Leishmania infantum asymptomatic infection in inflammatory bowel disease patients under anti-TNF therapy

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ABSTRACT

Background: In recent years anti-TNF therapy has been associated with leishmaniasis in immunocompromised patients from endemic areas. Nevertheless, data on asymptomatic Leishmania infection in such patients is scarce.

The aim of this study was to determine the prevalence of asymptomatic infection in inflammatory bowel disease (IBD) patients treated with TNF inhibitors living in an endemic area (Catalonia) and to follow up them to study how the infection evolved.

Methods: 192 IBD patients (143 Crohn’s disease; 49 ulcerative colitis) from Catalonia (Spain), an area endemic for L. infantum, were recruited. Peripheral blood samples were collected and tested for anti-Leishmania antibodies by Western blotting (WB). Leishmania kinetoplast DNA was detected in peripheral blood mononuclear cells (PBMC) by a quantitative PCR.

Results: Serology was positive in 3.1% and Leishmania DNA was found in 8.8%, with a low parasitic load and humoral response. The prevalence was 10.9%, patients being considered infected if they tested positive by at least one of the techniques. Eight out of the 21 patients with asymptomatic leishmaniasis were monitored for 3–8 months after the first test. None of them showed an increased parasitemia or humoral response, or developed Leishmania during the follow-up period.

Conclusion: The prevalence of Leishmania asymptomatic infection detected in our IBD cohort is similar to that found in healthy population in close endemic areas. Due to the short monitoring period, it is not possible to reach a conclusion about the risk of Leishmania reactivation from this study.

1. Introduction

Leishmaniasis is a vector-borne infectious disease caused by obligate intracellular parasites of the genus Leishmania. Three main clinical forms can be differentiated, visceral (VL), cutaneous (CL) and mucocutaneous, which develop according to the Leishmania species, strain virulence and host immune response. Leishmania infantum is endemic in the Mediterranean basin and causes cutaneous and visceral disease. It is well established that in this area there is a natural exposure to the parasite and asymptomatic Leishmania infection is present (Riera et al., 2008; Le Fichoux et al., 1999). Whereas most immunocompetent individuals will not develop the disease after Leishmania infection, immunosuppression is a recognized risk factor that may lead to either reactivation of a latent infection or failure to control a new infection. It can also alter the disease symptoms and the response to treatment (Van Griensven et al., 2014).

In recent years the number of reported cases of leishmaniasis in patients being treated with anti-TNF in endemic areas has increased, which suggests a possible heightened risk of developing VL or CL (Zanger and Gabrysch, 2013; Català et al., 2015). The development of unusually large and persistent cutaneous lesions and even visceralization of Leishmania in IBD patients treated with TNF inhibitors or immunosuppressant therapy has also been described (Marcoval et al., 2017; Delgado et al., 2017).

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However, literature on *Leishmania* infection in IBD patients treated with biological drugs is scarce.

The aim of this study was to determine the prevalence of asymptomatic infection in such patients living in an endemic area (Catalonia) and to follow up asymptomatic infected patients to study how the infection evolved.

### 2. Material and methods

#### 2.1. Patients: demographic characteristics and treatment

In a cross-sectional study, 192 IBD patients being treated with TNF inhibitors and living mostly in urban and peri-urban areas of Barcelona (Catalonia) were recruited at the Crohn’s and Colitis Attention Unit in Vall d’Hebron University Hospital from April to October 2016 (143 patients with Crohn’s disease (CD) and 49 with ulcerative colitis (UC)). The criteria for inclusion in this study were that they were adults of over 18 years, of either gender, and did not present any of the characteristic symptoms of leishmaniasis. All patients gave their written informed consent to participate in the study that was approved by the Ethics Committee of the Vall d’Hebron University Hospital. Epidemiological and clinical data were recorded in a clinical interview. The study was conducted according to the tenets of the Declaration of Helsinki. Out of the total, 180 patients had been previously treated or were being treated with biological drugs is scarce.

