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

M. Carmen Guillén, M. Magdalena Alcover, Natalia Borruel, Elena Sulleiro, Fernando Salvador, Diana Berenguer, Claudia Herrera-de Guise, Verónica Rodríguez, Zaira Moure, Adrián Sánchez-Montalvà, Israel Molina, Roser Fisà, Cristina Riera

Laboratory of Parasitology, Department of Biology, Health and Environment, Faculty of Pharmacy and Food Science, University of Barcelona, Avda Joan XXIII, 27-31, 08028, Barcelona, Spain
Crohn’s and Colitis Attention Unit, Vall d’Hebron University Hospital, Ps Vall d’Hebron, 119-129, 08035, Barcelona, Spain
Department of Microbiology, Vall d’Hebron University Hospital, PROSICS, Ps Vall d’Hebron, 119-129, 08035, Barcelona, Spain
Department of Infectious Diseases, Vall d’Hebron University Hospital, PROSICS Barcelona, Ps Vall d’Hebron, 119-129, 08035, Barcelona, Spain

ARTICLE INFO

Keywords:
Biological sciences
Health sciences
Immunology
Infectious disease
Parasitology
Leishmania
IBD
Anti-TNF

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 leishmaniasis 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).
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.

Demographic 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 different immunosuppressive drugs, including Azathioprine, 6-Mercaptopurine, Methotrexate, 6-Mercaptopurine, and Methotrexate, and only 12 had never been treated with immunosuppressants prior to anti-TNF therapy. 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 and only 12 had never been treated with immunosuppressants prior to anti-TNF therapy. Demographic and treatment data about the patients are summarized in Table 1.

| Table 1. Demographic characteristics and treatment of IBD patients studied. Non-infected and asymptomatic infected patients according to qPCR and WB results. |
|---|
| **Patients’ characteristics n = 192** | **Non-infected patients n = 171** | **Asymptomatic infected patients** n = 21 |
| **Age (years)** | 18-74 (Mdn 41) | 20-68 (Mdn 40) | 20-68 (Mdn 40) | 20-68 (Mdn 38.5) | 28-68 (Mdn 48) |
| **Gender (Male/Female)** | 84/87 | 8/13 | 6/9 | 2/4 |
| **Dog’s ownership (%)** | 50% | 48% | 40% | 67% |
| **Type of IBD** | | | | |
| CD | 132 (77%) | 14 (67%) | 9 (60%) | 5 (83%) |
| UC | 39 (23%) | 7 (33%) | 6 (40%) | 1 (17%) |
| **Biological Drugs** | | | | |
| IFX | 85 (50%) | 10 (48%) | 6 (40%) | 4 (67%) |
| ADA | 80 (47%) | 9 (43%) | 7 (47%) | 2 (33%) |
| GLM | 6 (3%) | 2 (9%) | 2 (13%) | - |
| **Time on BD** | 1-180 (Mdn 53) | 8-144 (Mdn 47) | 8-84 (Mdn 38.5) | 18-144 (Mdn 68) |
| **Concomitant IST** | | | | |
| AZA | 97 (57%) | 10 (48%) | 6 (40%) | 4 (67%) |
| 6-MP | 17 (10%) | 1 (4%) | 1 (7%) | - |
| MTX | 3 (2%) | - | - | - |
| AZA + MTX | 2 (1%) | - | - | - |
| 6-MP + Sirolimus | 2 (1%) | - | - | - |
| No conc. IST | 50 (29%) | 10 (48%) | 8 (53%) | 2 (33%) |

| Patients with at least one positive test were considered to be infected. Mdn: Median. IBD: Type of inflammatory bowel disease. CD: Crohn’s Disease. UC: Ulcerative Colitis. IFX: Infliximab, ADA: Adalimumab, GLM: Golimumab. BD: Biological drug. Time on BD: Time on biological treatment in months. Conc. IST: Concomitant immunesuppressant therapy. AZA: Azathioprine, 6-MP: 6-Mercaptopurine, MTX: Methotrexate. |
and were considered asymptotically 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. Berenguer: Analyzed and interpreted the data.
C. Riera and R. Fisa: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Funding statement
This work was supported by the Generalitat de Catalunya 2017 SGR Project (1008).

Competing interest statement
The authors declare no conflict of interest.

Additional information
No additional information is available for this paper.

Acknowledgements
The authors especially thank the patients for their participation in such an altruistic manner.

References
Carrillo, E., Carrasco-Antón, N., López-Medrano, F., Salto, E., Fernández, L., San Martín, J.V., et al., 2015. Cytokine release assays as tests for exposure to Leishmania, and for confirming cure from leishmaniasis, in solid organ transplant recipients. PLoS Neglected Trop. Dis. 9 (10), e0004179 eCollection 2015. 1(10):1282-1283.

Català, A., Roé, E., Dalmau, J., Pomar, V., Muñoz, C., Yelamos, O., et al., 2015. Anti-tumour necrosis factor-induced visceral and cutaneous leishmaniasis: case report and review of the literature. Dermatology 230 (3), 204–207.

Delgado, T.V., Ruiz, P.C., Munoz, F.B., 2017. Visceral leishmaniasis infection in a patient with crohn’s disease treated with azathioprine. J. Crohns Colitis 1.

