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Leishmaniasis in Patients With Inflammatory Bowel Disease: A National Multicenter Study of GETECCU

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ABSTRACT

Background: Leishmaniasis (LI) is a vector-borne illness caused by a protozoan of the genus *Leishmania*. Data on the features of LI in patients with inflammatory bowel disease (IBD) are scarce.

Aim: To describe the characteristics of patients with IBD who present with leishmaniasis, infection outcomes and the risk factors associated with developing visceral leishmaniasis (VL).

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Methods: An observational retrospective study performed in 26 hospitals in Spain, including all adult patients with IBD who developed Leishmaniasis from 2012 to 2022.

Results: A total of 73 patients were included [mean age 48 years; 65% male; 68% Crohn's disease]. Sixty patients (82.2%) presented localized cutaneous Leishmaniasis (CL), 2 (2.7%) diffuse CL, 3 (4.1%) mucocutaneous Leishmaniasis (MCL) and 8 (11%) VL. All patients were under biologicals (69 [94.5%]) or immunosuppressants (IMM) (4 [5.5%]) at Leishmaniasis diagnosis. AntiTNF was used in 97%, while 2 patients (3%) were receiving ustekinumab. Leishmaniasis resolution was achieved by 48% and 96% of the patients after 1 and 12 months, respectively. Biological withdrawal after Leishmaniasis diagnosis was not statistically related to increased rates of infection resolution among patients with localized CL. Age was the only risk factor associated with VL (OR 1.2, 95%CI 1.04–1.39; $p = 0.012$).

Conclusions: Leishmaniasis in patients with IBD doesn't seem to follow a complicated clinical course, even in those with localized CL who do not discontinue biological therapy after infection diagnosis. Age might be a risk factor for developing VL. This infection should be considered for immunosuppressed patients with IBD and suggestive symptoms dwelling or travelling to endemic areas.

1 | Introduction

Inflammatory Bowel Diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), are chronic, progressive and disabling diseases characterized by a remitting and relapsing course. Immunosuppression provides significant therapeutic benefits for treating IBD. Nonetheless, the prolonged use of drugs that cause immunosuppression can lead to a higher incidence of infections [1]. Therefore, patients with IBD who are on steroids, immunosuppressants, or advanced therapies should be regarded as immunosuppressed and susceptible to opportunistic infections [2]. In fact, an increased risk of infections caused by intracellular pathogens, notably *Mycobacterium tuberculosis*, *Listeria monocytogenes* and *Legionella pneumophila*, has been associated with antiTNF therapy [3]. *Leishmania* species have also been described as responsible of infections among antiTNF users [4].

Leishmaniasis (LI) is an opportunistic infection transmitted by vectors, specifically caused by protozoan parasites from the *Leishmania* genus. The vectors responsible are female sandflies belonging to the *Phlebotomus* and *Lutzomyia* genera [5]. Two species, *Leishmania infantum* and *Leishmania tropica*, are transmitted around the Mediterranean basin, one of the European endemic areas. Depending on the *Leishmania* species, strain virulence and host immune response, the illness can lead to three clinical presentations: cutaneous LI (CL) characterized by skin ulcers, sometimes accompanied by additional lesions and/or nodular lymphangitis; muco-cutaneous LI (MCL) affecting mucous membranes and surrounding connective tissues like cartilage structures along with cutaneous LI; and visceral leishmaniasis (VL) impacting internal organs such as the liver, spleen, and bone marrow [6]. VL can be fatal, similar to MCL although the latter occurs less frequently [7]. Although LI is an uncommon infection, in some endemic areas such as southern Europe [8–10] the prevalence of latent infection reaches up to 53% in the adult population. Interestingly, the prevalence of asymptomatic LI in patients with IBD receiving biological drugs is similar to that presented in healthy people from endemic regions [11]. However, screening for parasitic infections prior to immunosuppressive therapy is generally considered unnecessary, although this may be considered for

residents of endemic areas or for relevant travel history [2]. Therefore, it is crucial to increase the suspicion index of LI to make an accurate and early diagnosis. Some short case report series of LI in patients treated with antiTNF drugs have been reported [12, 13]. Nevertheless, the limited sample size of these studies hinders the ability to derive definitive conclusions about the clinical features and outcomes of this opportunistic infection in patients with IBD. Therefore, we aimed to offer a comprehensive clinical characterization of patients with IBD who contract LI, to describe the outcomes of LI and to evaluate the risk factors associated with developing VL in the IBD population.

2 | Methods

2.1 | Study Population/Study Design

We conducted a retrospective, nationwide study at 26 sites in Spain. We included all adult patients > 18 years old with a previously established diagnosis of CD, UC or unclassified IBD, according to international criteria [6, 14, 15] who had confirmed diagnosis of LI later than IBD diagnosis. The timeframe of the study comprised from 2012 to 2022 to ensure that medical records data were accessible and a follow-up of at least 12 months after LI diagnosis. All members of the Spanish Working Group on Crohn's Disease and Ulcerative Colitis (GETECCU) were invited to participate in the research. The study was approved by the Research Board of GETECCU, the local Ethics Committees of each participating centre and all patients gave their informed consent.

2.2 | Data Collection and Definitions

2.2.1 | Inflammatory Bowel Disease

Data collection included demographic and IBD-related features of all patients (age, sex, smoking status, date of IBD diagnosis, disease duration, age at IBD diagnosis, comorbidities and extra-intestinal manifestations). Therapeutic requirements were also recorded, including steroids, IMM (methotrexate, thiopurines

Summary

- Summarize the established knowledge on this subject:
 - Infection by *Leishmania* (LI) in patients diagnosed with inflammatory bowel disease (IBD) is uncommon, occurring in those who dwell or travel to endemic areas for this parasitic disease and who are receiving immunosuppressive treatment, mainly anti-TNF drugs.
 - Its optimal management has not been completely established, particularly regarding the need for the discontinuation of the immunosuppressive treatment that patients receive.
- What are the significant and/or new findings of this study?
 - LI develops not only in patients receiving anti-TNFs but also in those undergoing treatment with immunosuppressants or ustekinumab.
 - This infection in patients with IBD doesn't seem to follow a complicated clinical course, even in those with localized cutaneous LI who do not discontinue biological therapy after infection diagnosis.

and tacrolimus) and advanced therapies (infliximab, adalimumab, golimumab, ustekinumab, vedolizumab and tofacitinib). IBD-related surgeries were also collected. We used the Montreal classification of IBD to classify disease phenotypes according to the extent of UC and location and behaviour of CD [16]. Clinical activity of CD was quantified using the Harvey-Bradshaw index (HBI), while the partial Mayo score was employed to assess UC, both of them at the initial assessment and at 12 months. We documented whether IMM or advanced therapies were discontinued due to LI and whether these treatments were later resumed or substituted with an alternative therapy. Time lapse between LI diagnosis and the restarting of IBD therapy was also collected.

