Travel Medicine and Infectious Disease 53 (2023) 102570

Contents lists available at ScienceDirect

Travel Medicine and Infectious Disease

journal homepage: www.elsevier.com/locate/tmaid

Leishmania infantum infection after visiting southern Spain in patients on biological treatment; an observational, longitudinal, cohort study

Helena Hammarström [a,*], Javier Moreno [b], Leif Dotevall [c], Ann-Marie Calander [d]

[a] Department of Infectious Diseases, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden, and Department of Infectious Diseases, Region Västra Götaland, Sahlgrenska University Hospital, Gothenburg, Sweden
[b] WHO Collaborating Centre for Leishmaniasis, Centro Nacional de Microbiología, Instituto de Salud Carlos III, CIBERINFEC, Majadahonda, 28220, Madrid, Spain
[c] Department of Communicable Disease Control, Region Västra Götaland, Gothenburg, Sweden
[d] Department of Rheumatology and Inflammation Research, Sahlgrenska Academy at University of Gothenburg, Gothenburg, Sweden

ARTICLE INFO

Keywords:
Leishmaniasis
Immunosuppression
Rheumatic disease
Biological treatment
Tumour necrosis factor alpha inhibitor
Travel medicine

ABSTRACT

Background: Reports of leishmaniasis in immunosuppressed patients after visiting the Mediterranean Basin are becoming increasingly common. Still, awareness of the risk of infection and its clinical manifestations may be insufficient among healthcare professionals in the travellers' home countries.
Methods: This observational, longitudinal study included 47 patients from Sweden with rheumatic disease and ongoing immunomodulatory treatment, who visited a rehabilitation centre in southern Spain where leishmaniasis is endemic. Patients were evaluated for clinical signs of leishmaniasis at baseline and after three years. Patients with leishmaniasis were followed for 4–5 years. The treatment outcome was assessed by clinical evaluation and determination of the cell-mediated immunological response to *Leishmania* by a whole blood cytokine release assay.
Results: Seven patients (15%) were diagnosed with leishmaniasis. The median time from exposure to the onset of symptoms was 3 [1–17] months. The median delay between the onset of symptoms and treatment start was 9 [1–12] months. All patients with leishmaniasis responded well to treatment. Only one patient had a relapse, which occurred within the first year.
Conclusion: Healthcare professionals need to be aware of the increased risk of leishmaniasis for travellers who are immunosuppressed. Knowledge of the symptoms is crucial for a timely diagnosis and early treatment.

1. Introduction

Leishmaniasis is caused by protozoa belonging to the genus *Leishmania*. It is transmitted to humans through the bite of phlebotomine sand-flies. Leishmaniasis can manifest itself as cutaneous (CL), mucocutaneous (ML) or visceral (VL) disease. However, a large proportion of those who become infected develop no clinical symptoms at all, and the prevalence of asymptomatic leishmaniasis is high in endemic areas [1–3]. An impaired cellular immune response is a well-known risk factor for developing symptomatic leishmaniasis, and in recent years, the occurrence of leishmaniasis among immunosuppressed individuals has attracted growing attention [4,5].

Modern treatment algorithms for several rheumatic diseases include the use of biological disease-modifying antirheumatic drugs (bDMARDs). These are often initiated early in the course of the disease [6] and have become increasingly used over the years. Although the risk of certain viral and bacterial infections associated with bDMARD therapy is well known, and different preventive measures have been established [7,8], healthcare professionals may not be well aware of the increased risk of parasitic infections. A previous report suggests that patients treated with anti-TNFα monoclonal antibodies are at an increased risk of leishmaniasis [9], and in a systematic review leishmaniasis was found to be the most common parasitic infection in patients with rheumatic diseases undergoing immune modulatory treatment [10]. Despite this, and the increasing number of reports of leishmaniasis in patients treated with bDMARDs [11–20], this infection can be overlooked, especially in countries where it is not endemic.

Leishmaniasis occurs endemically in South America, Africa, the Middle East, Central Asia and the Mediterranean Basin. Indeed, the prevalence of leishmaniasis in southern Europe has increased over the

* Corresponding author. Infektion Sahlgrenska Universitetssjukhuset/Östra, 416 85, Gothenburg, Sweden.
E-mail address: helena.hammarstrom@infect.gu.se (H. Hammarström).

https://doi.org/10.1016/j.tmaid.2023.102570
Received 28 December 2022; Received in revised form 19 March 2023; Accepted 28 March 2023
Available online 29 March 2023

last decades [21], with Spain reporting the highest incidence [22]. *Leishmania infantum,* the dominant *Leishmania* species in the Mediterranean region, primarily causes CL or VL [23,24], and a small risk of acquiring travel-associated leishmaniasis with severe visceral manifestations does exist. Recently published surveillance data showed that among 190 cases of VL diagnosed in Europe, 88% were acquired in the Mediterranean Basin, with 23% originating from visits to southern European countries, mainly Spain [25]. Many patients with rheumatic disease receiving treatment with bDMARDs are likely to have low disease activity, facilitating their ability to travel. The emerging risk of acquiring leishmaniasis during visits to the Mediterranean Basin, particularly for immunosuppressed travellers, has already been highlighted [26,27], and 'leishmaniasis in the era of anti-TNF therapy' has been identified as an important research topic for Europe [28].

In 2018 our group published a short communication regarding an observation of a cluster of Swedish patients with rheumatic disease, all receiving ongoing treatment with bDMARDs, who became infected with leishmaniasis after visiting a rehabilitation centre in a *Leishmania*-endemic region in southern Spain [29]. A year later, a report was published describing seven Norwegian patients with VL acquired in southern Spain, among whom five were receiving biological treatment [18]. While the frequency of such reports appears to be increasing, studies describing the long-term outcome of immunosuppressed travellers exposed to or infected by *Leishmania* remain lacking.

This paper reports the results of an observational longitudinal study with a five year follow-up period, of a cohort of Swedish patients, all with ongoing bDMARD treatment for rheumatic disease, who had visited a rehabilitation centre in an area of southern Spain where transmission of *Leishmania* had been observed [29]. The aim was to assess the risk for these patients of developing symptomatic leishmaniasis over time, to describe the clinical manifestations and the treatment outcome among the patients who developed clinical disease, and to assess the risk of relapse after treatment.