Demographic and treatment data about the patients are summarized in Table 1.

#### 2.2. Antibody detection and PCR procedure

Peripheral blood samples were obtained by sterile venipuncture and the serum was separated by centrifugation and tested for anti- *Leishmania* antibodies by Western blotting (WB) using a whole *Leishmania* antigen according to Riera et al (1999). The WB assay was performed on 0.1% sodium dodecyl sulfate-13% polyacrylamide gel on a mini-gel system (Bio-Rad). Serum samples were diluted at 1/50, were assayed and a purified form of Pierce™ Protein A (Thermo Scientific) peroxidase conjugate (1/1000 dilution) was used. A molecular weight marker (BioRad) was used. Immunoreactivity against 14 and/or 16 kDa *L. infantum* specific antigen fractions was considered a positive result. *Leishmania* kinetoplast DNA was detected in peripheral blood mononuclear cells (PBMC obtained from 10 mL of EDTA-anticoagulated blood by density gradient centrifugation over Ficoll-Paque) by a quantitative PCR (qPCR) (Mary et al., 2004; Molina et al., 2013). Each amplification was performed in triplicate in a 20 μL reaction mixture containing 1 x iTaq supermix with Rox, 15 pmol direct primer (CCTITTCGTGCTCCGGGTAGG), 15 pmol reverse primer (CCACCGGCCCTATTATACCAA), 50 pmol labeled TaqMan probe (FAM-CCACCGGCCCTATTATACCAA), and 5 μL sample DNA. A non-template control was included in each run as the PCR negative control. Results were expressed as parasite equivalents per ml of blood (p.e./ml), considering values ≥0.001 p.e./ml (threshold cycle tC < 40) as positive (Mary et al., 2006).

A 10-fold dilution series of standard DNA from promastigotes (MHOM/ES/04/BCN-61; *L. infantum* ZMON-1) was used as a calibrator (serial dilution from 10⁷ parasites/mL to 10⁻³ parasites/mL), allowing for the plotting of a standard curve.

Patients with at least one positive test were considered infected and underwent further monitoring; a second blood sample was obtained and serology and qPCR was performed. The differences in characteristics between infected and non-infected patients were determined using Fisher’s test.

### 3. Results

Six out of 192 patients had positive serology results, showing immunoreactivity against 14 and/or 16 kDa antigen fractions (3.1% [95% CI 1.3–6.8%]). 171 patients out of the total could be tested by PCR. Parasite DNA was detected in 15 out of the 171 patients (8.8% [95% CI 5.3–14.1%]), even in the absence of a humoral response. Parasite load ranged between 0.001 and 1 p.e./ml as follows: 1 p.e./ml in one patient, 0.1 p.e./ml in another patient, 0.01 p.e./ml in four patients and 0.001 p.e./ml in nine patients. Twenty-one out of the 192 patients (10.9% [95% CI 7.2–16.2%]) tested positive by at least one of the assayed techniques.
and were considered asymptomatically infected; none of them presented clinical symptoms of active leishmaniasis.

Eight of the 21 positive patients were followed up and a second blood sample was collected between 3 and 8 months after the first test. Follow-up by qPCR was possible in the two patients with parasitemia between 0.1 and 1 p.e./ml. These results are shown in Table 2.

No statistically significant differences were found between the two groups of IBD patients (infected and non-infected) (Table 1) according to gender distribution, age or dog ownership, nor any association between infection and the type of IBD or the administered treatment (biological drugs/immunosuppressant therapy) (p > 0.05).

4. Discussion

*Leishmania* asymptomatic infection has been reported in healthy population living in Mediterranean endemic areas (Riera et al., 2008; Le Fichoux et al., 1999; Sakru et al., 2007). However, the prevalence of asymptomatic leishmaniasis in anti-TNF-treated IBD patients in these areas is unknown. In the absence of a gold standard to measure asymptomatic infection, it is hard to know whether these seropositive individuals who remained healthy were truly infected with *Leishmania*. Techniques such as WB and PCR are considered able to detect cryptic infection, in addition to others such as the skin test and cell-mediated immune response (Riera et al., 2008; Ibarra-Meneses et al., 2016; Carrillo et al., 2015).

