CASE REPORT

Polyclonal hypergammaglobulinemia and high smooth-muscle autoantibody titers with specificity against filamentous actin: consider visceral leishmaniasis, not just autoimmune hepatitis

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Summary Visceral leishmaniasis (VL) remains a public health problem in most countries bordering the Mediterranean basin. Its diagnosis is challenging and often delayed, as the main clinical picture is often indistinguishable from that of other infectious and non-infectious diseases. Herein, we report two unusual cases of VL that presented with several characteristics of autoimmune hepatitis (AIH). Neither patient had a history of fever, only generalized symptoms accompanied by polyclonal hypergammaglobulinemia, cytopenias, signs of portal hypertension, elevated transaminases, and high titers of antinuclear and smooth-muscle autoantibodies (SMA) with reactivity against filamentous actin (F-actin), which has been recognized as specific to AIH. A clinical diagnosis of AIH was considered, but a bone marrow biopsy was performed before a liver biopsy to exclude a primary bone marrow disease. The biopsy led to the diagnosis of VL. The diagnosis was further confirmed by IgG antibodies against Leishmania spp. using ELISA and PCR-based assays. Treatment with amphotericin in the first case and pentamidine in the second (because of a severe reaction to amphotericin) was effective. From the clinical point of view, it should be emphasized that, in cases with high titers of anti-F-actin AIH-specific SMA accompanied by polyclonal hypergammaglobulinemia, the possibility of AIH should be cautiously differentiated from VL; this distinction is of paramount importance because initiation of immunosuppression for
Introduction

Leishmaniasis is still a public health problem in most countries bordering the Mediterranean basin. Its diagnosis is challenging and often delayed, as the main clinical picture (fever, generalized symptoms, cytopenias, hypergammaglobulinemia and hepatosplenomegaly) is often indistinguishable from that of other infectious and non-infectious diseases.

Herein, we report two afebrile cases of visceral leishmaniasis (VL) that presented with several characteristics of autoimmune hepatitis (AIH). So far, only three cases have been published of VL presenting as presumed AIH for which immunosuppression had been initiated. The importance of the differential diagnosis between these two conditions is also described.

Case report

In November 2007, a 74-year-old male presented complaining of anorexia, abdominal pain, fatigue and weight loss of 15 kg during the past three months without fever. He suffered from coronary artery disease, arterial hypertension and diabetes mellitus. He was an alcohol consumer (30–50 g ethanol/day for the past 50 years), but not a smoker. Physical examination revealed petechiae in his legs and hepatosplenomegaly. Laboratory work-up showed: hemoglobin 9.2 g/dl; leukocytes 1900/μl; platelets 83,000/μl; a high erythrocyte sedimentation rate (ESR, 100 mm/hour); AST (100 IU/L); ALT (109 IU/L); albumin 2.4 g/dl; globulins 5.3 g/dl; serum immunoglobulin G (IgG) 6700 mg/dl (upper normal limit: 1690 mg/dl); serum immunoglobulin A (IgA) 630 mg/dl; IgM 290 mg/dl; IgE 140 KU/L; platelets 83,000/μl; a high erythrocyte sedimentation rate (ESR, 100 mm/hour); AST (100 IU/L); ALT (109 IU/L); albumin 2.4 g/dl; globulins 5.3 g/dl; serum immunoglobulin G (IgG) 6700 mg/dl (upper normal limit: 1690 mg/dl). Serum protein electrophoresis revealed a polyclonal IgG pattern with no monoclonal component detected.

Bence–Jones protein in urine was undetectable. Chest X-ray was normal. Repeated blood, urine and throat cultures, as well as antibodies against fungi, brucella and several viruses (including hepatitis B and C viruses), were negative. Upper gastrointestinal endoscopy revealed signs of portal gastropathy, suggesting portal hypertension. Serionmunological testing gave high positive results for antinuclear antibodies (ANA 1:640 of discrete speckled pattern by indirect immunofluorescence (IIF); positive titer >1:40), smooth-muscle autoantibodies (SMA 1:640; positive titer >1:40) with the characteristic glomerular/tubular (G/T) pattern in IIF on rodent multi-organ (kidney-liver-stomach) sections, and perinuclear staining of anti-neutrophil cytoplasmic antibodies (p-ANCA 1:80; positive titer >1:10). Further testing gave a high positive result for IgG-specific SMA against filamentous actin (IgG anti-F-actin SMA 118 units; positive titer >20 units) on a commercial internationally accepted ELISA (Quanta Lite Actin IgG assay; Inova Diagnostics, San Diego, CA, USA). A clinical diagnosis of probable AIH type-1 (AIH-1)-related cirrhosis leading to portal hypertension and hyperspleninic manifestations was made, based on the high IgG concentrations, high titers of specific anti-F-actin SMA and the absence of markers of viral hepatitis. Accordingly, a liver biopsy was planned to confirm the clinical diagnosis.