2. Material and methods

2.1. Inclusion of patients

An observation made in 2016 of three patients receiving anti-TNFα therapy who became infected with *L. infantum* during rehabilitation at a centre in the Alicante region of Spain led us to initiate an outreach investigation to identify possible cases of yet-undiagnosed leishmaniasis. This has been briefly described elsewhere [29].

All adult patients from the southwestern region of Sweden with a rheumatic disease and ongoing treatment with bDMARDs who had participated in a 4-week rehabilitation program at the centre in Spain between January 2014 and April 2017, were contacted by mail in May 2017. All received an informative letter along with a questionnaire to collect information on the presence of symptoms compatible with CL, ML and VL, and on details of current anti-rheumatic treatment, previous travel history, and activities undertaken during the stay at the rehabilitation centre. All patients who returned the questionnaire, along with the three who had previously been diagnosed with leishmaniasis, were included in the study.

2.2. Primary evaluation at baseline

The returned questionnaires were evaluated by a specialist in infectious diseases (H.H.), and all patients who had indicated any symptoms that might be associated with leishmaniasis were contacted by telephone. Patients for whom suspicion of leishmaniasis remained following the telephone interview underwent further medical examination at the nearest regional infectious disease clinic, in consultation with the university hospital responsible for this study. Clinical evaluation was performed according to local routines for the assessment of leishmaniasis, which include the examination of the patient's clinical history, and evaluation of typical skin or mucosal lesions indicating CL or ML, or fever, weight loss, hepatosplenomegaly and/or signs of bone marrow depression indicating VL. In all patients with signs and symptoms suggestive of leishmaniasis, a standard diagnostic work-up was performed including identification of the protozoa by tissue sampling for direct microscopic examination and for polymerase chain reaction (PCR) analysis plus sequencing to identify the responsible *Leishmania* species.

2.3. Follow-up

Patients with no clinical signs of leishmaniasis in the primary evaluation were followed up by telephone three years later. Information was collected regarding symptoms compatible with previous or current leishmaniasis, additional travel history, and changes in anti-rheumatic treatment. Patients with signs suggestive of leishmaniasis underwent further medical examination.

All who received a diagnosis of leishmaniasis were followed up systematically to assess the course of disease, the response to treatment and signs of relapse of infection. Clinical follow-up was performed at 1–2, 3, 6 and 12 months after completing treatment for leishmaniasis, and thereafter once yearly until the end of the study (i.e. a follow-up period of up to 5 years). Treatment response was assessed according to current guidelines [30,31]. For CL and ML, healing was defined as >50% reduction in lesion size along with a flattening of lesions and incipient re-epithelialisation. Complete clinical cure was defined as complete re-epithelialisation. Therapeutic failure was defined as incomplete healing three months after the end of treatment. For VL, healing was defined as the absence of fever and the improvement of laboratory variables. Clinical cure was deemed to have been achieved when laboratory variables normalized.

To determine the cell-mediated immunological response to infection in patients diagnosed with leishmaniasis, a blood sample was collected for analysis by the *Leishmania* cytokine release assay [32]. Whole blood samples were stimulated as previously described [33,34]. Briefly, aliquots of blood were added to an empty tube (negative control), and to another tube containing soluble *Leishmania* antigen (SLA). Both were incubated at 37° C for 24 h. After centrifugation, the supernatant plasma was collected and stored at −20° C prior to cytokine/chemokine analysis. IP-10, MIG, IL-2, IL-10 and IFN-γ were quantified in this plasma using the BD Cytometric Bead Array Human Flex Set (Becton Dickinson Biosciences, USA) following the manufacturer's instructions.

2.4. Ethical considerations

This study was approved by the Regional Ethics Committee of Gothenburg (Reg nr 759-17) and performed in accordance with the ethical standards of the 1964 declaration of Helsinki and its later amendments. Patients diagnosed with leishmaniasis gave informed written consent to be included in the clinical follow-up.

3. Results

3.1. Study cohort and primary evaluation at baseline

A total of 47 patients were included in the study (Fig. 1). In addition to the three patients (patients 1–3) who had already received a diagnosis of *leishmaniasis*, 66 eligible patients were identified by the above-described outreach. Among these, 44 (67%) returned a completed questionnaire. Thirteen of these 44 patients (30%) reported symptoms suggestive of leishmaniasis and were contacted by telephone for further evaluation. For eight patients the symptoms were not consistent with leishmaniasis. For five patients leishmaniasis could not be excluded by telephone and clinical assessment was performed at an infectious disease clinic. Four of these five patients showed a clinical picture compatible with CL and underwent a diagnostic skin punch biopsy.

