

Leishmania in SLE mimicking an exacerbation

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ABSTRACT

Systemic lupus erythematosus is a protean disease which may present manifestations that resemble other diseases posing serious problems of differential diagnosis. Visceral leishmaniasis is a parasitic infection, endemic in 88 countries, whose hallmarks may mimic a lupus flare. Fever, pancytopenia, splenomegaly, hypergammaglobulinemia, production of autoantibodies and complement consumption are some of the overlapping features between the two diseases. Thus, extra attention must be paid to patients with lupus who present with the mentioned symptoms. Diagnosis of visceral leishmaniasis relies on the detection of leishmania antibodies, on the presence of amastigotes in bone marrow aspirates, biopsies and cultures of the parasite. Treatment is based on the use of i.v. liposomal amphotericin B. The missed recognition of a leishmania infection in a lupus patient may lead to death, since both the omission of a specific anti-parasite treatment and the increase of the immunosuppressive therapy, in the conviction of a lupus flare, accelerate a fatal outcome. In this paper we present a case of visceral leishmaniasis occurring in a lupus patient. The clinical and laboratory features that overlap in the two diseases and the current literature on the topic were discussed.

Introduction

Visceral leishmaniasis (VL) or Kala-Azar is a systemic protozoan infection, transmitted to humans by sandflies of the *Phlebotomus* species. Dogs are the main host reservoir. Several clinical and laboratory features of the infection resemble SLE. Among them, fever, pancytopenia, hypergammaglobulinaemia, production of autoantibodies, and hepatosplenomegaly are the most frequent ones. Thus, in a patient with lupus, VL may be confused with a relapse of the autoimmune disease. The early response to corticosteroids and immunosuppressive treatment may mask the infection and further aggravate it. It is important for the clinician to know the characteristic features of VL and which symptoms and signs may be similar in the two diseases, because the correct

diagnosis may save the life of the patient, since VL is fatal without a specific treatment (1).

Herein, we describe a severe case of VL occurring in a SLE patient and discuss the overlapping clinical and laboratory features between the two diseases.

Case report

A 33-year-old male, living in central Italy, with a nine year history of SLE, was referred to our division because of high fever, nausea, vomiting, pancytopenia, splenomegaly, and impaired renal function. Clinical manifestations had begun 4 months earlier and had partially and intermittently responded to the increase of the corticosteroid and immunosuppressive treatment, based on the belief of a lupus flare.

Eight years before admission, when he was first diagnosed with SLE, high fever, malaise, butterfly rash, migrating arthralgia, anaemia, leucopenia, elevated acute phase reactants, hypergammaglobulinaemia, pleuropericarditis and moderate splenomegaly were present. At that time, serologic tests demonstrated positive ANA (homogeneous pattern), anti-dsDNA antibody, anti-Sm, anti-RNP, anti-Ro and anti-La antibodies and hypocomplementaemia. Remission was achieved by a combination therapy of i.v. methylprednisolone (MP) pulses, weekly i.v. methotrexate (MTX) and low doses of oral corticosteroids and cyclosporine A (CsA). Four years later, creatinine rose up to 2 mg/dl with proteinuria (6.6 g per 24 hours), haematuria and granular casts in the urine. Renal biopsy showed a diffuse proliferative glomerulonephritis (class IV by the WHO) with a low activity score (7/24) and a medium chronicity score (5/16). Deposition of IgM, IgG, IgA and C3 in the glomeruli, mesangia and basement membrane was shown by direct immunofluorescence assay (IFA). A slight enlargement of the spleen (bipolar diameter 11 cm) was detected by ultrasonography. A brief course of i.v. Cyclophosphamide (CY) and MP pulses was necessary to obtain remission. One year later, the patient experienced two additional renal flares, accompanied by the appearance of Jac-

could's arthropathy, which responded to the same treatment. One year before admission, he voluntarily discontinued the immunosuppressive treatment and remained only on oral corticosteroids. After 9 months, he started complaining of intermittent fever (up to 38°C), dry cough, tachycardia and malaise. Laboratory studies showed anaemia, leucopenia, elevation of acute phase reactants, a positive direct Coombs' test and a worsening of renal function with proteinuria and casts in the sediment. Ultrasonography showed splenomegaly (17 cm bipolar diameter). The patient was treated with MP pulses and CY with remission of fever and amelioration of renal function, pancytopenia and acute phase reactants. However, after a concomitant gastrointestinal infection, his general clinical condition progressively worsened. Immunosuppressive treatment was discontinued and the patient was referred to our hospital for further investigations.