2.2.2 | Leishmaniasis

All patients included in the study had been diagnosed by clinical features alongside one or more of the following methods: parasitological examination (histopathology, microscopy and/or parasite culture), serological technique and/or molecular diagnostics [17]. According to the clinical manifestations, LI was divided into (1) CL (localized and disseminated) (2) MCL and (3) VL. Disseminated CL was defined by the presence of more than 10 polymorphic skin lesions distributed on more than two non-contiguous parts of the body [18]. Resolution of LI was defined by lack of VL, MCL or CL signs and symptoms, and no requirement for rescue treatment. The date of LI diagnosis and time lapse from IBD diagnosis to LI, starting date of IBD therapies to LI and onset of symptoms to LI diagnosis were collected. The number and location of the skin lesions, the treatment used for LI, and the outcomes of the infection at 1 and 12 months of follow-up were recorded. Laboratory measurements at the time of LI diagnosis were also documented, including C-reactive protein (CRP), white blood cell and platelet counts, albumin, haemoglobin and calprotectin. LI-related hospitalizations and relapses of LI during 12 months of follow-up were also recorded.

2.3 | Statistical Analysis

Continuous variables were expressed as means with standard deviations and categorical variables as absolute and relative frequencies. Differences between the VL and MCL or CL groups were analysed using the two-tailed *t* test and Mann-Whitney *U* test for normally and non-normally distributed quantitative variables, respectively. Categorical variables were expressed as proportions and compared with the Chi-square test. Univariate and multivariate logistic regression analyses were performed to assess potential risk factors for developing VL. Only variables with a *p* value < 0.1 in the univariate analysis were entered into the regression model. Estimations of crude odds ratios (ORs) and their 95% confidence intervals (CIs) were calculated using logistic regression analysis. *p*-values < 0.05 were considered statistically significant.

3 | Results

3.1 | Patients' Characteristics

A total of 73 patients with IBD (48 male [66%] and 50 CDs [68%]) were diagnosed with LI from 2012 to 2022. Figure 1 shows the geographical distribution of the reported patients in Spain, highlighting that 88% (*n* = 62) lived in the Mediterranean basin area. In the whole cohort, mean age was 48.3 ± 15.06 years. Mean time from IBD diagnosis to LI was 10.5 ± 7.8 years. Patients' characteristics are described in Table 1. Extensive colitis was present in 78% of UC patients. Most patients were in clinical remission at the time of LI diagnosis (*n* = 65, 89%), as well as 12 months after LI (90%). Mean time lapse from the beginning of biological therapy to LI diagnosis was 3.9 ± 2.8 years. It was significantly shorter in the subgroup of patients with VL compared to the subgroup of patients with MCL/CL (1.2 ± 1.6 years vs. 4.1 ± 2.8 , *p* = 0.01).

Patients with VL were significantly older than those with MCL or CL (65.3 ± 7 vs. 46.2 ± 14.4 , *p* = 0.0001), were older at IBD diagnosis (50.6 ± 17.6 vs. 31.9 ± 13 , *p* = 0.0001) and showed a higher prevalence of comorbidities (87% vs. 41%, *p* = 0.014). In CD, age of IBD onset > 40 years was significantly more frequent among VL patients (80 vs. 17%, *p* = 0.008).

Regarding IBD therapy, all patients were immunosuppressed at LI diagnosis; 4 (5.5%) patients were on IMM, whereas 69 patients (94.5%) were under biological agents. No cases of LI were associated with vedolizumab or tofacitinib. In the CL or MCL group, biological agents were used more frequently than among VL patients (96.9% vs. 75%, *p* = 0.01). Seventeen out of 69 patients (24.6%) received an intensified biological regimen at baseline.

3.2 | Leishmaniasis' Features

Table 2 summarizes the main clinical and analytical features of LI. The time lapse between the beginning of symptoms and the LI diagnosis was 4.79 ± 4.25 months (range 0–24), without differences between patients with CL or MCL and VL (4.4 ± 6.2 vs. 4.8 ± 4.1 , *p* = 0.83). Figure 2 shows a representative lesion of

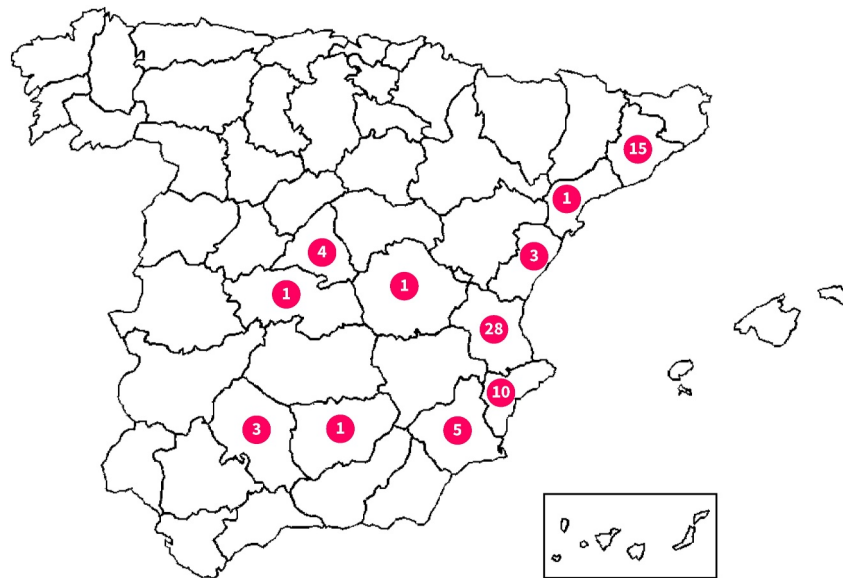


FIGURE 1 | Geographical distribution of leishmaniasis cases in patients with inflammatory bowel disease in Spain.