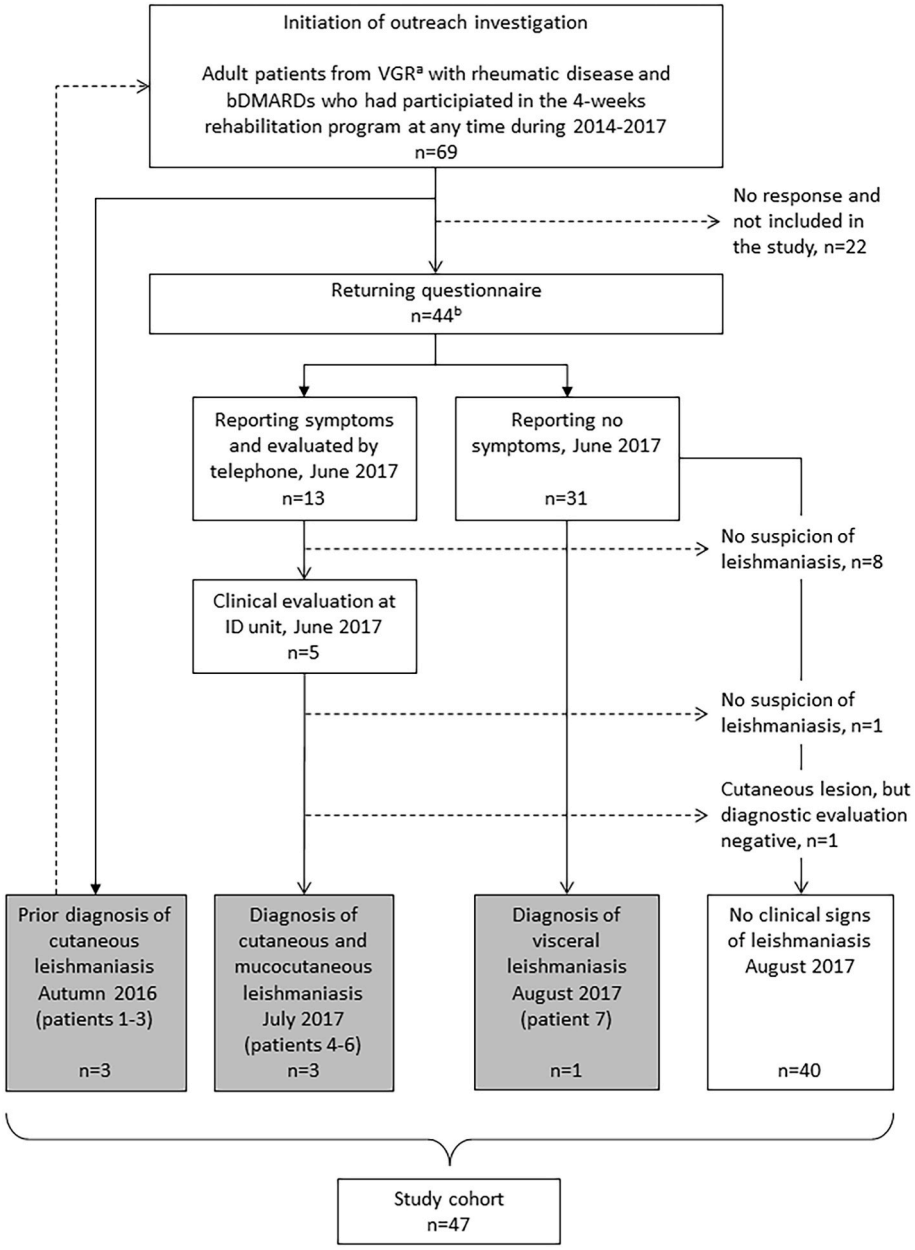

Fig. 1. Flow-chart showing the process of the outreach investigation and the primary evaluation at baseline.
[a]Västra Götalandsregionen, southwestern region of Sweden
[b]not including the three patients with prior diagnosis of leishmaniasis.

Three patients were found to be positive for *L. infantum* by PCR and subsequent sequencing (patients 4–6). One of the patients (patient 7) developed fever two months after the primary evaluation, and was subsequently diagnosed with VL. PCR analysis and sequencing from bone marrow aspirate revealed *L. infantum.* Thus, in total, seven out of 47 patients in this cohort were diagnosed with leishmaniasis in the primary evaluation. The median time elapsed between the visit to the area of transmission (time of possible exposure) and the primary evaluation was 20 [1–38] months. Table 1 provides background information and data regarding the stay at the rehabilitation centre for the whole study cohort.

3.2. Evaluation at follow-up after three years and overall frequency of leishmaniasis

The 40 patients with no clinical signs of leishmaniasis in the primary evaluation were followed up by telephone after three years for a second assessment of clinical signs of leishmaniasis. The median time elapsed between the latest possible exposure at the rehabilitation centre until this final assessment was 56 [37–74] months. Only three patients had stopped receiving bDMARD therapy. No patient had been diagnosed with leishmaniasis over the intervening three-year period. One had developed cutaneous lesions and had received a diagnosis of pemphigoid. The remainder reported no symptoms suggestive of leishmaniasis. The total number of patients with leishmaniasis thus remained at seven after the three years follow-up period. The attack rate of overt leishmaniasis was 7/47 (15%), or 7/28 (25%) when only contemplating those patients who had visited the rehabilitation centre during the late summer (Table 1), the season when *L. infantum* transmission is at its peak.

Table 1
Background data of the whole study cohort at the time point of the primary evaluation.

	All patients n = 47	Patients without clinical signs of leishmaniasis n = 40	Patients with diagnosed leishmaniasis n = 7
Female sex, n (%)	38 (81)	31 (78)	7 (100)
Age, median years (range)	61 (24–73)	60.5 (24–73)	61 (47–72)
Rheumatic diagnosis, n (%)			
rheumatoid arthritis[a]	28 (60)	25 (63)	3 (43)
ankylosing spondarthritis	7 (15)	7 (18)	0
psoriatic arthritis	11 (23)	7 (18)	4 (57)
other	1[b] (2)	1 (3)	0
Type of bDMARD, n (%)			
adalimumab	6 (13)	4 (10)	2 (29)
infliximab	16 (34)	14 (35)	2 (29)
etanercept	9 (19)	8 (20)	1 (14)
golimumab	10 (21)	9 (23)	1 (14)
other	6[c] (13)	5 (13)	1 (14)
Latest stay at rehabilitation centre in Southern Spain			
Time since the latest stay, median no of months (range)	20 (1–38)	20 (1–38)	32 (8–32)
Season during stay, n (%)			
Spring (March–May)	11 (23)	11 (28)	0 (0)
Late summer (August–September)	28 (60)	21 (53)	7 (100)
Autumn (October)	8 (17)	8 (20)	0 (0)
Long-term visit (≥4 weeks) to any other leishmania endemic region during the last 5 years	5 (11)	4[d] (10)	1[e] (14)
Prior visits to the same rehabilitation centre, n (%)	18 (38)	15 (38)	3 (43)

[a] Two patients with rheumatoid arthritis had another concomitant rheumatic diagnosis (ankylosing spondarthritis and psoriatic arthritis, respectively).
[b] SAPHO syndrome.
[c] Leflunomide (n = 1), tocilizumab (n = 1), abatacept (n = 1), tofacitinib (n = 1), ustekinumab (n = 1) and rituximab (n = 1).
[d] Dominican republic (n = 1), Thailand (n = 2), Southern Spain, other region than location of rehabilitation centre (n = 1).
[e] Northern Spain.

3.3. Patients with leishmaniasis

3.3.1. Clinical details prior to treatment

Of the seven patients with leishmaniasis caused by *L. infantum*, five had CL, one had ML, and one had VL. One of the patients with CL had 13 skin lesions; the remaining four had between one and three lesions. No lesion was larger than 15 mm. The patient with ML had an ulcerative lesion in the nostril, but no further mucosal involvement. The patient with VL presented with long term fever, weight loss, pancytopenia and hepatosplenomegaly.