On admission, laboratory parameters showed WBC count 1.440/mm³, neutrophils 770/mm³, lymphocytes 500/mm³, Hb 6.9 g/dl, platelet count 88.000/mm³, ESR 84 mm/h, CRP 12 mg/l, gammaglobulins 3.1 g/dl, IgM 707 mg/dl, IgG 3000 mg/dl and IgA within the normal range. An IgGκ monoclonal component of 2840 mg/dl and an IgGλ monoclonal component of 1710 mg/dl were also detected by immunoelectrophoresis. Renal functional parameters revealed a creatinine of 1.65 mg/dl, a creatinine clearance of 55 ml/min, a proteinuria of 1.6 gr per 24 hours and 30-40 red blood cells in the sediment. Serologic evaluation detected the presence of ANA (1:160; homogeneous pattern), a positive direct Coombs' test, the presence of cryoglobulins (cryocrit 6%) and negativity for anti-dsDNA, anti-ENA and anti-cardiolipin antibodies. C3 and C4 were decreased 31 mg/dl and 8 mg/dl, respectively. Ultrasonography of the abdomen demonstrated a marked spleen enlargement (longitudinal diameter 20 cm, antero-posterior diameter 12 cm) and a moderate hepatomegaly, which were confirmed by computed tomography (CT). A Giemsa-stained bone marrow aspirate demonstrated myriads of *Leishmania* amastigotes

inside and outside phagocytic cells and reactive plasmacells actively synthesising immunoglobulins (Fig. 1). Anti-leishmania antibody titre was 1:640 by indirect IFA and *Leishmania* *Donovani* Infantum was the species revealed. IgG anti-*Leishmania* antibodies were also detected in the cryoprecipitate 1:400 (Fig. 2) by indirect IFA as previously described (2). Treatment with i.v. liposomal amphotericin B (Ambisome: Nextar Pharmaceuticals, San Dimas, CA) at the dosage of 3 mg/kg/day for 5 consecutive days, and a single additional dose on day 10 was started. The

patient's general condition quickly improved with remission of fever. Five days after the last dose of Ambisome laboratory evaluation showed WBC count 5000/mm³, neutrophils 2440/mm³, lymphocytes 2130/mm³, Hb 9.4 g/dl, platelet count 220.000/mm³, ESR 40mm/h, CRP < 5 mg/l, gammaglobulins 3 g/dl, with a IgG monoclonal component of 0.6 g/dl. Renal functional parameters remained constantly altered with a proteinuria of 1.1 g per 24 hours. The patient was finally discharged with a maintenance therapy of prednisone 25 mg/day. A month later,

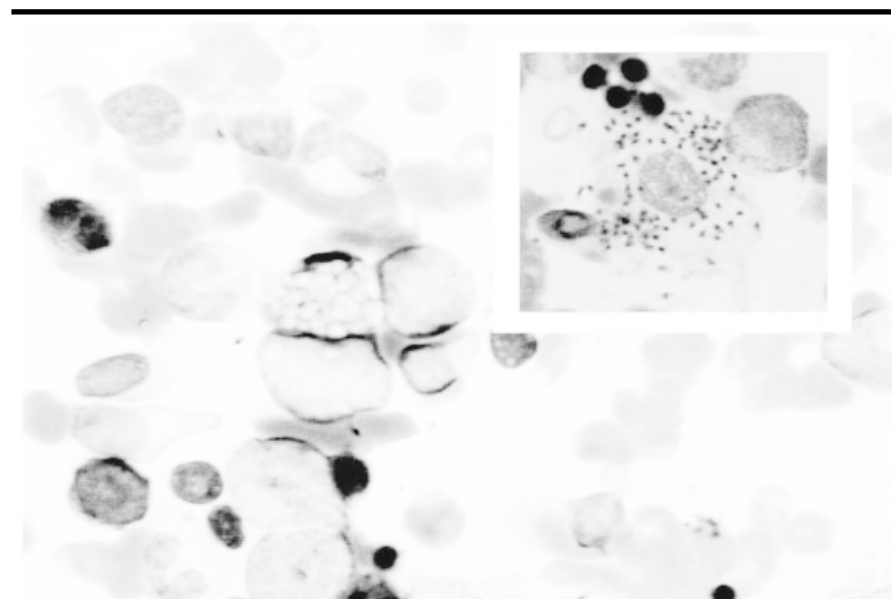


Fig. 1. Giemsa-stained bone marrow aspirate shows myriads of *Leishmania* amastigotes inside and outside phagocytic cells. In the box, plasmacells actively synthesising immunoglobulins.



Fig. 2. Indirect immunofluorescence of anti-*Leishmania* antibodies in the cryoprecipitate. Titre 1:400.

