Visceral Leishmaniasis in a patient with common variable immunodeficiency and Evans syndrome: clinical remarks

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SUMMARY

Visceral Leishmaniasis (VL) is a vector-borne zoonosis endemic in Southern Italy whose usual clinical features include fever, splenomegaly, pancytopenia and hypergammaglobulinemia. The clinical and biochemical picture may be misleading in patients with immunodeficiency diseases hampering the diagnosis. We describe a VL case in a patient whose spleen had been removed and who had Common Variable Immunodeficiency and Evans syndrome.

KEY WORDS: Visceral leishmaniasis, Common variable immunodeficiency, Evans syndrome, Splenectomy, FUO

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Visceral Leishmaniasis (VL) is a potentially life-threatening disease caused by the protozoan *Leishmania infantum* which infects macrophages through the reticuloendothelial system. It is a vector-borne zoonosis transmitted by the bites of phlebotomine sandflies with canids as a common reservoir host and humans as incidental hosts (Gradoni *et al.*, 2003). Sporadic transmission by blood transfusions or needle sharing among intravenous drug addicts is also described. Infections by *Leishmania infantum* are widespread in the Mediterranean basin and are endemic in Southern Italy due to the closeness of dogs, vectors and human habitations. In Italy two hundred cases per year and in the Campania region forty up to eighty cases per year are reported (Gradoni *et al.*, 1996). In humans, the course of the illness depends on both the infectant parasite and the host's immune status, being more severe in immunosuppressed patients such as HIV-infected subjects and transplant recipients or patients treated with corticosteroid/immunosuppressive drugs (Hernández-Pérez *et al.*, 1999; World Health Organization, 1997). A favourable clinical outcome is determined by the T_H1 immune response, with γIFN and IL-2 release (Kemp, 2000).

We describe a case of VL in a splenectomized patient with Common Variable Immunodeficiency (CVID) and Evans syndrome (autoimmune hemolytic anemia plus immune thrombocytopenia) (Evans *et al.*, 1951).

In 2007 a 46-year-old man was referred to our Infectious Diseases Department because of an irregular fever that had started 10 days before, with muscle weakness and no other relevant clinical symptom. In the past he developed autoimmune cytopenias, firstly immune thrombocytopenia (ITP) which required splenectomy, and then autoimmune hemolytic anemia (AIHA) treated with steroid therapy. Subsequently, he was found to have CVID and started cyclic intravenous immunoglobulin therapy carried on discontinuously. Some months before our clinical examination,

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he had been admitted to hospital for pneumonia. At that time, laboratory investigations demonstrated anemia (with positive direct and indirect Coombs tests, decreased haptoglobin, rising unconjugated bilirubin, serum iron and LDH) and thrombocytopenia. The finding of ongoing AIHA plus thrombocytopenia, related to a history of ITP allowed the hematologist to diagnose Evans syndrome, just consisting of AIHA plus ITP. Therapy was carried on with prednisone for 8 weeks with dose tapering. Clinical conditions and blood tests improved but fever reappeared when the therapy stopped.

On admission, he was pale, asthenic, feverish (39°C) and complained of mild headache. On examination only mild hypotension and hepatomegaly were found. A complete blood count showed mild anemia, leucocytosis with lymphocytosis and severe thombocytopenia. Red blood cell (RBC) count was $3.8x10^6$/µL, hemoglobin 9.1 g/dl, white blood cells (WBC) count was $13.1x10^3$/µL with 53% lymphocytes and 40% neutrophils, platelet (PLT) count was $4.9x10^3$/µL. Direct and indirect Coombs tests were positive. Liver biochemical tests showed moderately elevated values (AST 58 IU/L, ALT 105 IU/L, LDH 800 IU/L). Serum proteins, albumin and gammaglogulin value were reduced (5.08 g/dl, 2.9 g/dl, 0.4 g/dl, respectively). All immunoglobulin subclasses were reduced (IgA: 0.20 g/l, IgM: 0.34 g/l, IgG 5.70 g/l). The systemic inflammation indices were abnormal (CRP 65 mg/dl, ESR 42 mm/h, fibrinogen 525 mg/dl) and serum iron was 21 µg/dl. Renal function was normal. Blood culture for bacteria and mycetes, urine culture and stool culture were negative. HIV-RNA, CMV-DNA, EBV-DNA, HCV-RNA, HBV-DNA were absent in his blood. A bone marrow aspirate and biopsy demonstrated *Leishmania* amastigote, inside and outside the hystiocytes. Serum *Leishmania* antibodies were detectable on IFAT, even though with low titre (1/80). Therapy with Liposomal Amphotericin B (LAB) was started, 4 mg/kg IV q24h on days 1-5, 10, 17, 24, 31, 38. On the tenth day PLT count further decreased to $4.3x10^3$/L and bleeding began from gums, nose and rectum. PLT transfusions were provided while LAB therapy was continued in accordance with the scheduling and the patient recovered with normal RBC and PLT count restored. CRP returned to normal value within 40 days. A second bone aspirate was performed 10 days after the therapy stopped, demonstrating lack of *Leishmania*. The patient has remained free of VL in the following ten months.