Fig. 2 shows the time axis from exposure until start of treatment for the seven patients with leishmaniasis. The median time from the latest possible *Leishmania* exposure to the onset of clinical symptoms was 3 [1–17] months. In the three patients (patients 1–3) diagnosed prior to the initiation of the study, the median time between the first contact with healthcare until treatment was started was 8 [2–10] months. For the four cases identified in the outreach investigation, the median time until treatment was started following the first contact with healthcare was 2 [1–3] months.

3.3.2. Follow-up and clinical outcome

bDMARD treatment was discontinued in all patients with leishmaniasis at the time of diagnosis. All were treated with liposomal amphotericin B (AmB) according to current guidelines [30]. One patient

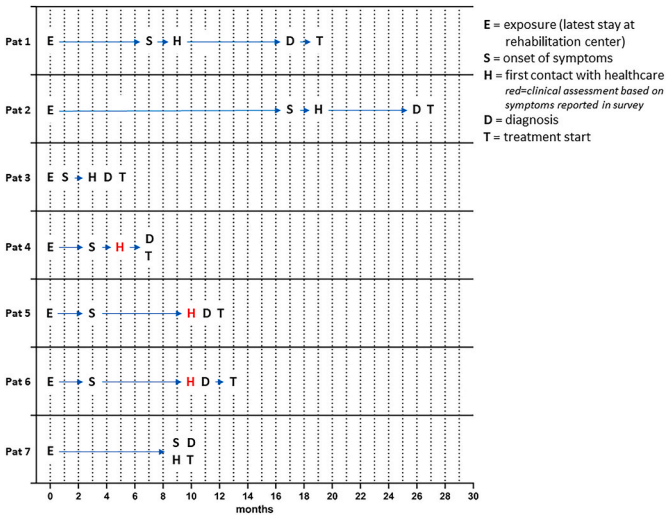

Fig. 2. Time-line showing months from exposure (i.e. last visit to rehabilitation centre) until onset of symptoms, contact with healthcare, diagnosis and start of treatment for leishmaniasis.
Patients 1, 2 and 3 were diagnosed with leishmaniasis based on a routine clinical assessment prior to the initiation of the questionnaire survey. Patients 4, 5 and 6 reported symptoms in the survey and were subsequently subjects to a clinical evaluation. Patient 7 developed symptoms after completion of the survey and was diagnosed based on a routine clinical assessment.

was treated with cryotherapy prior to receiving AmB. The patients were followed until the end of the study, which led to a follow-up period of 4 years for three patients and between 4.5 and 5 years for four patients. Table 2 shows the results of the clinical follow-up. In 6/7 patients, treatment success was confirmed 3 months after the end of treatment. The patient with ML (patient 2) showed incomplete healing at 3 months, defined as treatment failure, but responded well to a new course of treatment with AmB. For all patients, the median time from the end of treatment until complete healing of the infection was 3 [1–6] months. In 3/7 patients (patients 3, 4 and 5), treatment with bDMARD was restarted during the follow-up period, all within three months of the end of treatment for leishmaniasis. Patient 3 developed a relapse infection one year after the initial treatment; cure was achieved three months after cryotherapy. The remaining six patients showed no signs of relapses during the time of follow-up.

3.3.3. Whole blood cytokine release assay

Blood samples for the *Leishmania* cytokine release assay were collected at a median time of 7 [1–10] months after completed treatment for leishmaniasis (Table 2). Determination of IP-10 and MIG concentrations after stimulation with SLA revealed the production of high levels of these two chemokines in all patients. The expression of IFN-γ and IL-2 was variable among the patients. No patient showed any expression of IL-10 induced by SLA stimulus.

4. Discussion

We describe the results of a systematic follow-up study of a cohort of 47 Swedish patients, with rheumatic disease and ongoing treatment with bDMARDs, who had visited a rehabilitation centre in southern Spain where leishmaniasis is endemic. We found a high attack rate of leishmaniasis in this cohort of immunosuppressed individuals, and for the majority of the patients, there was a substantial delay between the onset of symptoms and the confirmation of the diagnosis of leishmaniasis. This highlights the importance of being aware of the risk of contracting this infection in southern Europe, and the need to provide adequate pre-travel advice to immunosuppressed individuals from non-*Leishmania*-endemic countries travelling to the Mediterranean Basin.

Table 2
Clinical follow-up of patients with leishmaniasis.

Pat	Sex/ age	rheumatic disease	DMARD	type of leishmaniasis[a]	treatment	follow-up visits	end of study	time point of sampling for cytokine release assay
						months after the end of the first treatment		
1	F/66	RA	infliximab	CL	AmB 18 mg/kg	complete cure at 3 months	no relapse at 54–60 months	7
2	F/67	PsA	infliximab	MCL	AmB 18 mg/kg	treatment failure at 3 months[b] complete cure at 6 months	no relapse at 54–60 months	8
3	F/50	PsA	adalimumab	CL	AmB 18 mg/kg	complete cure at 3 months relapse at 12 months[c] complete cure at 15 months	no relapse at 54–60 months	10
4	F/61	RA	etanercept	CL	AmB 18 mg/kg	complete cure at 1–2 months	no relapse at 54–60 months	9
5	F/47	PsA	adalimumab	CL	Cryotherapy + AmB 18 mg/kg	complete cure at 1–2 months	no relapse at 48 months	2
6	F/72	PsA	golimumab	CL	AmB 9 mg/kg	complete cure at 3 months	no relapse at 48 months	1
7	F/61	RA	Rituximab[d]	VL	AmB 40 mg/kg	complete cure at 3 months	no relapse at 48 months	7

RA Rheumatoid arthritis; *PsA* Psoriatic arthritis; *CL* cutaneous leishmaniasis; *MCL* mucocutaneous leishmaniasis; *VL* visceral leishmaniasis; *AmB* liposomal amphotericin B.
[a] PCR analysis of biopsy specimen (pats 1–6) and bone marrow aspirate (pat 7) revealed *L infantum*.
[b] The patient received a new course of treatment with AmB, total dose 15 mg/kg.
[c] The patient received cryotherapy.
[d] Previous treatment with abatacept and before that different anti-TNF drugs.