MCL affecting the palate area in a patient with inactive UC under infliximab treatment. The mean number of cutaneous lesions was 2 ± 2.6 (range 1–15). Among those patients diagnosed with VL, fever was present in up to 75% (6 out of 8), followed by weight loss and malaise ($n = 4$, 50%) and abdominal pain ($n = 2$, 25%). Splenomegaly was found in 4 out of 8 patients with VL (50%) and hepatomegaly in 1 patient.

3.3 | Treatment and Outcomes of Leishmaniasis

In the whole cohort, 25% of patients were hospitalized due to LI, more frequently among patients with VL versus those with CL or MCL (87.5% vs. 16.9%, $p = 0.04$). A detailed description of treatment requirements and clinical outcomes regarding the type of LI is provided in Table 3.

Forty-eight percent of patients achieved LI resolution 1 month after diagnosis, whereas 96% resolved the infection at 12 months without significant differences between the CL or MCL and VL groups. In addition, no differences in the rate of LI resolution at 1 and 12 months between patients with standard versus intensified biological therapy (46 vs. 35%, $p = 0.43$; 97.4% vs. 100%, $p = 0.57$, respectively) (Supporting Information S1; Table S1) or in patients under biologicals plus IMM versus those on biological monotherapy (Supporting Information S1; Table S2) were observed.

Relapse of LI during 12 months of follow up occurred in 8 patients, 6 with localized CL, 1 with disseminated CL, and 1 with VL, all of those under antiTNF therapy. Two out of 8 patients who relapsed (25%) had continued antiTNF after LI diagnosis. Nevertheless, 75% of patients who suffered a previous relapse had LI resolution 12 months after LI diagnosis. Patients under monotherapy with biologicals relapsed less frequently than those treated with a combination of biologicals plus IMM and/or

steroids at baseline (5.1% vs. 20.7, $p = 0.049$, Supporting Information S1; Table S2).

Following the diagnosis of LI, baseline biological treatment was temporarily stopped in 71% of cases (49 out of 69 patients). Of these, 28 patients eventually resumed treatment after suspension. Treatment was permanently withdrawn in approximately 30% of cases (21 out of 69 patients). The time lapse between LI and the restarting of the biological agent was 6 ± 7 months (range 1–29 months) in the whole cohort.

Among patients who withdrew biologicals, the new started therapies were infliximab ($n = 1$, 5.3%), adalimumab ($n = 1$, 5.3%), ustekinumab ($n = 7$, 36.8%), vedolizumab ($n = 3$, 15.8%), mesalamine ($n = 4$, 21%), whereas no maintenance treatment was prescribed in 3 patients (15.8%).

Three out of 4 patients (75%) under IMM monotherapy at baseline in the whole cohort permanently withdrew this kind of therapy and started vedolizumab, mesalamine and no IBD maintenance therapy, respectively. The other patient stopped azathioprine temporarily and restarted it after healing of localized skin lesions.

All patients with VL receiving antiTNF ($n = 6$) stopped these drugs after LI diagnosis. Four out of these 6 patients (66.7%) received combination therapy with IMM and 3 (50%) received antiTNF plus IMM and steroids. Four out of 6 patients with VL on antiTNF therapy resumed this drug after 10 ± 7 months without relapse of the infection. Regarding the 2 remaining patients with VL on antiTNF who permanently withdrew this treatment, one started mesalamine and the other one did not receive any IBD maintenance treatment.

Twenty patients (29%) among the whole cohort continued biologicals despite LI diagnosis; 19 of them presented localized

TABLE 1 | Patients' baseline characteristics.

	Whole cohort, <i>n</i> = 73	MCL and CL, <i>n</i> = 65	VL, <i>n</i> = 8	<i>p</i>
Male gender, <i>n</i> (%)	48 (66)	41 (63)	7 (87)	0.17
Age, years (mean ± SD)	48.3 ± 15.06	46.2 ± 14.4	65.3 ± 7.2	0.0001
Active smoking, <i>n</i> (%)	14 (19)	12 (18)	2 (25)	0.9
Comorbidities, <i>n</i> (%)	34 (47)	27 (41)	7 (87)	0.014
IBD type, <i>n</i> (%)				0.6
CD	50 (68)	45 (69)	5 (62)	
UC	23 (31)	20 (31)	3 (37)	
Age at IBD diagnosis, years (mean ± SD)	34.1 ± 14.7	31.9 ± 13	50.6 ± 17.6	0.0001
Disease duration, years (mean ± SD)	14.3 ± 8.08	16 ± 14.1	14.5 ± 7.1	0.63
Montreal Classification CD, <i>n</i> (%)				
A1/A2/A3	3 (6)/35 (70)/12 (24)	3 (6.7)/34 (75)/8 (17)	0/1 (20)/4 (80)	0.008
L1/L2/L3/L4	22 (44)/6 (12)/22 (4)/ 5 (6)	20 (44)/5 (11)/20 (44)/ 5 (7)	2 (40)/1 (20)/2 (40)/0	0.84
B1/B2/B3	34 (68)/8 (16)/8 (16)	31 (69)/7 (15)/7 (15)	3 (60)/1 (20)/ 1 (20)	0.92
Perianal disease, <i>n</i> (%)	21 (42)	19 (42)	2 (40)	0.92
Extraintestinal manifestations, <i>n</i> (%)	23 (31)	20 (31)	3 (37)	0.69
Montreal UC extent, <i>n</i> (%)				0.32
E2/E3	5 (21)/18 (78)	5 (25)/15 (75)	0/3 (100)	
History of abdominal IBD surgery, <i>n</i> (%)	14 (19)	12 (18)	2 (25)	0.65
History of perianal IBD surgery, <i>n</i> (%)	13 (26)	12 (27)	1 (20)	0.74
IBD clinical activity at LI diagnosis, <i>n</i> (%)	8 (11)	7 (11)	1 (12)	0.88
Biologicals or IMM 6 months prior LI, <i>n</i> (%)	70 (96)	62 (95)	8 (100)	0.53
IMM monotherapy at LI diagnosis, <i>n</i> (%)	4 (5.5)	2 (3.1)	2 (25)	0.01
Azathioprine	2 (3)	1 (50)	1 (50)	
Methotrexate	1 (1)	0	1 (50)	
Tacrolimus	1 (1)	1 (50)	0	
Biologicals at LI diagnosis, <i>n</i> (%)	69 (94.5)	63 (96.9)	6 (75)	0.01
Infliximab, <i>n</i> (%) of patients on biologicals)	39 (56.5)	36 (57.1)	3 (50)	
Adalimumab, <i>n</i> (%) of patients on biologicals)	26 (37.7)	23 (36.5)	3 (50)	
Golimumab, <i>n</i> (%) of patients on biologicals)	2 (2.9)	2 (3.2)	0	
Ustekinumab, <i>n</i> (%) of patients on biologicals)	2 (2.9)	2 (3.2)	0	
Biologicals plus IMM, <i>n</i> (%) of patients on biologicals)	27 (39.1)	23 (36.5)	4 (66.7)	0.14
Biologicals plus IMM and steroids, <i>n</i> (%) of patients on biologicals)	4 (5.7)	1 (1.5)	3 (37)	0.0001
Biologicals plus steroids, <i>n</i> (%) of patients on biologicals)	2 (2.8)	2 (3.1)	0	NA