VL is a cause of "fever of unknown origin" (FUO) in immunocompetent subjects in variable percentages depending on geografic area, patients' age and diagnostic means available. In the Mediterranean basin VL is reported in 4.5% up to 17.1% of FUO (Pasic *et al.*, 2006; Saltoglu *et al.*, 2004). It has been also diagnosed, increasingly, in immunocompromised hosts, like splenectomized, transplantated, HIV-infected, immunosuppressive drug-treated patients and those suffering from hematological malignancies (Bada *et al*., 1979; Basset *et al.,* 2005; Fernandez-Guerrero *et al.*, 2004; Lozano *et al.*, 1996; Pati *et al.*, 1999; Pavone *et al.*, 2008).

In immunocompetent hosts VL generally causes fever, splenomegaly, hepatomegaly, weight loss, pancytopenia-anemia, leukopenia, thrombocytopenia and hypergammaglobulinemia.

Pancytopenia is caused partially by medullary parasitic infiltration and mainly by increased cellular destruction in the enlarged and congested spleen. Hypergammaglobulinemia, mostly IgG, results from polyclonal-B cell activation by *Leishmania* antigens (Berman, 1997). Serum *Leishmania* antibodies on IFAT (≥1/40) are found in more than 90% of the patients. Diagnosis is confirmed by *Leishmania* detection in bone marrow specimens with microscopic examination or, lately, molecular amplification assay (PCR). The protozoan can be also found in liver and spleen tissue or in peripheral blood . In immunocompromised hosts clinical signs and symptoms closely resemble those observed in immunocompetent patients but may be misleading or delayed, allowing a low index of suspicion and a misdiagnosis. Moreover, opportunistic coexistent or superimposed infections from bacteria and viruses, mostly CMV, can modify the clinical picture. To date, transplantated and HIV-infected patients have been the most widely studied among immunocompromised hosts (Antinori *et al.,* 2008; Basset *et al.,* 2005). Fever is the most common symptom. In a recent review concerning VL in immunocompromised hosts, fever was described in 94% of the transplantated and 81% of the HIV infected patients, in comparison with 99% of immunocompetent patients. Splenomegaly was re-

ported in 75% and 74% respectively, *vs.* 98% of immunocompetent patients, hepatomegaly in 42% and 77% *vs.* 86% (Antinori *et al.*, 2008). Among the laboratory findings, leucopenia was the most frequently observed abnormality (93% in transplantated and 90% in HIV-infected patients), followed by anemia (86% and 88%) and thrombocytopenia (85% and 79%). In one liver transplantated patient, Basset reported an isolated AST and ALT increase with no blood cell count abnormality (Basset *et al.,* 2005). *Leishmania* serology was found positive more often in transplanted patients than in HIV-infected patients (92% *vs.* 48%) (Antinori *et al.*, 2008). Direct microscopic examination of bone marrow smears was the most frequently used diagnostic procedure in immunocompromised hosts as well as in immunocompetent ones. Its sensitivity was higher in transplantated than in HIV-infected patients (98% *vs.* 81%), probably due to the bone marrow hypoplasia in advanced HIV disease subjects.