A leishmaniasis attack rate of 15% was recorded for the present cohort of immunosuppressed patients as a whole. The patients who developed leishmaniasis had all stayed at the rehabilitation centre during the season of late summer (August–September), which is the peak season of activity of phlebotomine vectors of *L. infantum* in the Mediterranean region and consequently the period of highest risk of transmission [35,36]. When only considering the 28 patients who had visited the area during this period of high risk of transmission, the attack rate was as high as 25%. It should be noted, however, that 25 of the 69 patients included in the initial outreach investigation did not respond to the questionnaire and were not included in the study. It is unlikely that any of these patients developed leishmaniasis since all received the letter with information regarding symptoms that should warrant contact with an infectious disease clinic. Moreover, in our region, patients with suspected leishmaniasis are attended to in consultation with the university hospital responsible for this study. Any additional cases among the patients not included in the study would therefore very likely have come to our attention. Consequently, if all 69 eligible subjects were included in the study, the attack rate as a whole would presumably have been somewhat lower, yet not less than 10%.

Previous studies on outbreaks of CL among immunocompetent military personnel from non-*Leishmania*-endemic areas deployed to *Leishmania*-endemic regions in Latin America and Afghanistan, report attack rates of 13–25% [37–41]. Although immunocompromised individuals are expected to be at greater risk of developing clinical leishmaniasis after infection [4,42], the attack rate in our study was not higher than that reported in these studies on immunocompetent individuals. This may be explained by differences in the activities undertaken between the studied cohorts, resulting in different degrees of exposure to phlebotomine sand-flies. In a previous study of an urban community outbreak of *L. infantum* infections near Madrid in Spain, seven cases of VL were detected among 68 solid organ transplant recipients living at a median distance of 1.2 km from the focus of the outbreak [43], giving an attack rate of 10% for this group of immunocompromised individuals. However, contrary to our study, this study only included cases of VL, and not CL or ML. The results are, therefore, not readily comparable. To the best of our knowledge, no previous report describes the leishmaniasis attack rate for a cohort of immunocompromised individuals as homogenous as that described here. The present individuals were all *Leishmania*-naïve individuals, all were treated with bDMARDs, and all had an identical duration of stay, under comparable living conditions, in a restricted area with ongoing leishmania transmission. Although the sample size was small, the homogeneity of the studied cohort is a strength that increases the validity of the results.

The median time elapsed between exposure and the onset of symptoms of leishmaniasis was three months and in one case as long as 17 months. Before the patients and clinicians were notified of the ongoing *Leishmania* transmission, there was a substantial delay until a final diagnosis was reached and treatment was started. Other studies describing cases of CL imported into non-*Leishmania*-endemic regions also report long diagnostic delays of 5–84 months [44–46]. Clearly, there is a pressing need for a higher level of awareness among healthcare professionals regarding the risk of travel-related leishmaniasis.

Six patients in our study had CL, out of which one also had mucocutaneous involvement. One of the patients with CL had more than ten lesions, but the rest had few lesions and relatively mild manifestations.

In a recent report on patients with CL in Europe, 36 patients with *L. infantum* infection acquired in the Mediterranean region were described. The patients presented with one to two lesions with a median size of 20 mm. Twenty-two percent of these patients had mucosal involvement [47]. Despite being immunocompromised due to bDMARD therapy, the patients in our study had clinical manifestations similar to those described in the latter report, in which 95% of the patients were fully immunocompetent. Initial treatment for leishmaniasis was successful in six of the seven present patients with complete cure recorded within 1–3 months after the end of treatment. One patient experienced initial treatment failure, but responded well to a repeated course of treatment. The results of the *Leishmania* cytokine release assay performed after completing treatment for leishmaniasis showed that all patients had high levels of IP-10 and MIG after whole blood stimulation with SLA. In a study that assessed the value of this assay in patients that had been treated for VL, high levels of IP-10 and MIG were found to be the most reliable markers of cure [32]. These results support the interpretation of favourable treatment outcomes for the immunosuppressed patients with leishmaniasis in our study. One patient had a relapse of leishmaniasis despite showing high levels of IP-10 and MIG after the first treatment. However, for this patient, bDMARD treatment had been reintroduced prior to the relapse, which might have inhibited the *Leishmania*-specific immune response. Despite ongoing treatment with bDMARDs at the time of *Leishmania* infection, the patients showed a typical clinical picture and a high rate of treatment success was achieved. Although our work is limited by the small number of patients with leishmaniasis, the results agree with those of a systematic review in which a majority of 189 patients with drug-induced immunosuppression and leishmaniasis exhibited a typical clinical presentation and responded well to treatment [48].

As far as we know, this is the first study of a cohort of immunocompromised patients with leishmaniasis that includes a systematic follow-up for as long as five years. Although four patients were restarted on bDMARDs after finishing treatment for leishmaniasis, only 1/7 experienced a relapse infection within the follow-up period, which occurred one year after the end of the initial treatment. In a case series on patients with rheumatic disease treated with anti-TNFα who developed CL, 2/13 patients (15%) for whom follow-up data was available experienced a relapse infection. The median follow-up time in that study was 12 months [46]. In another retrospective study, including information from a case series as well as from a review of published cases of leishmaniasis among patients receiving anti-TNFα treatment, 4/28 patients with CL (14%) relapsed within one year despite etiological treatment [19]. Although our study included a follow-up period of 4–5 years, the relapse rate recorded was no higher than that of the other studies that included follow-up periods of only one year. Moreover, the only case of relapse in our study did indeed occur within the first year. Relapses of leishmaniasis occurring more than one year after treatment thus seem to be rare.