Note: E: CU extent; E2: left-sided; E3: extensive; A: age on CD onset; A1: < 16 years; A2: 17–40 years; A3: > 40 years; L: CD location; L1: ileum; L2: colon; L3: ileocolonic; L4: upper tract; B: CD behaviour; B1: Inflammatory; B2: stenosing; B3: penetrating.

Abbreviations: IBD, inflammatory bowel disease; CD, Crohn's disease; UC, Ulcerative colitis; IMM, immunosuppressants; LI, Leishmaniasis; SD, standard deviation.

CL, and 1 MCL. When we analysed the outcomes in the subgroup of patients with localized CL according to biological discontinuation after LI diagnosis, no differences regarding IBD activity, LI resolution and rate of LI relapse at 12 months were observed (Table 4). Cutaneous lesions resolved more frequently

1 month after LI among patients who continued biological therapy versus those who discontinued therapy (63% vs. 36%, $p = 0.048$), even in the subgroup of patients with more than 1 skin lesion, although this difference did not reach statistical significance (71% vs. 31%, $p = 0.081$).

TABLE 2 | **Leishmaniasis' features.**

	MCL and CL (<i>n</i> = 65)	VL (<i>n</i> = 8)	<i>p</i>
Type of LI, <i>n</i> (%)			
Localized CL	60 (82.2)	NA	NA
Disseminated CL	2 (2.7)	NA	NA
MCL	3 (4.1)	NA	NA
VL	NA	8 (11)	NA
Number of skin lesions, mean $\pm$ SD (range)	2 $\pm$ 2.6 (1–15)	NA	NA
Location of skin lesions, <i>n</i> (%)		NA	NA
upper extremities	26 (40)	NA	NA
lower limbs	22 (33.8)	NA	NA
face	17 (26.1)	NA	NA
trunk	7 (10.7)	NA	NA
Location of mucocutaneous lesions, <i>n</i> (%)			
nasal	2 (66.7)	NA	NA
palate	1 (33.3)	NA	NA
Symptoms, <i>n</i> (%)			
Fever	NA	6 (75)	NA
Weight loss and malaise	NA	4 (50)	NA
Abdominal pain	NA	2 (25)	NA
Signs, <i>n</i> (%)			
Splenomegaly/hepatomegaly	NA	5 (62)	NA
Lymph node involvement	NA	0	NA
Serum parameters at baseline, mean $\pm$ SD			
Albumin, g/dL	4.2 $\pm$ 0.47	3.1 $\pm$ 0.56	0.0001
Haemoglobine, g/dL	14.03 $\pm$ 1.4	9.7 $\pm$ 1.45	0.0001
White blood cells/uL	7439 $\pm$ 9217	2072 $\pm$ 1696	0.16
Platetets/uL	249,000 $\pm$ 63,550	100,857 $\pm$ 49,522	0.0001
CRP, mg/dL	2.3 $\pm$ 4.9	2.3 $\pm$ 4.18	0.54
Calprotectin, ug/g	281.2 $\pm$ 517.15	224.8 $\pm$ 137.59	0.61

Abbreviations: CL, cutaneous leishmaniasis; CRP, C-reactive protein, LI, leishmaniasis; MCL, mucocutaneous leishmaniasis; SD, standard deviation; VL, visceral leishmaniasis.

3.4 | Risk Factors Associated to Visceral Leishmaniasis

Table 5 shows the results of the univariate and multivariate analysis of the predictors for developing VL among patients with IBD. Univariate analysis found that the presence of comorbidities was more frequent in patients with VL vs. those with CL or MCL (87.5% vs. 41.5%, $p = 0.014$). On the other hand, VL patients were significantly older and were diagnosed with IBD later than patients with CL or MCL. Biological therapy was more frequent among patients who suffered CL or MCL (96.9% vs. 75%, $p = 0.01$).

Finally, the multivariate analysis revealed that age was the only risk factor for developing VL (OR 1.2, 95%CI:1.04–1.3, $p = 0.012$), while biological therapy was a factor associated with an increased risk of cutaneous or mucocutaneous disease vs. VL

(OR 39.7, 95%CI:1.2–1220, $p = 0.035$). The width of 95%CI may be due to the small size of the VL subgroup.

