

CASE REPORT

A case of visceral leishmaniasis in Oltenia region (Romania)

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Abstract

Visceral leishmaniasis is produced by a protozoan parasite that belongs to the genus *Leishmania*. Transmission is made through sting, the vector being represented by a species of the genus *Phlebotomus*. The first case of visceral leishmaniasis in Romania was reported by Manicatide (1912). In 1934, it was described a focus of visceral leishmaniasis in Oltenia region (24 cases). The symptoms of disease are unspecific: fatigue, feverishness, cephalalgia, anorexia, nausea, obnubilation status. The fever is irregular, with high oscillations. Clinical, a sallow pallor of the skin, enlarge lymph nodes, hepatomegaly, splenomegaly, weight loss have been observed. Laboratory exams showed frequently severe anemic syndromes or other cytopenias, erythrocytes sedimentation rate was increased, hypergammaglobulinemia with monoclonal peak has been found. Immunoelectrophoresis showed hyper-IgG and hyper-IgM. Bone marrow biopsy showed lympho-plasmocyte infiltration, histiocytes, Leishman–Donovan bodies intracellular or extracellular. The prognosis of the disease is unfavorable in the absence of specific treatment with antimony. In case of resistance, it is used immunotherapy, amphotericin or miltefosine.

Keywords: leishmaniasis, *Phlebotomus*, hypergammaglobulinemia, Leishman–Donovan bodies, antimony.

□ Introduction

Leishmaniasis is a disease caused by protozoan parasites that belongs to the genus *Leishmania*. The vector is a species of *Phlebotomus*, who transmits disease to human through sting [1, 2]. Visceral leishmaniasis is a systemic chronic disease induced by species of *L. donovani*, *L. infantum*, *L. chagasi*, *L. mexicana*, *L. tropica*, *L. major*, *L. aetiopica* and subgenus *Viannia* (*L. brasiliensis*, *L. guayanensis*, *L. panamensis*, *L. peruviana*) [3, 4]. Subgenera are morphologically indistinguishable; they can be differentiated by isoenzyme analysis, DNA-sequence analysis, or monoclonal antibodies. There are more types of leishmaniasis: cutaneous, muco-cutaneous, visceral. The first observation about visceral leishmaniasis is from the XIX-th century in the Middle East and India, where the disease has an endemic character.

In Mediterranean countries, the disease is frequently described, and sporadic cases were reported in Bulgaria (1941), Hungary (1949) [5]. The first case of visceral leishmaniasis was published in Romania by Manicatide (1912). In 1954, Minculescu M described a focus of visceral leishmaniasis in Oltenia region [6, 7]. The visceral leishmaniasis is the most severe and the evolution is potentially fatal without treatment.

Leishmania parasites the reticulo-endothelial system and the patient presents irregular fever, progressive fatigue, anemia, hepatomegaly, splenomegaly and adenopathy.

Laboratory exams frequently show severe anemia of

mixed etiology (bone marrow inhibition, hypersplenism and iron deficiency) or cytopenias through hematologic hypersplenism (leucopenia with neutropenia, thrombocytopenia); erythrocyte sedimentation rate was increased and electrophoresis showed hypergammaglobulinemia with monoclonal peak. Immunoelectrophoresis showed hyper-IgG and hyper-IgM. Bone marrow biopsy showed lympho-plasmocyte infiltration, macrophages and visualization of the amastigotes; Leishman–Donovan bodies, intracellular and/or extracellular. Differential diagnosis is made by chronic myeloproliferative syndromes, especially with myeloid metaplasia with myelofibrosis, chronic malaria, Waldenström's disease, tuberculosis, which affects liver and spleen, malignant lymphomas stage IV, histoplasmosis, brucellosis. Prognosis is unfavorable in the absence of a specific treatment (antimony) [9–11]. In case of resistance, it is used immunotherapy, amphotericin or miltefosine [12, 13].

