages of different diagnostic methods. The IDSA recommends using a multiple diagnostic approach starting with histopathology and molecular methods, preferably on bone marrow samples rather than on spleen samples. If not feasible or negative with a high suspicion, serologies can be useful.

If these methods fail to identify parasites, serologies could be useful. The diagnosis by serological testing is based on the immune response. A range of serological methods exist with variable sensitivity and specificity: enzyme-linked immunosorbent assay (ELISA), indirect immunofluorescence antibody (IFA), immunoblotting, direct agglutination test, strip test. A positive serological test does not definitely confirm a diagnosis of active visceral leishmaniasis since antibodies can persist for years after an infection. This is the reason why serological testing cannot be used to assess the response to treatment (2,3,5,9,10). The sensitivity is lower in immunocompromised patients (5,10). IDSA guidelines (Infectious Diseases Society of America) recommend serological testing if microscopic examination, culture and molecular tests cannot be realized or have negative results despite a high clinical suspicion of visceral leishmaniasis (5).

In our case, molecular testing was not performed on the first two bone marrow samples. Diagnosis could not be confirmed by direct examination alone but visceral leishmaniasis was highly suspected due to typical clinical symptoms and a positive serology. This suspicion led us to take a third bone marrow sample to confirm the diagnosis by PCR. A molecular test on the first bone marrow aspiration would probably have allowed a faster diagnosis.

Symptomatic patients with visceral leishmaniasis should be treated. Pentavalent antimonial compounds have been the first line treatment during the last 7 decades and are still widely used. The main concerns which led to a change of care were their toxicity (cardiac, hepatotoxicity, nephrotoxicity and pancreatitis) and the apparition of therapeutic failure due to resistance in certain area in India (3). Intravenous liposomal amphotericin B (total dose (18)-21 mg/kg: 3mg/kg/day on days 1-5, 14 and 21) is currently the recommended treatment for immunocompetent patients with visceral leishmaniasis due to *Leishmania infantum* or *donovani*, (3,5). Immunocompromised patients require a higher dose of liposomal amphotericin B (total dose 40 mg/kg) combined with a longer treatment duration because of a high risk of relapse (5). The most frequent side effects of liposomal amphotericin B are fever, flushing, nausea and headache that may occur 1 to 2 hours after the infusion is started. Close monitoring is necessary during treatment. In some cases, acute renal failure or hypokalemia occur. Preexisting renal failure or ionic disorder should be excluded before treatment administration (5). Mortality is high without treatment (10-20%) and death often results from hemorrhagic or infectious complications (12). Clinical and hematological evolutions are used to assess the treatment effectiveness because it correlates with parasitological clearance. Fever should resolve in less than one week after treatment. Organomegaly can take up to 10 days to decrease and up to 3 to 6 months to resolve completely. Leukopenia and thrombopenia usually normalize within 1 month and anemia within 6 to 12 months (5). Patients should have a long term follow up since relapses can occur up to 6 to 12 months after treatment. Post kala-azar dermal leishmaniasis (PKDL) has also been observed up to 12 months after visceral leishmaniasis. Clinical manifestations of PKDL are papules, nodules or hypopigmented lesions around the nose and mouth. It occurs more frequently post *Leishmania donovani* infections. The diagnosis can be confirmed with a skin biopsy showing amastigote infiltrated lesions (1-3,5).

There is no vaccine or prophylaxis to prevent the infection. For people travelling to endemic areas preventive measures are important and include reduced contact with sandflies, use of bed nets and insecticide sprays (3,6).

Conclusion

Visceral leishmaniasis is an infrequent diagnosis in Belgium but it should be considered in the differential diagnosis of children with persistent fever, hepatosplenomegaly and pancytopenia with travel history to endemic areas. Definitive diagnosis requires demonstration of parasites in affected organs. Serological tests have a high sensitivity but are not stage specific and remain positive for months or years after treatment. Molecular methods have remarkable sensitivity and specificity and allow species identification (2,3,5-9). Combination of these multiple diagnostic tools is suggested for accurate diagnosis (5). The IDSA recommends using a multiple diagnostic approach starting with histopathology and molecular methods. Spleen biopsy is the gold standard but because of a high risk of complications, bone marrow aspiration or biopsy is preferred. If not feasible or negative with a high suspicion, serologies can be useful (5-8).

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