4 | Discussion

This study shows the largest cohort of patients with IBD who develop LI reported to date. We describe that patients with IBD who develop LI live in endemic regions and are immunosuppressed, mostly due to anti-TNF, though some are on immunosuppressants or ustekinumab. The clinical outcomes of both IBD and LI are favourable in the short- and long-term. Localized CL is the most common clinical presentation, solving in almost 50% of patients after 1 month, regardless of biological withdrawal. In addition, older patients bear a higher risk of developing VL. Nevertheless, these data should be interpreted with caution, as our study only detected 8 cases of VL.



FIGURE 2 | A mucocutaneous leishmaniasis lesion on the palate area in a patient with ulcerative colitis under infliximab monotherapy.

Predictably, almost 90% of the reported cases of our research involved patients dwelling in the Mediterranean basin area. In fact, LI is endemic in large parts of southern Europe [8–10, 19]. Similarly, a recent brief case report series and literature review including a total of 19 patients diagnosed with IBD and LI, described that 68% of them resided in the Mediterranean basin [12].

Based on clinical symptoms, it can be categorised into (1) CL (localized and disseminated), (2) MCL, and (3) VL or ‘kala-azar’. We found that more than 80% of IBD patients presented localized CL, whereas VL was only diagnosed in 11% of our cohort. These findings are consistent with the previous series of patients with IBD and LI [12], highlighting that CL was the most common clinical presentation, reaching more than 75% of patients. Conversely, data from patients diagnosed with inflammatory arthritis [13] under antiTNF therapy and LI yielded that VL was more frequent than cutaneous involvement (17 out of the 32 reported cases). Younger age may be a potential explanation for the lower frequency of patients with IBD developing VL compared with rheumatological patients. In fact, the mean age of patients diagnosed with VL in our cohort was 65 years-old, and age constituted the single independent risk factor for developing VL in the multivariable analysis. Regardless, caution is warranted due to the small size of the subgroup of patients with VL in the present study.

On the other hand, the clinical features of VL in our patients were typical, with prolonged fever in up to 75% of the patients,

TABLE 3 | Treatment and outcomes regarding the type of leishmaniasis.

	MCL and CL (n = 65)	VL (n = 8)	p
Clinical resolution of LI at 1 month, n (%)	29 (44.6)	6 (75)	0.1
Clinical resolution of LI at 12 months, n (%)	62 (95.4)	8 (100)	0.53
Relapse n (%)	7 (11)	1 (12.5)	0.89
Hospitalization, n (%)	11 (16.9)	7 (87.5)	0.04
Treatment LI, n (%)			
Intralesional meglumine antimoniate	26 (40)	NA	NA
Liposomal amphotericine B	36 (55.4)	8 (100)	
Miltefosine	1 (1.5)	NA	NA
Topical antibiotic	2 (3.1)	NA	NA
Number of intralesional injections, mean ± SD (range)	5 ± 4 (1–21)	NA	NA
Temporarily discontinuation of biologicals, n (%)	43 (66.15)	6 (75)	0.1
Definitive discontinuation of biologicals, n (%)	19 (29.2)	2 (25)	0.87

Abbreviations: CL, cutaneous leishmaniasis; LI, leishmaniasis; MCL, mucocutaneous leishmaniasis; SD, standard deviation; VL, visceral leishmaniasis.

which is consistent with previous series [12, 20]. Nevertheless, CL in immunosuppressed patients can lead to atypical presentations. In fact, most patients with CL in our cohort presented more than 1 cutaneous injury, with a mean number of skin lesions of 2 ± 2.6 , ranging from 1 to 15. In line with our results, Marcoval [4] et al. described 3 patients diagnosed with CD and CL, pointing out that the cutaneous lesions were atypical, being unusually large and multifocal. Moreover, other Spanish study [21] included 38 patients diagnosed with CL or MCL while being treated with anti-TNF and compared them with 76 patients with LI without antiTNF exposure. They found that CL, among antiTNF users, presented more frequently as a plaque with a larger median lesion size, ulceration and required a greater median number of intralesional meglumine antimoniate infiltration than in non-exposed patients. However, disseminated CL and MCL, which represent more aggressive forms of infection presentation, were infrequent in our study, occurring in just 3% and 4% of patients, respectively.

The median time lapse from the beginning of symptoms to LI diagnosis was 4 months, without significant differences between patients with VL versus CL or MCL. This diagnostic delay even reached 24 months in one case. Undoubtedly, diagnosing CL can be challenging since it can be misdiagnosed for inflammatory, malignant, or other infectious diseases [22–24]. Therefore, it is worth advising clinicians that persistent skin lesions in immunosuppressed patients with IBD dwelling or travelling to endemic areas should include LI as part of the differential diagnosis. Figure 3 summarizes the risk factors and diagnosis of LI in patients with IBD.

TABLE 4 | Localized cutaneous leishmaniasis outcomes regarding withdrawal of biological therapy after LI diagnosis.

	Patients who maintained biologics, <i>n</i> = 19	Patients who discontinued biologics, <i>n</i> = 39	<i>p</i>
Clinical resolution of LI at 1 month, <i>n</i> (%)	12 (63)	14 (36)	0.048
Clinical resolution of LI at 12 months, <i>n</i> (%)	18 (95)	38 (97)	0.59
Relapse, <i>n</i> (%)	2 (10)	4 (10)	0.97
Need for hospitalization, <i>n</i> (%)	2 (10)	8 (20)	0.34
Clinical remission of IBD 12 months after LI, <i>n</i> (%)	19 (100)	32 (94)	0.28

Abbreviations: IBD, inflammatory bowel disease; LI, leishmaniasis.

TABLE 5 | Predictive risk factors for developing visceral leishmaniasis.