□ Patient and Methods

We present the case of a female patient (A.C.), 28-year-old, from Sopot, Dolj County. Anamnesis showed that the patient made a long trip (fourteen months) in Greece. At the presentation in the Clinic of Hematology from Craiova, the patient presented fatigue, irregular fever, weight loss, transient periods of obnubilation, left bacterial otitis. The examination was made and there were determined: hemoglobin value, leukocyte count, leukocyte formula, platelets count, peripheral blood

smear, bone marrow biopsy, erythrocyte sedimentation rate, glycemia, hepatic and renal tests, viral markers, electrophoresis, immunoelectrophoresis.

Results

The patient presented organomegaly (enlarged lymph nodes, hepatomegaly, splenomegaly), irregular fever, transient periods of obnubilation, pancytopenia. The examination showed a sallow pallor of the skin, enlargement latero-cervically and axillary lymph nodes (1.5–2 cm diameter, firm consistency, without adherence to superficial and profound plans, painless), moderate hepatomegaly and important splenomegaly.

Laboratory exams showed: hemoglobin 6 g/dL, leukocyte count 830/cmm (NS=2%, S=15%, Lf=27%, Mo=3%, lymphoplasmocyte 3%, leukocyte formula based on 50 cellular elements), thrombocyte count 96 000/cmm; peripheral blood smear showed erythrocytes in rouleaux; erythrocyte sedimentation rate 135 mm/1 hr., seric uric acid, seric urea, seric creatinin, glycemia were normal. Viral markers showed presence of HBs antigen and anti-virus C antibodies. HIV-test was negative. ALAT 8.9 iu/L, ASAT 19.2 iu/L. Electrophoresis showed: total proteins 8.2 g%, albumin 23.8%, α_1 -globulin 3.2%, α_2 -globulin 7.2%, β -globulin 6.6%, γ -globulin 59.2% (Figure 1).

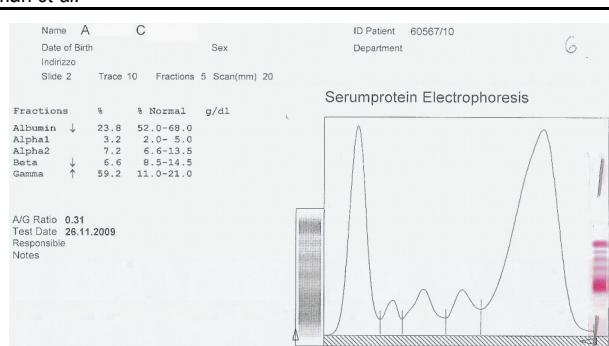


Figure 1 – Serum protein electrophoresis for patient A.C.

Immunoelectrophoresis: IgA 111.6 iu/L, IgG 6929 iu/L, IgM 552.3 iu/L. The Montenegro reaction (IDR to *Leishmania*) was not performed. Bone marrow exam showed bone marrow smears rich in mononucleates cells. Erythroid series 35%, uncoiled, with erythroblastic anisocytosis. Granulocytic series 30%, light deviation to the left. Lympho-plasmocyte series 33%, heteromorphic plasmocytes uni- and binucleous, lympho-plasmocytes, lymphocytes. Megakaryocyte series was of 1–2%, with frequent thrombocytogenic megakaryocyte. Bone marrow biopsy showed hypercellular bone marrow (Figure 2) with numerous histiocytes containing amastigotes (Leishman–Donovan bodies) (Figures 3–7).

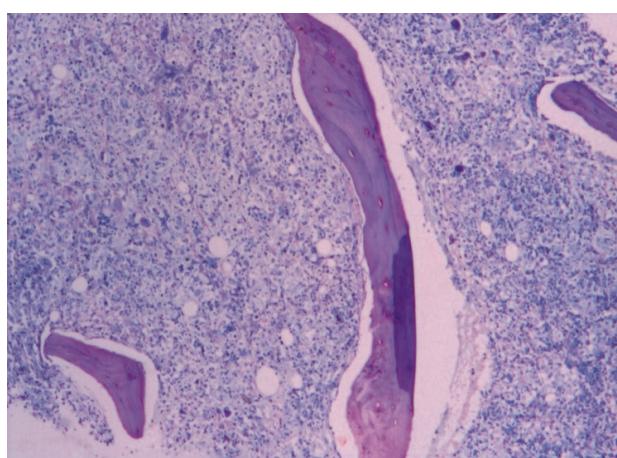


Figure 2 – BMB: hypercellular marrow (Giemsa stain, ob. 4×).