Table I. Characteristics of systemic lupus erythematosus patients with visceral leishmaniasis.

Reference	Country	Fever	ESR	CRP	P	γ	HA	S	Diagnosis	Anti-leishmania	Treatment	Outcome
Wallis	England	High*	140	+	Yes	27 g/L	H	Yes	BM smears +	1/80	SS 600 mg/day i.m. for 25 days	Cured
Altozano	Spain	High*	80	NR	Yes	74 g/L	H	Yes	2 BM smears - Culture +	1/160	3 cycles with MA 20 mg/kg/day i.m. for 15 days	Cured
Fernandez-Guerrero	Spain	High*	NR	NR	Yes	64.6 g/L	H	Yes	BM smears +	NR	2 cycles with SS 600 mg/day i.v. for 2 weeks and then MA 100 mg/kg/day	Exitus
Fernandez-Guerrero	Spain	NR	NR	NR	NR	NR	NR	NR	1 BM smears - Culture +	1/160	MA**	Cured
Braun	Spain	High	40	18	Yes	NR	No	No	BM smears +	1/2560	MA 60 mg/kg/day in 3 daily doses	Exitus
Capell	Spain	High	90	NR	Yes	40 g/L	NR	Yes	BM smears +	1/160	MA 800 mg/day i.v. for 3 weeks	Cured
Granel	France	High	NR	118	Yes	24 g/L	NR	Yes	BM smears +	1/1600	2 cycles of MA**, substituted by LAB i.v. and oral allopurinol	Cured
Our case	Italy	High	84	12	Yes	31 g/L	H	Yes	BM smears +	1/640	LAB 3 mg/kg/die for 5 days + single additional dose on day 10	Cured

*Two daily peak fever (bimodal); **One daily peak fever; †††Dosage not reported; -: negative; +: positive.

P: pancytopenia; γ : gamma globulins; HA: hepato-adenopathy; H: hepatomegaly; S: splenomegaly; BM: bone marrow; SS: Sodium Stibogluconate; MA: Meglumine antimonate; LAB: Liposomal amphotericin B; NR: not reported; ESR: erythrocyte sedimentation rate (mm/h); CRP: C-reactive protein (mg/l).

an abdomen ultrasound scan showed reduction of the enlarged spleen (bipolar diameter of 15 cm). Anti-leishmania antibodies detected by indirect IFA were reduced to 1:100 and a bone marrow aspirate documented the eradication of the infection. Six months later, gammaglobulins returned into the normal range and cryoglobulins and monoclonal components disappeared in the serum. On follow up, as the patient showed persisting proteinuria, mycophenolate mofetil (1.5 g/day) was added. Eight months later, creatinine raised again to 1.7 mg/dl and proteinuria to 2 g per 24 hours. The patient was treated with i.v. CY added to MP pulses with a good control of the disease. To date, no signs suggesting a reactivation of VL have appeared since the patient's discharge from our institution.

Discussion

To date, only 7 cases of VL occurring in SLE patients have been reported (Table I) (2-6). Our patient, who lives in a rural area in central Italy (Abruzzo), endemic for the parasite (7), represents the first case described in Italy. VL is endemic in 88 countries and has an estimated incidence of 500,000 cases per year (8). The Mediterranean Basin, Latin America and Asia are mostly affected. *Leishmania Infantum* is the anthroponotic parasite transmitted in the Mediterranean Basin. Since the incubation period lasts between 2 and 8 months (range 10 days to 2 years) and sandflies life cycle is seasonal, late summer months are the period of highest transmission, with symptoms manifesting mostly from December to April (9).

Humans infected may remain asymptomatic, develop classic Kala-azar or a sub-clinical disease, which resolves in the majority of patients and confers immunity (10-12). Resolution of the infection is highly dependent on the species involved and on efficient host cell-mediated immune responses. Anti-leishmania antibodies are positive in most of the cases; they do not seem to confer protection or to be involved in the direct control of the infection, but they have a major role in the diagnosis of the infection. *Leishmania amastigotes* are often revealed by Giemsa-

stained bone marrow aspirates or by culturing the parasite. They are obligate intracellular parasites of the mononuclear phagocyte system, able to evade the macrophage microbicidal activity and to successfully proliferate within the acidic, hydrolase parasitophorous vacuole (13).