			Univariate analysis	Multivariate analysis	
	MCL and CL (<i>n</i> = 65)	VL (<i>n</i> = 8)	<i>p</i>	OR (95% CI)	<i>p</i>
Comorbidities, <i>n</i> (%)	27 (41)	7 (87)	0.014		
Arterial hypertension	8 (12.3)	4 (50)	0.007		
Diabetes mellitus	2 (3)	2 (25)	0.01		
Age, years ± SD	46.2 ± 14.4	65.3 ± 7.2	0.0001	1.2 (1.04–1.3)	0.012
Age at IBD diagnosis, years ± SD	31.9 ± 13	50.6 ± 17.6	0.0001		
Time lapse between IBD diagnosis and LI, years ± SD	1.2 ± 1.6	4.9 ± 2.8	0.023		
Biologics at LI diagnosis, <i>n</i> (%)	63 (96.9)	6 (75)	0.01	39.7 (1.2–1220.2)	0.035

Abbreviations: CL, cutaneous leishmaniasis; IBD, inflammatory bowel disease; IMM, immunosuppressants; LI, leishmaniasis; MCL, Mucocutaneous leishmaniasis; SD, standard deviation; VL, visceral leishmaniasis.

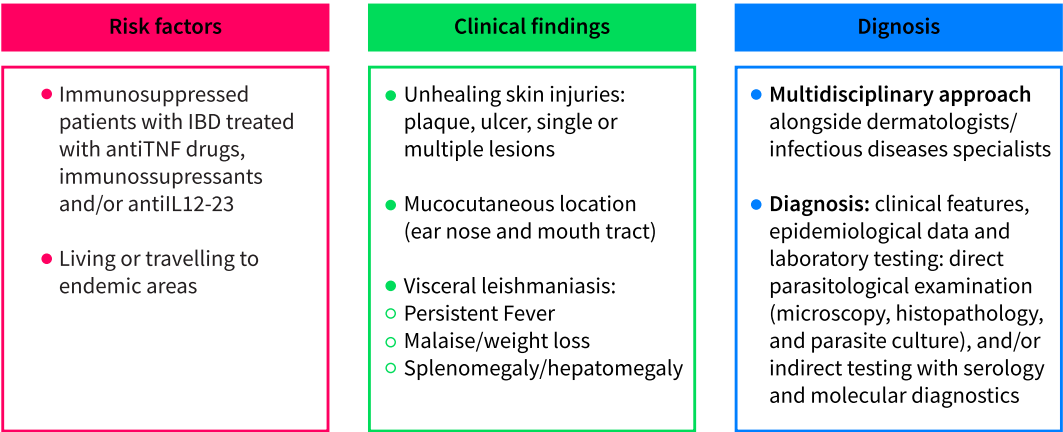


FIGURE 3 | Risk factors and diagnosis of LI in patients with IBD.

Immuno-suppression is a key factor associated with the risk of opportunistic LI, as it has been demonstrated in patients with human immunodeficiency virus (HIV) co-infection [25, 26] or in subjects treated with anti-TNF drugs [27, 28]. Apart from the evidence supported by the series of patients treated with anti-TNF that present LI, several studies carried out in animal models have demonstrated that TNF plays a crucial role in

mediating host protection against CL [29]. In accordance, our results show that 97% of patients under biologics were on anti-TNF therapy at the time of LI diagnosis.

Our patients developed LI between 1 and 4 years of starting biologics, similar to other studies' [13, 27, 28]. Although most of the patients receiving biologics in our cohort were on anti-TNF

agents, it is noteworthy that 2 patients became infected while on ustekinumab monotherapy. A previous case of diffuse CL in a patient being treated with ustekinumab, an anti-IL12/23 inhibitor, had been reported [30], highlighting the potential role of IL-12 in maintaining resistance against some species of *Leishmania* [31]. Nevertheless, anti-IL12-23 drugs have been considered to date as options with a lower risk of developing the infection and its complications. Interestingly, ustekinumab was the drug chosen to restart IBD therapy in 36% of the patients who discontinued their biological therapy in our study. However, considering our findings, further clinical experience and accurate reporting of all clinical forms of LI in patients receiving biological agents other than anti-TNFs are necessary to determine if these treatments truly present a lower risk of LI.

Otherwise, a French nation-based cohort study found that patients with IBD treated with combination of anti-TNF plus IMM had a higher risk of serious and opportunistic infections than monotherapy with anti-TNF [1]. Despite this, we did not find a relationship between the addition of IMM or steroids to biologicals or intensified regimens and worse LI outcomes in terms of cure. On the other hand, combination of biologicals plus IMM and/or steroids was associated with a higher risk of relapse (20.7 vs. 5.1%). However, the small number of patients who experienced relapse of LI prevents us to draw robust conclusions. Nevertheless, these findings emphasise that the specific role of anti-TNF in promoting LI is undeniable, as widely proven through both in vitro and in vivo studies [32, 33], regardless of the addition of other immunosuppressive drugs or the dose of anti-TNF received. In fact, in the present study, 58% of patients received biological monotherapy at the time of LI diagnosis.

On the other hand, LI had a favourable clinical course, with a rate of resolution at 1 and 12 months of almost 50% and higher than 95% respectively. Interestingly, these results did not differ among patients with localized CL who never stopped biologicals compared with those who withdrew. In fact, data supporting the need of withdrawal of biologicals after CL diagnosis are conflicting. Bosch-Nicolau [27] found non-significantly higher rates of LI cure (both VL and CL) among patients withdrawing anti-TNF (94% vs. 70%, $p = 0.13$). Palacios-Díaz [21] also reported a non-significant difference in the cure rate of CL for patients who suspended anti-TNF or not (61% vs. 36%). Nevertheless, some authors argue in favour of stopping biological treatment after LI diagnosis [12].

Other issues regarding the LI therapeutic strategy are the potential relapses associated with immunosuppression. We found a low rate of relapse affecting only 11% of patients, with no significant differences depending on whether they maintained or not anti-TNF in the case of patients with localized CL. In addition, our results showed that IBD clinical remission 12 months after LI was similar among patients with localized CL who withdrew anti-TNF and those who did not stop the treatment. Bearing this in mind, we consider that biological therapy could be maintained in patients with localized CL if necessary for a better IBD control, individualising by LI and IBD severity.