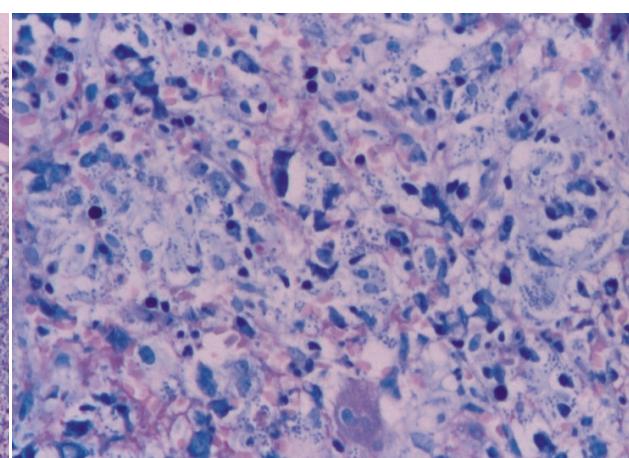


Figure 3 – BMB: numerous histiocytes including amastigotes (Giemsa stain, ob. 20×).

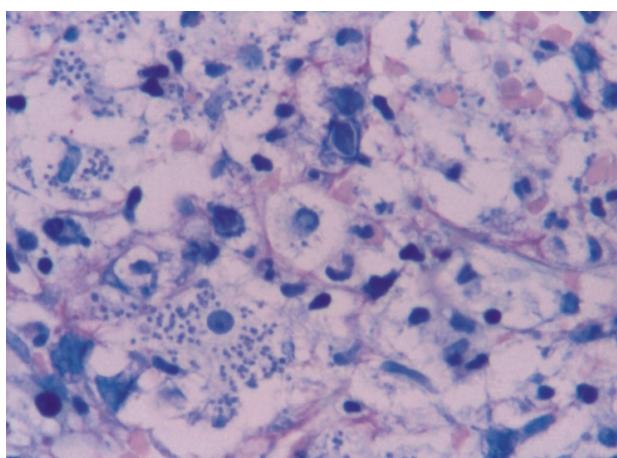


Figure 4 – BMB: amastigotes are intracellular and on extracellular areas (Giemsa stain, ob. 40×).

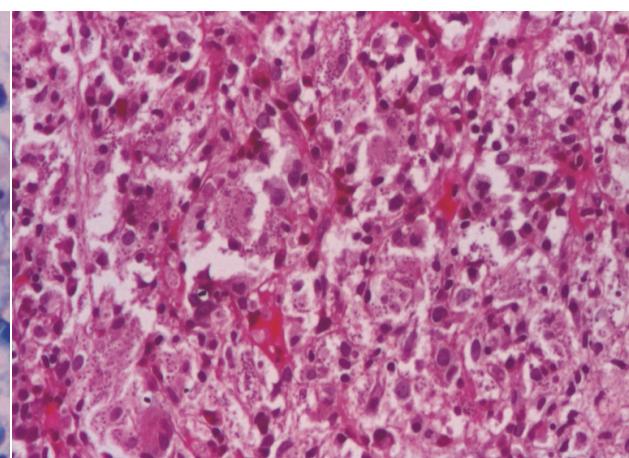


Figure 5 – BMB: the inclusions are visible on PAS stain, ob. 20×.