VL clinical features resemble closely some SLE signs and symptoms. Moreover, in immunosuppressed patients, VL may present atypically (9), with less severe clinical and laboratory findings and this may render the two diseases even more alike. Most clinical VL features are the consequence of the host immunologic responses against the parasite (14). Hyperplasia of the infected reticulo-histiocytic system is responsible for the characteristic splenomegaly, which can reach very large dimensions, often accompanied by hepatomegaly and lymphadenopathy. Pancytopenia, a constant haematologic feature, is caused by hypersplenism, bone marrow haemophagocytosis (15), and partly by secondary autoimmune activity (16). A high irregular fever, always present, is resistant to antibiotics, and may present with two daily spikes or in an anarchic manner. Hypergammaglobulinaemia, reflects both polyclonal and specific B cell activation. Autoantibodies such as ANA, anti-Sm, anti-RNP, anti-Ro, anti-La, rheumatoid factor, anti-phospholipid antibodies, anti-platelets, anti-smooth muscle, and cryoglobulins have been detected in the sera of leishmania patients. Positive Coombs' test and complement consumption have also been reported (16-20). The production of autoimmune antibodies in VL seems to be due to polyclonal B cell activation and to molecular mimicry between leishmanial membrane antigens and ribonucleoproteins, as suggested by competition experiments (18). Molecular resemblance may also trigger activation of autoimmune cytotoxic T-cell responses with consequent tissue damage (21). The control of the infection relies on a successful cell-mediated response. In particular, IL-12 driven CD4⁺Th1 responses lead to the production of interferon-gamma which activates macrophages enhancing their

oxygen-dependent and -independent killing parasites capacities (22). While in cutaneous leishmaniasis the polarisation towards an IL-4 driven CD4⁺Th2 response leads to a non-healing outcome, in VL there seems to be a mixed Th1/Th2 activation with Th2 cytokines possibly bearing a protective role and influencing treatment response (23, 24). Whether SLE itself may alter, in some way, the course of LV is still to be determined. Theoretically, unbalanced Th1/Th2 responses occurring in lupus (25) may modify the immune reaction against the parasite.

Other symptoms described in VL (9) are dry intermittent cough, oedema, diarrhoea, abdominal distension, weight loss and renal involvement, all of which can be present in lupus. In particular, in VL patients, proliferative glomerulonephritis with immune deposits and tubulo-interstitial nephritis have been reported (26, 27). Our patient had a previous kidney biopsy that demonstrated a diffuse proliferative glomerulonephritis with immune deposits. After the burst of leishmaniasis his renal function worsened and responded irregularly to corticosteroids and immunosuppressive therapy. In this particular case, both VL and SLE, may have played a role.

In the cases we reviewed from the literature, (see Table) the hallmarks of LV (high fever, pancytopenia, hypergammaglobulinaemia, augmentation of inflammatory indexes) were present in all patients. Interestingly, Braun and colleagues (4) described the case of a patient who died from fulminant pneumococci septicemia after treatment but did not present splenomegaly.

Hepatomegaly was found in 3 of the reported cases, while lymphadenopathy or renal involvement was never reported. Anti-leishmania antibodies were demonstrated, at different titres, in all patients except patient described by Fernandez-Guerrero (1). The presence of leishmania amastigotes was revealed by bone marrow aspirates in 5 cases, in the two remaining patients confirmation of leishmaniasis was possible only by culture of the parasite. This is an important issue, since it underlies the importance of requiring

bone marrow cultures in suspicious cases.

The role of immunosuppressive therapy in uncovering latent leishmaniasis acquired sub-clinically months or years before the appearance of symptoms is supported by several reports (1, 11, 28). Control of the infection is highly dependent on cell-mediated immune responses, that are impaired to some extent in all immunosuppressed patients. Moreover, the initial response of some VL symptoms to corticosteroids and immunosuppressants dosage increase, may further delay a correct diagnosis as it erroneously confirms the suspicion of a lupus relapse.

VL has been described in patients with vasculitidis such as polyarteritis nodosa (28), Wegener disease (29) and type II mixed cryoglobulinemia (16). As in our case, the problem was to recognise the infection as well as to give the right treatment instead of increasing immunosuppression. Modern therapy of VL relies on liposomal amphotericin B for its high effectiveness and low toxicity with respect to parental pentavalent antimonials (30). The reticulo-endothelial system, where the protozoa reside, exhibits a specific uptake of the drug and acts by interrupting the parasite cell wall synthesis. Multiple courses of therapy may be required in immunosuppressed patients. In conclusion, it is essential to suspect a VL in a lupus patient, who presents high irregular fever, pancytopenia, hypergammaglobulinaemia, and splenomegaly. Diagnosis can be made by the presence of anti-leishmania antibodies and the Giemsa-stained bone marrow smears and cultures positivity. The correct therapy may save the patient, while the omission of a specific treatment, with concomitant immunosuppression, may be life-threatening.

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