Ibarra-Meneses, A.V., Carrillo, E., Sánchez, C., García-Martínez, J., López Lacomba, D., San Martín, J.V., et al., 2016. Interleukin-2 as a marker for detecting asymptomatic individuals in areas where Leishmania infantum is endemic. Clin. Microbiol. Infect. 22 (8), 739.e1–739.e4. Epub 2016 Jun 3.

Jiménez-Marco, T., Fisa, R., Girona-Llobera, E., Cancino-Faure, B., Tomás-Pérez, M., Berenguer, D., et al., 2016. Transfusion-transmitted leishmaniasis: a practical review. Transfusion 56 (Suppl 1), S45–S51.

Le Fichoux, Y., Quaranta, J.F., Aufereuve, J.P., Lelievre, A., Marty, P., Sufia, I., et al., 1999. Occurrence of Leishmania infantum parasitemia in Asymptomatic blood donors living in an area of endemicity in southern France. J Clin Microbiol 37 (6), 1953-1957.

Marcovál, J., Penin, R.M., Sábe, N., Valentí-Medina, F., Bonfily-Ortí, M., Martínez-Molina, L., 2017. Cutaneous leishmaniasis associated with anti-tumour necrosis factor-a drugs: an emerging disease. Clin. Exp. Dermatol.

Maritati, M., Trentini, A., Michel, G., Bellini, T., Almugadam, S., Hanau, S., et al., 2018. Subclinical Leishmania infection in patients with rheumatic diseases under biological drugs. Infection [Epub ahead of print].

Mary, C., Faraot, L., Laumonier, R., Dumon, H., 2004. Quantification of Leishmania infantum DNA by a real-time PCR assay with high sensitivity. J. Clin. Microbiol. 42 (11), 5249-5255.

Mary, C., Faraot, L., Droguot, M.P., Xeridat, B., Schleinitz, N., Cuivenier, B., et al., 2006. Reference values for Leishmania infantum parasitemia in different clinical presentations: quantitative polymerase chain reaction for therapeutic monitoring and patient follow-up. J. Infect. (19), 30291–30299 [Epub ahead of print].

Ortalli, M., De Pascale, A.M., Longo, S., Pascarella, N., Porcellini, A., Ruggieri, D., et al., 2019. Asymptomatic Leishmania infantum infection in blood donors living in an endemic area, northeastern Italy. pii: S0163-4453 J. Infect. (19), 30291–30299 [Epub ahead of print].

Pérez-Catillas, P., Goyena, E., Chitimia, L., De la Rúa, P., Bernal, L.J., Fisa, R., et al., 2015. Spatial distribution of human asymptomatic Leishmania infantum infection in southeast Spain: a study of environmental, demographic and social risk factors. Acta Trop. 146, 127–134. Epub 2015 Mar 20.

Rahier, J.F., Magro, F., Abreu, C., Amuzzi, A., Ben-Horin, S., Chowers, Y., et al., 2014. European Crohn’s and Colitis Organisation (ECCO) Second European evidence-based consensus on the prevention, diagnosis and management of opportunistic infections in inflammatory bowel disease. J. Crohns Colitis 8 (6), 443–468. Epub 2014 Mar 6.

Riera, C., Valladares, J.E., Rieradevall, J.E., Gallego, M., Aisa, M.J., Castillejo, S., Fisa, R., et al., 1999. Serological and parasitological follow-up in dogs experimentally infected with Leishmania infantum and treated with meglumine antimoniate. Vet. Parasitol. 84 (1–2), 33–47.

Riera, C., Fisa, R., Lopez-Chejade, P., Serra, T., Girona, E., Jiménez, M., et al., 2008. Asymptomatic infection by Leishmania infantum in blood donors from the Balearic Islands (Spain). Transfusion 48 (7), 1383–1389. Epub 2008 Apr 18.

Sakri, N., Korkmaz, M., Ozbel, Y., Erbakanlar, H., Sengul, M., Toz, S.O., 2007. Investigation of asymptomatic visceral leishmaniasis cases using western blot in an endemic area in Turkey. New Microbiol. 30 (1), 15–18.

Tordini, G., Puntini, C., Rossetti, B., Samburro, G., Fanetti, A., Cianchini, S., et al., 2011. Which screening for Leishmania infantum in asymptomatic blood donors? Inf. Med. 19 (3), 152–156 [Article in Italian].

Van Griensven, Carrillo E., López-Vélez, R., Lynen, L., Moreno, J., 2014. Leishmaniasis in immunosuppressed individuals. Clin. Microbiol. Infect. 20 (4), 286–299.

www.isciii.es (Instituto de Salud Carlos III), 2017. Quienes somos. Centros propios. Centro Nacional de Epidemiología. Servicios. Protocolos RENAVE. Informes. Enfermedades EDO.

Zanger, P., Gabrysch, S., 2013. Leishmaniasis in the era of tumor necrosis factor alpha antagonist therapy-a research agenda for Europe. Am. J. Trop. Med. Hyg. 89 (1), 105–110. Euro Surveill. 2013 Jul 25;18(30):20542.