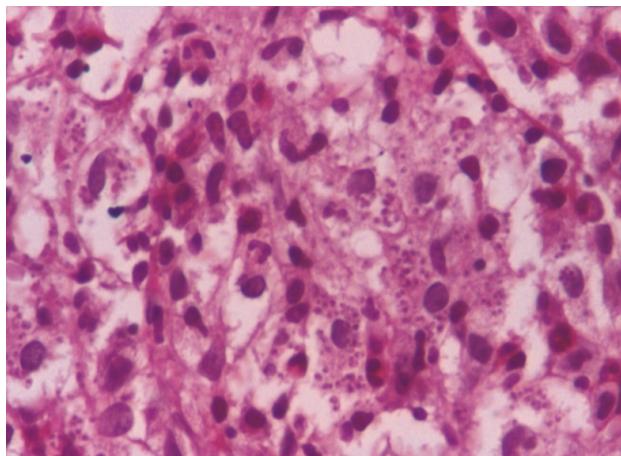


Figure 6 – BMB: the inclusions are visible on PAS stain, ob. 40×.

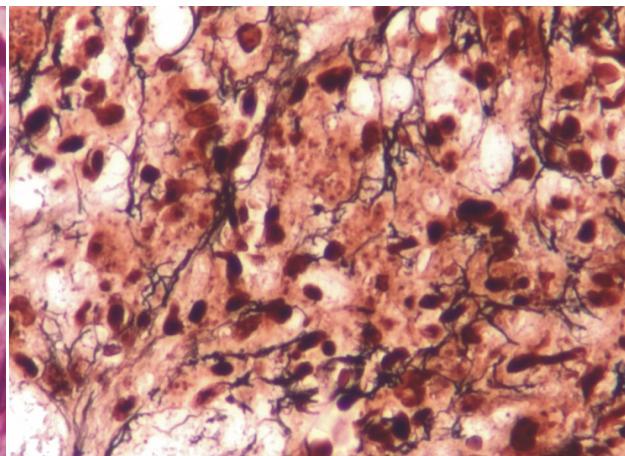


Figure 7 – BMB: amastigotes are weakly positive on Gömöri silver stain, ob. 40×.

Discussion

Positive diagnosis was difficult because of the rarity of leishmaniasis in temperate zones. Anamnesis was important because we found out of the long trip of the patient in a Mediterranean zone where phlebotomus is present. Organomegaly (enlarged lymph nodes, hepatomegaly, splenomegaly), irregular fever, transient periods of obtundation, pancytopenia, electrophoresis showed hypergammaglobulinemia and immunoelectrophoresis with hyper-IgG and hyper-IgM made positive diagnosis difficult. Bone marrow biopsy showed Leishman–Donovan bodies, intracellular and/or extracellular had a decisive role in establishing the diagnosis.

Differential diagnosis was made with: myeloid metaplasia with myelofibrosis (important splenomegaly, pancytopenia, white bone marrow punctuation; collagenic sclerosis to bone marrow biopsy); chronic malaria (similar evolution, without epidemiologic context, fever with ague); Waldenström's disease (difficult differential diagnosis, physic examination and the similar evolution, bone marrow infiltration with lymphoplasmacytes more than 20%, electrophoresis with hypergammaglobulinemia, IgM monoclonal peak to immunoelectrophoresis, hepatosplenic tuberculosis (rarely, positive IDR to PPD, in figurative epidemiological context), Hodgkin's or non-Hodgkin's malignant lymphomas stage IV (excluded through lymph node biopsy, histopathologic exam and immunohistochemistry). Histoplasmosis and brucellosis were offcast through specific serologic tests.

The feature of the case was represented by the rarity of the disease in our geographical area (the last case of visceral leishmaniasis was described in Romania, in 1954), difficult differential diagnosis with Waldenström's disease, which is rarely at the younger patients.

The prognosis of the patient was unfavorable in the absence of specific treatment, especially in the presence of associate comorbidities (presence of anti-virus C antibodies and HBs antigen). In the literature, there are quoted healing until 100% in case of utilization of antimony, miltefosine.

Our patient was symptomatic treated (erythrocyte transfusion, granulocytic growth factors, B-group vitamins), the case was stated nominally and coordinated to Infectious Diseases Clinic.

Conclusions

The case report presents a rare form of visceral leishmaniasis in our geographical area, with severe evolution in the absence of specific treatment and in context of associated comorbidities, in which bone marrow biopsy through the prominence of numerous histiocytes containing amastigotes (Leishman–Donovan bodies), had a decisive role in establishing the diagnosis.

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