The cohort of patients in our study had acquired leishmaniasis in an area of frequent international tourism in southern Spain. The prevalence of leishmaniasis around the Mediterranean Basin has increased [21], and the risk of travel-related infections is emerging. In recently published data from the LeishMan group of leishmaniasis surveillance in Europe, 799/1044 cases of leishmaniasis diagnosed in Europe (77%) were travel-related, of which at least 39% were acquired during tourist travelling, mainly to Spain [25]. The last decades extensive use of biological treatment for patients with rheumatic diseases has greatly contributed to low disease activity or even remission in many patients, facilitating their ability to travel. In a survey of 273 Danish patients with rheumatic disease treated with bDMARDs, 74% reported a history of frequent travel, and for 81% of these the frequency of travel had remained unchanged or even increased after the initiation of biological treatment [49]. Thus, the risk of travel-associated leishmaniasis in this patient group, also when travelling within Europe, needs to be strongly considered [50]. Pre-travel counselling should include information on symptoms of leishmaniasis that should warrant contact with healthcare providers in order to allow for a timely diagnosis being made and early treatment being started.

A limitation of our study is the small number of patients with leishmaniasis, something that has constrained any in-depth statistical analyses. However, the number of cases could not be influenced as the study comprised a cohort of immunocompromised patients exposed to *Leishmania*, during a specific time period, in a given place. Another limitation is that only 67% of eligible patients responded to the questionnaire and were included in the study. Despite these limitations, the descriptive findings of this study are remarkable in the perspective of a non-endemic country and may also serve to provide guidance for future prospective studies with larger cohorts of patients.

5. Conclusions

A leishmaniasis attack rate of 15% was recorded for this cohort of immunocompromised patients with rheumatic disease who had visited a rehabilitation centre in southern Spain. This finding highlights the risk of contracting leishmaniasis faced by immunosuppressed travellers visiting areas with high rates of *Leishmania* transmission. Although clinical cure was achieved in all patients, the implications of the infections were significant. One patient had severe VL, a condition associated with a high rate of mortality, one had cumbersome mucosal manifestations, and all had to be admitted to hospital for intravenous treatment. The clinical outcome for patients with leishmaniasis is dependent upon timely diagnosis and prompt treatment. Awareness and proper knowledge of leishmaniasis among healthcare professionals in non-endemic countries is thus of greatest concern. Given the increasing incidence of leishmaniasis in the Mediterranean Basin, along with a growing population of immunocompromised patients with frequent travelling, an increasing prevalence of travel-related leishmaniasis is to be expected.

CRediT authorship contribution statement

Helena Hammarström: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Javier Moreno:** Formal analysis, Investigation, Resources, Writing – review & editing. **Leif Dotevall:** Conceptualization, Methodology, Writing – review & editing. **Ann-Marie Calander:** Conceptualization, Methodology, Investigation, Writing – review & editing.

References

[1] Singh OP, Hasker E, Sacks D, Boelaert M, Sundar S. Asymptomatic Leishmania infection: a new challenge for Leishmania control. Clin Infect Dis 2014;58(10):1424–9.

[2] Sisko-Kraljevic K, Jeroncic A, Mohar B, Punda-Polic V. Asymptomatic Leishmania infantum infections in humans living in endemic and non-endemic areas of Croatia, 2007 to 2009. Euro Surveill : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin 2013;18(29):20533.

[3] Michel G, Pomares C, Ferrua B, Marty P. Importance of worldwide asymptomatic carriers of Leishmania infantum (L. chagasi) in human. Acta Trop 2011;119(2–3):69–75.

[4] van Griensven J, Carrillo E, Lopez-Velez R, Lynen L, Moreno J. Leishmaniasis in immunosuppressed individuals. Clin Microbiol Infect 2014;20(4):286–99.

[5] Herrador Z, Gherasim A, Jimenez BC, Granados M, San Martin JV, Aparicio P. Epidemiological changes in leishmaniasis in Spain according to hospitalization-based records, 1997-2011: raising awareness towards leishmaniasis in non-HIV patients. PLoS Neglected Trop Dis 2015;9(3):e0003594.

[6] Smolen JS, Landewé RBM, Bijlsma JWJ, Burmester GR, Dougados M, Kerschbaumer A, McInnes IB, Sepriano A, van Vollenhoven RF, de Wit M, Aletaha D, Aringer M, Askling J, Balsa A, Boers M, den Broeder AA, Buch MH, Buttgereit F, Caporali R, Cardiel MH, De Cock D, Codreanu C, Cutolo M, Edwards CJ, van Eijk-Hustings Y, Emery P, Finckh A, Gossec L, Gottenberg JE, Hetland ML, Huizinga TWJ, Koloumas M, Li Z, Mariette X, Müller-Ladner U, Mysler EF, da Silva JAP, Poór G, Pope JE, Rubbert-Roth A, Ruyssen-Witrand A, Saag KG, Strangfeld A, Takeuchi T, Voshaar M, Westhovens R, van der Heijde D. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2019 update. Annals of the rheumatic diseases 2020;79(6):685–99.

[7] Sepriano A, Kerschbaumer A, Smolen JS, van der Heijde D, Dougados M, van Vollenhoven R, McInnes IB, Bijlsma JW, Burmester GR, de Wit M, Falzon L, Landewé R. Safety of synthetic and biological DMARDs: a systematic literature review informing the 2019 update of the EULAR recommendations for the management of rheumatoid arthritis. Annals of the rheumatic diseases 2020;79(6):760–70.

[8] Furer V, Rondaan C, Heijstek MW, Agmon-Levin N, van Assen S, Bijl M, Breedveld FC, D'Amelio R, Dougados M, Kapetanovic MC, van Laar JM, de Thurah A, Landewé RB, Molto A, Müller-Ladner U, Schreiber K, Smolar L, Walker J, Warnatz K, Wulffraat NM, Elkayam O. 2019 update of EULAR recommendations for vaccination in adult patients with autoimmune inflammatory rheumatic diseases. Annals of the rheumatic diseases 2020;79(1):39–52.

[9] Zanger P, Kotter I, Kremsner PG, Gabrysch S. Tumor necrosis factor alpha antagonist drugs and leishmaniasis in Europe. Clin Microbiol Infect 2012;18(7):670–6.

[10] Fabiani S, Bruschi F. Rheumatological patients undergoing immunosuppressive treatments and parasitic diseases: a review of the literature of clinical cases and perspectives to screen and follow-up active and latent chronic infections. Clin Exp Rheumatol 2014;32(4):587–96.

[11] Guedes-Barbosa LS, Pereira da Costa I, Fernandes V, Henrique da Mota LM, de Menezes I, Aaron Scheinberg M. Leishmaniasis during anti-tumor necrosis factor therapy: report of 4 cases and review of the literature (additional 28 cases). Semin Arthritis Rheum 2013;43(2):152–7.