We describe a case of VL in a splenectomized patient with CVID and Evans syndrome, which hampered the diagnosis. CVID is the most prevalent of the primary immunodeficiency diseases and is often associated with infections, mostly of the respiratory tract, malignancies and autoimmune disorders. Evans syndrome is an autoimmune pathology consisting of AIHA plus ITP and has been reported in CVID patients (Chapel *et al.*, 2008; Garcia-Munoz *et al.*, 2008; Wang *et al.*, 2005). Our patient showed anemia and thrombocytopenia associated with leucocytosis and lymphocytosis. The latter features must be related to the previous splenectomy. Anemia with positive Coombs test and thrombocytopenia could have been judged, at the beginning, as an expression of Evans syndrome's relapse. In fact, the patient was Evans syndrome diagnosed on a previous hospital admission. Moreover, as expected, he showed hypogammaglobulinemia with IgA, IgM and IgG subclass deficiency because of his CVID (Schroeder *et al.*, 2007). A bone marrow biopsy was performed because of suspected lymphoid malignancy. The bone marrow specimen disclosed no abnormality in any cell line but heavy infiltration of bone marrow by *Leishmania* amastigotes, thereby establishing the diagnosis. Anti-*Leishmania* serology was also positive (IFAT 1/80). The recovery of immunoglobulin production transiently or permanently following HCV and HIV infection, as well as humoral vaccination response have been reported in patients with CVID (Goldacker *et al.*, 2007; Wright *et al.*, 1987) indicating that this immunodeficiency is associated with potentially reversible defects in immunoregolatory factors and B cell systems. *Leishmania* antibody response in our patient could confirm this concept.

Through a careful review of the scientific literature, this is the first VL case associated with CVID in adult host, few cases of VL having been reported in patients with different kinds of hypogammaglobulinemia (Martin *et al.*, 1996;

TABLE 1 - *Visceral Leishmaniasis in patients with hypogammaglobulinemia.*

Authors	*Years*	*Epidemiology*	*Clinical/laboratory features*	*Treatment*	*Outcome*
Martin JC	1996	24 yrs; X-linked hyper IgM syndrome	Fever, weight loss, hepato-splenomegaly, elevated ALT, GGT, bilirubin; reduced WBC, RBC, total proteins, IgA (on IV IG therapy).	Meglumine antimoniate 20 mg/kg/die for 30 days then amphotericin B for 4 weeks	Recovery
Voutsinas D	2001	26 yrs; drug induced hypogammaglobulinemia	Fever, hepatosplenomegaly, palpable nodes, elevated ALT, GGT, LDH, reduced WBC, PLT, total proteins, γ-globulines, IgG, IgA	LAB	Recovery

Mendoza *et al.,* 1997; Voutsinas *et al.,* 2001; Wright, 1959). Epidemiological and clinical features, therapy and outcome of two of these patients are reported in Table 1. The outcome in treated patients was good. Not many data on VL clinical course in CVID-affected patients are available. It is known that cell mediated immunity and not humoral immunity is the most important protective mechanism against *Leishmania* spp. A consistent principle is that healing and resistance to reinfection are associated with an intact T_H1 cell response essentially based on γIFN- and IL-2 release as well as activation of macrophages able to kill intracellular amastigotes (Kemp, 2000). In patients with CVID the most common abnormality is defective antibody formation but numerous immune system abnormalities have been reported and both humoral and cell-mediated lymphocytic responses are affected. Moreover, our patient had been treated in the past with steroid therapy for Evans syndrome. Probably, this treatment played an additional favourable leading role in protozoan spread and disease development as corticosteroids inhibit T lymphocytes allowing an increasing susceptibility to infections, especially from intracellular pathogens like *Leishmania* (Boumpas *et al.*, 1993; Stuck *et al.*, 1989).

Therefore we had a patient who had undergone spleen removal, had CVID and received steroid therapy for autoimmune complications. So, we chose an anti-*Leishmania* strengthened therapeutic regimen, approved for immunosuppressed patients, consisting of LAB, 4 mg/kg IV q24h on days 1-5, 10, 17, 24, 31, 38 (Meyerhoff, 1999). This therapy proved to be well tolerated and effective. Clinical symptoms passed off within some days. RBC and PLT count normalized and CRP, initially remarkably raised, also reached the normal value within 40 days, confirming it is a reliable marker of the disease's good course**.** In fact, CRP is an acute phase protein that proved of good prognostic value in VL (Bern *et al.*, 2007). In a case control study in children with VL at different stages of the disease, Singh showed that during treatment, mean serum CRP levels were significantly higher in late responders than in early responders ($p<0.001$) (Singh *et al.*, 1999). In another study, Gasim observed that plasma CRP was a serum simple marker useful to identify patients with a high risk of developing post kala-azar dermal leishmaniasis after treatment (Gasim *et al.*, 2000). Unfortunately, there are no data in the literature on the role of PCR in the follow-up of VL in immunocompromised hosts.

Our patient has remained free of Leishmaniasis in the following ten months. We conclude that VL has to be taken into account for diagnosis of FUO in immunocompromised hosts, above all in endemic areas.

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