However, a bone marrow biopsy that was carried out first to exclude a primary bone marrow disease showed numerous protozoan parasites (Leishmania spp.) within macrophages (Figure 1). Subsequently, whole blood testing by PCR using specific primers for Leishmania spp. and IgG antibodies against Leishmania spp. in serum by semi-quantitative ELISA (Leishmania Ab, Cypress Diagnostics, Langdorp, Belgium; positive sample ratio >1.1) proved to be positive (sample ratio: 5.2). According to the manufacturer’s instructions, the sample ratio is calculated by dividing the sample OD = optical density by the cut-off value obtained by adding 0.15 to the average OD of two negative controls. For the PCR procedure, 200 μl of peripheral blood, which was collected in EDTA tubes, was processed for DNA extraction using the QIAmp DNA mini-kit (QIAGEN, Hilden, Germany) according to the manufacturer’s instructions. PCR for the amplification of the small subunit ribosomal RNA gene (ssu-rRNA) of all Leishmania species was carried out using the following primers, designed by us (GenBank accession number X07773): 5′-ttagaccgaccaagcagact-3′ (sense; nucleotides 1158–1178) and 5′-gatcccaatctaggtggt-3′ (anti-sense; nucleotides 1281–1300). 5 μl of template corresponding to 200 ng of total DNA was then analyzed in a 50 μl reaction volume containing 2 mM MgCl2, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 0.4 mM of each primer, 1.25 U of Taq DNA polymerase and 0.2 mM dNTP mixture. The reaction was carried out through cycles in a DNA thermocycler at the following specifications: initial cycle at 95 °C for 4 min followed by 40 cycles at 94 °C for 30 s, 60 °C for 30 s and 72 °C for 30 s, with a final extension period at 72 °C for 10 min. A band of approximately 142 base pairs was obtained, corresponding to the...
specific amplification of the ssu-rRNA gene. Each amplification run contained several negative controls (heat detergent buffer) and a positive control, including DNA prepared from promastigotes of *Leishmania panamensis* strain MHOM/CO/86/1166.

Therefore, the liver biopsy was postponed and the patient was then treated for VL with liposomal amphotericin B 3 mg/kg/day on days 1–5, 14 and 21 (total dose 21 mg/kg) intravenously. Prompt resolution of his symptoms and complete regression of liver and spleen size were recorded at the end of treatment. Of note, a sharp decrease in the titers of non-organ-specific autoantibodies was also recorded, namely, ANA 1:80, SMA 1:80, negative p-ANCA and IgG anti-F-actin SMA (14.6 units by ELISA). At an eight-month follow up, there was no recurrence of his symptoms. When seen in June 2008, he had remained well, with complete correction of previous anemia, leukopenia and thrombocytopenia.

In January 2008, an 81-year-old male presented complaining of paroxysmal cough, constipation and malaise for the past two months, but no fever. His past medical history included splenectomy due to an accident, appendectomy, Parkinson’s disease, arterial hypertension and Billroth II gastrectomy due to peptic ulcer disease. He neither smoked nor consumed alcohol. On physical examination, the patient was pale and the liver was palpable 4 cm below the right costal margin. Laboratory work-up showed: hemoglobin 9.5 g/dl; leukocytes 1300/μl; ESR 93 mm/hour; albumin 2.9 g/dl; globulins 7 g/dl; IgG 5350 mg/dl; rheumatoid factors (RF) 117 IU/ml (upper normal limit: 15 IU/ml) and presence of serum cryoglobulins. Serum protein electrophoresis revealed a polyclonal γ-globulin pattern with no monoclonal component detected, and absence of Bence–Jones protein in urine. Chest X-ray was normal. Clinical and laboratory tests showed no evidence of infectious diseases, including hepatitis B and C viruses, brucellosis, fungi and tuberculosis. Seroimmunological testing gave positive results for ANA and G/T SMA by IIF (1:640 and 1:320, respectively). Further testing gave a high positive result for IgG-specific anti-F-actin SMA by ELISA (81.6 units). A clinical diagnosis of probable AIH-1-related burn out cirrhosis was made, based on recently published simplified criteria for the diagnosis of AIH. As in the previous case, a bone marrow biopsy that was carried out to exclude a primary bone marrow disease showed the presence of protozoan parasites (*Leishmania* spp.) either extracellular or within bone marrow macrophages. Subsequently, both PCR and IgG antibodies in his serum proved to be positive (sample ratio 4.8) for *Leishmania* spp. Treatment with liposomal amphotericin B was started, but it was discontinued because of a severe anaphylactic reaction. The patient was then treated efficiently with pentamidine (4 mg/kg every other day for 15 doses totally) without side effects. He responded rapidly with complete clinical improvement. Hematological parameters returned to normal within two months, and RF activity and serum cryoglobulins disappeared. A sharp decrease in the titers of non-organ-specific autoantibodies was again recorded 20 days after the completion of treatment, namely, ANA 1:80, SMA 1:80, p-ANCA 1:20 and negative IgG anti-F-actin SMA (17.3 units by ELISA). When seen in June 2008, he had remained well, with normal hemoglobin, leukocytes and platelet counts.