This collaborative study has several strengths to be highlighted. First of all, this is the largest reported cohort of

patients with IBD developing LI to date. Indeed, the sample size enables us to clinically characterise patients who develop this opportunistic infection. In addition, we ensure a follow-up of 12 months allowing evaluation of the outcomes of both IBD and LI in the short- and long-term. Nonetheless, we are aware of some limitations. These limitations are mainly those associated with the study's retrospective design, including the potential for missing data in clinical records. Nevertheless, the collection of data from multiple centres reduces this potential bias. Other limitation to be acknowledged is the absence of a control group that could help better define potential risk factors associated with LI in immunosuppressed patients with IBD. However, the size of our series reinforces clinical data previously reported in case reports and literature reviews, adding valuable and innovative information to the management of this infection.

In conclusion, we present the largest cohort to date of patients with IBD who developed LI. This infection occurs not only in patients receiving anti-TNFs but also in those undergoing treatment with immunosuppressants or ustekinumab. LI doesn't seem to follow a complicated clinical course, even in those with localized CL who do not discontinue biological therapy after infection diagnosis. Age might be a risk factor for developing VL. This opportunistic infection should be considered as part of the differential diagnosis of immunosuppressed IBD patients exhibiting suggestive symptoms, particularly those living or travelling to endemic areas.

Author contributions

LMV and AG: Concept and design, analysis and interpretation of data, drafting of the manuscript. All authors: Data acquisition. AG: Critical revision of the manuscript for important intellectual content. All the authors approved the final version of this article.

Ethics Statement

This study involving human participants was reviewed and approved by the Ethics Committee from Hospital General Universitario Dr Balmis of Alicante (LMV-LEIBD-2023), and the local Ethics Committees of each participating centre. All the patients provided written informed consent to participate in this study.

Conflicts of Interest

AM-C has received financial support for conference attendance, educational activities, and research support from AbbVie, Biogen, Faes Farma, Ferring, Janssen, MSD, Pfizer, Takeda, Dr. Falk Pharma, Lilly and Tillotts. CRC has received education funding from Galápagos, Lilly, Ferring, Tillotts Pharma, AbbVie, Norgine, MSD, Pfizer, Takeda and Janssen. YZ has served as a speaker, consultant and advisory member for or has received research funding from Adacety, Almirall, Amgen, FAES Pharma, Ferring, Janssen, Kern Pharma, Lilly, MSD, Otsuka, Pfizer, Sanofi, Shire Pharmaceuticals, Takeda, Galapagos, Boehringer Ingelheim and Tillotts. The remaining authors declare no conflicts of interest related to this manuscript.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Autorship

Guarantor of the article: Ana Gutiérrez.