[12] Xynos ID, Tektonidou MG, Pikazis D, Sipsas NV. Leishmaniasis, autoimmune rheumatic disease, and anti-tumor necrosis factor therapy, Europe. Emerg Infect Dis 2009;15(6):956–9.

[13] De Leonardis F, Govoni M, Lo Monaco A, Trotta F. Visceral leishmaniasis and anti-TNF-alpha therapy: case report and review of the literature. Clin Exp Rheumatol 2009;27(3):503–6.

[14] Guarneri C, Bevelacqua V, Patterson JW, Tchernev G. Cutaneous and visceral leishmaniasis during anti-TNFalpha therapy. Wien Med Wochenschr 2017;167(3–4):78–82. 1946.

[15] Marcoval J, Penin RM, Sabe N, Valenti-Medina F, Bonfill-Orti M, Martinez-Molina L. Cutaneous leishmaniasis associated with anti-tumour necrosis factor-alpha drugs: an emerging disease. Clinical and experimental dermatology; 2017.

[16] Valdés Delgado T, Rodríguez Delgado C, Pérez-Pérez M. Mucocutaneous leishmaniasis associated with anti-TNF therapy: a case report. Inflamm Bowel Dis; 2021.

[17] Cerro PA, Sánchez-Hernández M, Morales-Callaghan AM, Estrada-Mallarino N, Gilaberte Y. Successful treatment with photodynamic therapy in a patient with nasal mucocutaneous leishmaniasis undergoing treatment with TNFα inhibitor. Eur J Dermatol 2020;30(6):750–1.

[18] Schwartz T, Jensenius M, Blomberg B, Fladeby C, Maeland A, Pettersen FO. Imported visceral leishmaniasis and immunosuppression in seven Norwegian patients. Tropical diseases, travel medicine and vaccines 2019;5:16.

[19] Bosch-Nicolau P, Ubals M, Salvador F, Sánchez-Montalvá A, Aparicio G, Erra A, Martinez de Salazar P, Sulleiro E, Molina I. Leishmaniasis and tumor necrosis factor alpha antagonists in the Mediterranean basin. A switch in clinical expression. PLoS Neglected Trop Dis 2019;13(8):e0007708.

[20] Martínez-Doménech A, García-Legaz-Martínez M, Magdaleno-Tapial J, Torrecillas M, Hernández-Bel P, Ocete Mochón MD, Pérez-Ferriols A, de Miquel VA. Anti-tumour necrosis factor-associated cutaneous leishmaniasis: a single-institution experience. Br J Dermatol 2019;181(2):403–5.

[21] Ready PD. Leishmaniasis emergence in Europe. Euro Surveill : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin 2010;15(10):19505.

[22] Humanes-Navarro AM, Herrador Z, Redondo L, Cruz I, Fernández-Martínez B. Estimating human leishmaniasis burden in Spain using the capture-recapture method, 2016-2017. PLoS One 2021;16(10):e0259225.

[23] Gradoni L. Epidemiological surveillance of leishmaniasis in the European Union: operational and research challenges. Euro Surveill : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin 2013;18(30):20539.

[24] Gradoni L, Gramiccia M. Leishmania infantum tropism: strain genotype or host immune status? Parasitol Today 1994;10(7):264–7.

[25] Van der Auwera G, Davidsson L, Buffet P, Ruf MT, Gramiccia M, Varani S, Chicharro C, Bart A, Harms G, Chiodini PL, Brekke H, Robert-Gangneux F, Cortes S, Verweij JJ, Scarabello A, Karlsson Söbirk S, Guéry R, van Henten S, Di Muccio T, Carra E, van Thiel P, Vandeputte M, Gaspari V, Blum J. Surveillance of leishmaniasis cases from 15 European centres, 2014 to 2019: a retrospective analysis. Euro Surveill : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin 2022;27(4).

[26] Franco-Paredes C. The growing challenge of leishmaniasis in travelers. Trav Med Infect Dis 2014;12(6 Pt A):559–60.

[27] Ehehalt U, Schunk M, Jensenius M, van Genderen PJ, Gkrania-Klotsas E, Chappuis F, Schlagenhauf P, Castelli F, Lopez-Velez R, Parola P, Burchard GD, Cramer JP. Leishmaniasis acquired by travellers to endemic regions in Europe: a EuroTravNet multi-centre study. Trav Med Infect Dis 2014;12(2):167–72.

[28] Zanger P, Gabrysch S. Leishmaniasis in the era of tumor necrosis factor alpha antagonist therapy–a research agenda for Europe. Euro Surveill : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin 2013;18(30):20542.

[29] Hammarström H, Dotevall L, Calander AM. A cluster of intracellular parasitic infections among patients on biological DMARDs - the tip of the iceberg? Rheumatol Adv Pract 2018;2(2):rky048.

[30] Aronson N, Herwaldt BL, Libman M, Pearson R, Lopez-Velez R, Weina P, Carvalho EM, Ephros M, Jeronimo S, Magill A. Diagnosis and treatment of leishmaniasis: clinical practice guidelines by the infectious diseases society of America (IDSA) and the American society of tropical medicine and hygiene (ASTMH). Clin Infect Dis 2016;63(12):e202–64.

[31] Blum J, Buffet P, Visser L, Harms G, Bailey MS, Caumes E, Clerinx J, van Thiel PP, Morizot G, Hatz C, Dorlo TP, Lockwood DN. LeishMan recommendations for treatment of cutaneous and mucosal leishmaniasis in travelers. J Trav Med 2014;21(2):116–29. 2014.

[32] Ibarra-Meneses AV, Ghosh P, Hossain F, Chowdhury R, Mondal D, Alvar J, Moreno J, Carrillo E. IFN-Γ, IL-2, IP-10, and MIG as biomarkers of exposure to leishmania spp., and of cure in human visceral leishmaniasis. Front Cell Infect Microbiol 2017;7:200.

[33] Carrillo E, Carrasco-Anton N, Lopez-Medrano F, Salto E, Fernandez L, San Martin JV, Alvar J, Aguado JM, Moreno J. Cytokine release assays as tests for exposure to leishmania, and for confirming cure from leishmaniasis, in solid organ transplant recipients. PLoS Neglected Trop Dis 2015;9(10):e0004179.