To our knowledge, the present study is the first asymptomatic *Leishmania* infection survey in IBD patients receiving biological drugs. Herein, a significant proportion of IBD patients had positive results for *Leishmania* by WB and/or qPCR, with a low and intermittent parasitic load and low humoral response, which is characteristic of asymptomatic infection (Riera et al., 2008; Le Fichoux et al., 1999; Mary et al., 2006). Some studies point out that the presence of antibodies does not always come with a positive PCR in asymptomatic population (Ortalli et al., 2019). This absence of correlation between serological and molecular methods may be explained by a previous resolved infection by *Leishmania* which would show anti-*Leishmania* antibodies but would not detect DNA.

Leishmaniasis is of public health significance and a notifiable disease in Spain. The zones of Spain with the highest incidence rate of leishmaniasis are Catalonia, the Balearic Islands, Madrid, Valencia and the Mediterranean areas of Andalusia. According to the official data (www.isciii.es. Enfermedades EDO, 2017), the overall rate of leishmaniasis in Catalonia was 0.82 cases/100.000 inhabitants in 2017 but the prevalence of asymptomatic infection in this area is unknown.

Although it is difficult to distinguish between a new and a latent infection in patients living in an endemic area, the prevalence detected was similar to that reported in other studies on healthy individuals from close endemic areas in Spain and in other endemic Mediterranean countries (Riera et al., 2008; Le Fichoux et al., 1999; Sakru et al., 2007; Tordini et al., 2011; Pérez-Cutillas et al., 2015; Jiménez-Marco et al., 2016; Maritati et al., 2018).

The main limitations of our study were the difficulty of monitoring all cryptically infected patients and the short duration of the follow-up period since only 8 patients with asymptomatic leishmaniasis were followed up and for a short period of time. During the monitoring period none of our patients showed an increased parasitemia or humoral response, or any reactivation. On the contrary, they became negative or maintained the low results.

In our study, no associations were found between infection and demographical and clinical characteristics. Administered biological medication did not appear to influence infection.

There is controversy about the usefulness of conducting studies to detect the infection status in such patients. Some authors suggest that exposure to *Leishmania* should be determined in patients who need to be kept in an immunosuppressed state (Carrillo et al., 2015). Others recommend serological and molecular screening before initiating anti-TNF therapy (Marcoval et al., 2017; Maritati et al., 2018). In contrast, ECCO Guidelines (Rahier et al., 2014) do not support a general policy of screening for parasitic infections prior to initiating biological treatment, with certain exceptions, such as Strongyloides stercoralis.

Due to the lack of information on subclinical infection in such patients, Zanger et al. (Zanger and Gabrysch, 2013) stress the importance of providing prospective data from a well-defined population to be able to estimate the incidence of clinical infection due to anti-TNF use. Studies like ours carried out on IBD patients and others on patients with rheumatic diseases (Maritati et al., 2018) provide information on the prevalence of *Leishmania* subclinical infection in these specific patients treated with anti-TNF in endemic areas.

5. Conclusions

The prevalence found was similar to that reported in other studies on healthy individuals from close endemic areas. It is not possible to reach a conclusion about the risk of *Leishmania* reactivation from this study due to the short monitoring period. A more complete and longer follow-up would have strengthened our results and would have provided more information about how the infection evolves and if the risk of developing clinical infection is higher than in asymptomatic healthy subjects.

Declarations

Author contribution statement

Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.
M. Guillén: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

N. Borruel, E. Sulleiro, F. Salvador, C. Herrera, V. Rodríguez, Z. Moure, A. Sánchez-Montalvá and I. Molina: Contributed reagents, materials, analysis tools or data.

M. Alcover and D. Berengué: Analyzed and interpreted the data.

C. Riera and R. Fisa: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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