References

1. J. Kirchgesner, M. Lemaitre, F. Carrat, M. Zureik, F. Carbone, and R. Dray-Spira, "Risk of Serous and Opportunistic Infections Associated With Treatment of Inflammatory Bowel Diseases," *Gastroenterology* 155, no. 2 (2018 Aug): 337–346.
2. T. Kucharzik, P. Ellul, T. Greuter, et al., "ECCO Guidelines on the Prevention, Diagnosis, and Management of Infections in Inflammatory Bowel Disease," *J Crohns Colitis* 15, no. 6 (2021 Jun 22): 879–913, <https://doi.org/10.1093/ecco-jcc/jjab052>.
3. S. Singh, A. Facciorusso, P. S. Dulai, V. Jairath, and W. J. Sandborn, "Comparative Risk of Serious Infections With Biologic And/or Immunosuppressive Therapy in Patients With Inflammatory Bowel Diseases: A Systematic Review and Meta-Analysis," *Clinical Gastroenterology and Hepatology* 18, no. 1 (2020 Jan): 69–86.
4. J. Marcoval, R. M. Penín, N. Sabé, F. Valentí-Medina, M. Bonfill-Orti, and L. Martínez-Molina, "Cutaneous leishmaniasis Associated With Anti-tumour Necrosis Factor- α Drugs: An Emerging Disease," *Clinical and Experimental Dermatology* 42, no. 3 (2017 Apr): 331–334.
5. B. S. McGwire and A. R. Satoskar, "Leishmaniasis: Clinical Syndromes and Treatment," *QJM Mon J Assoc Physicians* 107, no. 1 (2014): 7–14.
6. E. Torres-Guerrero, M. R. Quintanilla-Cedillo, J. Ruiz-Esmenjaud, and R. Arenas, "Leishmaniasis: A Review," *F1000Res* 6 (2017 May 26): 750.
7. M. S. Bailey and D. N. Lockwood, "Cutaneous Leishmaniasis," *Clinical Dermatology* 25, no. 2 (2007): 203–211.
8. E. Diza, A. Kansouzidou, S. Gerou, E. Veziri, S. Metallidis, and A. Antoniadis, "Leishmaniasis in Northern Greece: Seroprevalence of the Infection and Incidence of the Disease During the Period 2001–2006," *European Journal of Clinical Microbiology & Infectious Diseases* 27, no. 10 (2008): 997–1003.
9. G. Federico, F. Damiano, G. Caldarola, C. Fantini, V. Focchi, and L. Ortona, "A Seroepidemiological Survey on Leishmania Infantum Infection," *European Journal of Epidemiology* 7, no. 4 (1991): 380–383.
10. P. D. Ready, "Leishmaniasis Emergence in Europe," *Euro Surveill* 15, no. 10 (2010): 19505.
11. M. C. Guillén, M. M. Alcover, N. Borrue, et al., "Leishmania Infantum Asymptomatic Infection in Inflammatory Bowel Disease Patients under Anti-TNF Therapy," *Heliyon* 6, no. 5 (2020 May 8): e03940.
12. L. Gimeno-Pitarch, P. Almela, and P. Nos, "Leishmania Infection in Patients With Inflammatory Bowel Disease: Case Series and Literature Review," *Gastroenterology and Hepatology*, English, Spanish 47, no. 1 (2024 Jan): 82–92; Epub 2023 Apr 13. PMID: 37061089, <https://doi.org/10.1016/j.gastrohep.2023.04.001>.
13. L. S. Guedes-Barbosa, I. Pereira da Costa, V. Fernandes, L. M. Henrique da Mota, I. de Menezes, and M. Aaron Scheinberg, "Leishmaniasis During Anti-tumor Necrosis Factor Therapy: Report of 4 Cases and Review of the Literature (Additional 28 Cases)," *Seminars in Arthritis and Rheumatism* 43, no. 2 (2013 Oct): 152–157.
14. H. Gordon, S. Minozzi, U. Kopylov, et al., "ECCO Guidelines on Therapeutics in Crohn's Disease: Medical Treatment," *J Crohns Colitis* (2024 Jun 15): jjae091.
15. T. Raine, S. Bonovas, J. Burisch, et al., "ECCO Guidelines on Therapeutics in Ulcerative Colitis: Medical Treatment," *J Crohns Colitis* 16, no. 1 (2022 Jan 28): 2–17.
16. M. S. Silverberg, J. Satsangi, T. Ahmad, et al., "Toward an Integrated Clinical, Molecular and Serological Classification of Inflammatory Bowel Disease: Report of a Working Party of the 2005 montreal world Congress of Gastroenterology," *Canadian Journal of Gastroenterology* 19, no. Suppl A (2005): 5–36.
17. S. Thakur, J. Joshi, and S. Kaur, "Leishmaniasis Diagnosis: An Update on the Use of Parasitological, Immunological and Molecular Methods," *Journal of Parasitic Diseases* 44, no. 2 (2020 Jun): 253–272.
18. G. U. Machado, F. V. Prates, and P. R. L. Machado, "Disseminated Leishmaniasis: Clinical, Pathogenic, and Therapeutic Aspects," *Anais Brasileiros de Dermatologia* 94, no. 1 (2019 Jan-Feb): 9–16, <https://doi.org/10.1590/abd1806-4841.20198775>.
19. L. Moral, E. M. Rubio, and M. Moya, "A Leishmanin Skin Test Survey in the Human Population of l'Alacantí Region (Spain): Implications for the Epidemiology of Leishmania Infantum Infection in Southern Europe," *Transactions of the Royal Society of Tropical Medicine and Hygiene* 96, no. 2 (2002 Mar-Apr): 129–132.
20. L. Horrillo, A. Castro, B. Matia, et al., "Clinical Aspects of Visceral Leishmaniasis Caused by L. Infantum in Adults. Ten Years of Experience of the Largest Outbreak in Europe: What Have We Learned?," *Parasites & Vectors* 12, no. 1 (2019 Jul 24): 359.
21. R. D. Palacios-Díaz, A. Sahuquillo-Torralba, V. Rocamora-Durán, et al., "Clinicopathological Characteristics of Cutaneous and Mucocutaneous Leishmaniasis in Patients Treated With TNF- α Inhibitors," *JDDG: Journal der Deutschen Dermatologischen Gesellschaft* 21 (2023): 473–480.
22. H. Al-Dwibe, A. Amro, A. Gashout, et al., "A Pyoderma Gangrenous-like Cutaneous Leishmaniasis in a Libyan Woman With Rheumatoid Arthritis: A Case Report," *BMC Research Notes* 11, no. 1 (2018 Mar 1): 158.
23. M. Z. Handler, P. A. Patel, R. Kapila, Y. Al-Qubati, and R. A. Schwartz, "Cutaneous and Mucocutaneous Leishmaniasis: Differential Diagnosis, Diagnosis, Histopathology, and Management," *Journal of the American Academy of Dermatology* 73, no. 6 (2015 Dec): 911–26–927–8.
24. A. Di Altobrando, C. Misciali, B. Raone, L. Attard, and V. Gaspari, "Case Report: Cutaneous Leishmaniasis Misdiagnosed as Pyoderma Gangrenosum," *American Journal of Tropical Medicine and Hygiene* 104, no. 2 (2020 Dec 14): 640–642.
25. J. van Griensven, E. Carrillo, R. Lopez-Velez, et al., "Leishmaniasis in Immunosuppressed Individuals," *Clinical Microbiology and Infections* 20, no. 4 (2014): 286–299.
26. C. B. Meireles, L. C. Maia, G. C. Soares, et al., "Atypical Presentations of Cutaneous Leishmaniasis: A Systematic Review," *Acta Tropica* 172 (2017): 240–254.
27. P. Bosch-Nicolau, M. Ubals, F. Salvador, et al., "Leishmaniasis and Tumor Necrosis Factor Alpha Antagonists in the Mediterranean Basin. A Switch in Clinical Expression," *PLoS Neglected Tropical Diseases* 13 (2019): e0007708.
28. A. Martínez-Doménech, M. García-Legaz-Martínez, J. Magdaleno-Tapia, et al., "Anti-tumour Necrosis Factor-Associated Cutaneous Leishmaniasis: A Single-Institution Experience," *British Journal of Dermatology* 181, no. 2 (2019): 403–405.
29. F. Y. Liew, C. Parkinson, S. Millott, A. Severn, and M. Carrier, "Tumour Necrosis Factor (TNF Alpha) in Leishmaniasis. I. TNF Alpha Mediates Host Protection Against Cutaneous Leishmaniasis," *Immunology* 69, no. 4 (1990 Apr): 570–573.
30. L. Rakotonarivo, D. Lons-Danic, and M. Janier, "Plurifocal Cutaneous Leishmaniasis During Treatment With Ustekinumab," *JAAD Case Rep* 4, no. 4 (2018): 298–300.
31. A. Y. Park, B. Hondowicz, M. Kopf, and P. Scott, "The Role of IL-12 in Maintaining Resistance to Leishmania Major," *Journal of Immunology* 168, no. 11 (2002): 5771–5777.

32. R. G. Titus, B. Sherry, and A. Cerami, "Tumor Necrosis Factor Plays a Protective Role in Experimental Murine Cutaneous Leishmaniasis," *Journal of Experimental Medicine* 170, no. 6 (1989): 2097–2104.
33. H. W. Murray, A. Jungbluth, E. Ritter, et al., "Visceral Leishmaniasis in Mice Devoid of Tumor Necrosis Factor and Response to Treatment," *Infection and Immunity* 68, no. 11 (2000): 6289–6293.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.