[34] Ibarra-Meneses AV, Carrillo E, Sánchez C, García-Martínez J, López Lacomba D, San Martin JV, Alves F, Alvar J, Moreno J. Interleukin-2 as a marker for detecting asymptomatic individuals in areas where Leishmania infantum is endemic. Clin Microbiol Infect 2016;22(8):739.e1-739.e4.

[35] Alten B, Maia C, Afonso MO, Campino L, Jiménez M, González E, Molina R, Bañuls AL, Prudhomme J, Vergnes B, Toty C, Cassan C, Rahola N, Thierry M, Sereno D, Bongiorno G, Bianchi R, Khoury C, Tsirigotakis N, Dokianakis E, Antoniou M, Christodoulou V, Mazeris A, Karakus M, Ozbel Y, Arserim SK, Erisoz Kasap O, Gunay F, Oguz G, Kaynas S, Tsertsvadze N, Tskhvaradze L, Giorgobiani E, Gramiccia M, Volf P, Gradoni L. Seasonal dynamics of phlebotomine sand fly species proven vectors of mediterranean leishmaniasis caused by leishmania infantum. PLoS Neglected Trop Dis 2016;10(2):e0004458.

[36] Benabid M, Ghrab J, Rhim A, Ben-Romdhane R, Aoun K, Bouratbine A. Temporal dynamics and Leishmania infantum infection prevalence of Phlebotomus perniciosus (Diptera, Phlebotominae) in highly endemic areas of visceral leishmaniasis in Tunisia. PLoS One 2017;12(9):e0184700.

[37] Henry K, Mayet A, Hernandez M, Frechard G, Blanc PA, Schmitt M, André N, Loreau JM, Ginouves M, Prévot G, Couppié P, Demar M, Blaizot R. Outbreak of Cutaneous Leishmaniasis among military personnel in French Guiana, 2020: clinical, phylogenetic, individual and environmental aspects. PLoS Neglected Trop Dis 2021;15(11):e0009938.

[38] Oré M, Sáenz E, Cabrera R, Sanchez JF, De Los Santos MB, Lucas CM, Núñez JH, Edgel KA, Sopan J, Fernández J, Carnero AM, Baldeviano GC, Arrasco JC, Graf PCF, Lescano AG. Outbreak of cutaneous leishmaniasis in Peruvian military personnel undertaking training activities in the amazon basin. Am J Trop Med Hyg 2015;93(2):340–6. 2010.

[39] Sanchez JL, Diniega BM, Small JW, Miller RN, Andujar JM, Weina PJ, Lawyer PG, Ballou WR, Lovelace JK. Epidemiologic investigation of an outbreak of cutaneous leishmaniasis in a defined geographic focus of transmission. Am J Trop Med Hyg 1992;47(1):47–54.

[40] van Thiel PP, Leenstra T, de Vries HJ, van der Sluis A, van Gool T, Krull AC, van Vugt M, de Vries PJ, Zeegelaar JE, Bart A, van der Meide WF, Schallig HD, Faber WR, Kager PA. Cutaneous leishmaniasis (Leishmania major infection) in Dutch troops deployed in northern Afghanistan: epidemiology, clinical aspects, and treatment. Am J Trop Med Hyg 2010;83(6):1295–300.

[41] van Thiel PP, Zeegelaar JE, van Gool T, Faber WR, Kager PA. Cutaneous leishmaniasis in three Dutch military cohorts following jungle training in Belize. Trav Med Infect Dis 2011;9(3):153–60.

[42] Saporito L, Giammanco GM, De Grazia S, Colomba C. Visceral leishmaniasis: host-parasite interactions and clinical presentation in the immunocompetent and in the immunocompromised host. Int J Infect Dis 2013;17(8):e572–6.

[43] Carrasco-Antón N, López-Medrano F, Fernández-Ruiz M, Carrillo E, Moreno J, García-Reyne A, Pérez-Ayala A, Rodríguez-Ferrero ML, Lumbreras C, San-Juan R, Alvar J, Aguado JM. Environmental factors as key determinants for visceral leishmaniasis in solid organ transplant recipients, Madrid, Spain. Emerg Infect Dis 2017;23(7):1155–9.

[44] El Hajj L, Thellier M, Carrière J, Bricaire F, Danis M, Caumes E. Localized cutaneous leishmaniasis imported into Paris: a review of 39 cases. Int J Dermatol 2004;43(2):120–5.

[45] Harms G, Schönian G, Feldmeier H. Leishmaniasis in Germany. Emerg Infect Dis 2003;9(7):872–5.

[46] Neumayr AL, Morizot G, Visser LG, Lockwood DN, Beck BR, Schneider S, Bellaud G, Cordoliani F, Foulet F, Laffitte EA, Buffet P, Blum JA. Clinical aspects and management of cutaneous leishmaniasis in rheumatoid patients treated with TNF-α antagonists. Trav Med Infect Dis 2013;11(6):412–20.

[47] Guery R, Walker SL, Harms G, Neumayr A, Van Thiel P, Gangneux JP, Clerinx J, Söbirk SK, Visser L, Lachaud L, Bailey M, Bart A, Ravel C, Van der Auwera G, Blum J, Lockwood DN, Buffet P. Clinical diversity and treatment results in Tegumentary Leishmaniasis: a European clinical report in 459 patients. PLoS Neglected Trop Dis 2021;15(10):e0009863.

[48] Kurizky PS, Marianelli FF, Cesetti MV, Damiani G, Sampaio RNR, Gonçalves LMT, Sousa CAF, Martins SS, Vernal S, Mota L, Gomes CM. A comprehensive systematic review of leishmaniasis in patients undergoing drug-induced immunosuppression

for the treatment of dermatological, rheumatological and gastroenterological diseases. Rev Inst Med Trop Sao Paulo 2020;62:e28.

[49] Szabados F, Kragh J, Rasmussen C, Leutscher PDC. Biological therapy and international travel: a questionnaire survey among Danish patients with rheumatic disease. Eur J Rheumatol 2020;7(4):158–63.

[50] Hall V, Johnson D, Torresi J. Travel and biologic therapy: travel-related infection risk, vaccine response and recommendations. J Trav Med 2018;25(1).