**Discussion**

AIH is a relatively rare chronic necroinflammatory disease of the liver characterized by hypergammaglobulinemia even in the absence of cirrhosis, characteristic autoantibodies and an unfavorable outcome if untreated. The diagnosis is based on a combination of demographic, clinical, laboratory (especially the absence of viral markers of hepatitis B and C), histological and immunological parameters. The detection of non-organ-specific and liver-related autoantibodies remains the hallmark for the diagnosis of the disease. Furthermore, antibodies against F-actin, particularly in high titers, have a close relationship with the SMA G/T pattern on IIF, also known as the microfilament pattern, which is mostly seen in patients with AIH-1. This suggests F-actin as the predominant, if not the sole, target of AIH-1-specific SMA reactivity. However, recent studies have produced conflicting results concerning the specificity of these antibodies in AIH-1 diagnosis. Our cases further support this notion, as both highly anti-F-actin-specific SMA-positive patients were shown to suffer from VL.

Interestingly, there was no history of fever in either of our patients. However, bone marrow biopsies revealed the presence of the parasite, demonstrating the known propensity of *Leishmania* to occur in the reticuloendothelial system. Notably, one of our patients presented with subclinical cryoglobulinemia of RF activity, which has been recently recognized as part of the clinical picture of VL. Therefore, both physician experience and the availability of appropriate diagnostic and therapeutic tools seem to determine the outcome of this disease.

The presence of anti-F-actin AIH-1-specific autoantibodies in VL deserves a brief comment. Evidence exists that soluble parasite-derived antigens from *Leishmania* spp. are mitogenic and trigger production of immunoglobulins with autoantibody activity. The release of sequestered neo-antigens that surround host tissues and cells after cell damage by the parasite could be another possible explanation, as F-actin is a ubiquitous antigen in the human body. Finally, in genetically susceptible patients, molecular mimicry via cross-reactivity of *Leishmania* spp. antigen cannot be excluded. The absence of reactivity against F-actin after efficient treatment for VL in both of our patients further supports these explanations.

The immune response to the infection caused by *Leishmania* spp. includes both T-cell and B-cell activation. However, the pathogenesis of the disease appears to be related to T-cell cytotoxicity, and control of VL depends on the magnitude of T helper 1 (Th1) and multicytokine responses early in the course of the infection. Indeed, it has been shown during progressive infection in mice that Th2 CD4+ T cells expand and secrete interleukin-4, resulting in polyclonal B-cell activation. Later on, in fully established VL, a cellular anergy is established. Inappropriate antigen presentation and communication between the antigen-presenting cells and T cells, as well as the induction of interleukin-10 and interleukin-4, might be the reason for this anergy. The absence of fever in our two elderly cases might be due to the above-mentioned dysregulation of the immune system caused by the infection and also to the known decreased capacity of cytotoxic responses during senescence.

From the clinical point of view, it should be emphasized that, in cases with high titers of anti-F-actin AIH-1-specific...
autoantibodies accompanied by polyclonal hypergammaglobulinemia, transaminasemia and hepatosplenomegaly, the possibility of AIH-1 should be cautiously differentiated from VL. This distinction is of paramount importance because initiation of immunosuppression for AIH-1 treatment will be detrimental to a patient with underlying leishmaniasis. Therefore, in such cases and in areas where the disease is still present, it seems rational to exclude VL before starting any immunosuppressive therapy. Perhaps the very high levels of γ-globulins, although not a surprising finding in AIH, could be used as a surrogate marker to confirm VL in these unusual cases. The use of rapid and specific molecular-based assays, such as PCR, could also be helpful to achieve a correct and timely diagnosis. 7,15

Conflict of interest: No conflict of interest to declare.

References