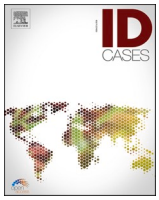
IDCases 32 (2023) e01753

Contents lists available at ScienceDirect

IDCases

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



Case report

A difficult to treat *Leishmania infantum* relapse after allogeneic stem cell transplantation

Rob J.W. Arts [a], Geneviève I.C.G. Ector [b], Pau Bosch-Nicolau [c,d], Isreal Molina [c,d], Matthew B.B. McCall [e], Walter J.F.M. van der Velden [b], Arjan van Laarhoven [a], Quirijn de Mast [a], Suzanne van Dorp [b,*]

[a] *Department of Internal Medicine, Radboud Institute of Molecular Life Sciences (RIMLS) and Radboudumc Center for Infectious Diseases (RCI), Radboud University Medical Center, Geert Grooteplein Zuid 8, 6525 GA Nijmegen, the Netherlands*
[b] *Department of Hematology, Radboud University Medical Center, Radboud Institute of Molecular Life Sciences (RIMLS), Geert Grooteplein Zuid 8, 6525 GA Nijmegen, the Netherlands*
[c] *International Health Unit Vall d'Hebron-Drassanes, Infectious Diseases Department, Vall d'Hebron University Hospital, Barcelona, Spain*
[d] *PROSICS Barcelona, CSUR Imported Tropical Diseases, Center for Biomedical Research in Infectious Diseases Network (CIBERINFEC), Institute of Health Carlos III, Av de Monforte de Lemos 3-5, 28029 Madrid, Spain*
[e] *Department of Medical Microbiology, Radboud Institute of Molecular Life Sciences (RIMLS) and Radboudumc Center for Infectious Diseases (RCI), Radboud University Medical Center, Geert Grooteplein Zuid 8, 6525 GA Nijmegen, the Netherlands*

ARTICLE INFO

Keywords:
Miltefosine
Amphotericin B
Pancytopenia
Leishmania

ABSTRACT

Here we describe a complicated case of a relapsed *Leishmania infantum* infection after an allogeneic stem cell transplantation (allo-SCT) for primary myelofibrosis. Three years earlier the patient had been diagnosed with a hemophagocytic lymphohistiocytosis secondary to a visceral *Leishmania infantum* infection, for which he was effectively treated with a cumulative dose of 40 mg/kg liposomal amphotericin B. During the first disease episode he was also diagnosed with primary myelofibrosis for which he received medical follow-up. One year later ruxolitinib was started due to progressive disease. No *Leishmania* relapse occurred. Nevertheless, the marrow fibrosis progressed, and an allo-SCT was performed. Two months after allo-SCT prolonged fever and a persistent pancytopenia occurred, which was due to a relapse of visceral Leishmaniasis. The infection was refractory to a prolonged treatment with liposomal amphotericin B with a cumulative dose up to 100 mg/kg. Salvage treatment with miltefosine led to reduction of fever within a few days and was followed by a slow recovery of pancytopenia over the following months. The *Leishmania* parasite load by PCR started to decline and after 3.5 months no *Leishmania* DNA could be detected anymore and follow-up until ten months afterwards did not show a relapse.

Introduction

Leishmania is a protozoon that is transmitted by the bite of a female sand fly. Different subtypes of *Leishmania* are endemic throughout the world and cause different types of disease, e.g. visceral, cutaneous, or mucocutaneous leishmaniasis. In Southern Europe the main subspecies is *L. infantum*, which causes visceral leishmaniasis and occasionally cutaneous leishmaniasis [1].

L. infantum can cause an asymptomatic infection and can have a long latency period. Patients with visceral leishmania, suffer from leukocytopenia secondary to the infection, and a proportion of them has a cellular immune disorder as predisposing condition [1,2]. *Leishmania* infections in immunocompromised hosts, such as HIV-infection [3], or patients with solid organ or allogeneic stem cell transplantation (allo-SCT) [4,5], warrant extra care since the immunocompromised status complicates the treatment greatly and might necessitate prolonged treatment, higher dose administration of antiparasitic drugs, and secondary prophylaxis.

Here we discuss a case of a relapsed *L. infantum* visceral leishmaniasis after allo-SCT that did not respond to the first line treatment with liposomal amphotericin B (L-amB) but did respond to oral miltefosine.

* Correspondence to: Department of Hematology, Radboud University Nijmegen Medical Center, Geert Grooteplein Zuid 8, 6525 GA Nijmegen, the Netherlands.
E-mail address: Suzanne.vanDorp@radboudumc.nl (S. van Dorp).

https://doi.org/10.1016/j.idcr.2023.e01753
Received 22 February 2023; Received in revised form 28 March 2023; Accepted 28 March 2023
Available online 29 March 2023

Case report

This concerns a 49-year-old male, who almost three years prior to this episode was diagnosed with a secondary hemophagocytic lymphohistiocytosis (HLH), provoked by a visceral *L. infantum* infection. *Leishmania* PCRs on peripheral blood and bone marrow were positive. Most likely, he was infected with the parasite a half year earlier during a holiday at the Spanish coast near Barcelona. He received treatment with L-amB with, a cumulative dose of 40 mg/kg, with good clinical effect. The leishmania PCR on peripheral blood became negative after 17 days (Fig. 1A). During this admission a reactivation of Epstein-Barr virus also occurred, for which he received rituximab twice (Fig. 1B). He received dexamethasone to dampen the hyperinflammation, but as it was considered to be secondary to the leishmania infection, he no additional etoposide treatment was started.

Apart from the HLH and leishmania, bone marrow examination also showed signs of a myeloproliferative neoplasm, at an early prefibrotic stage. A mutation in the calreticulin gene (CALR type 1 mutation) was found, which is consistent with the diagnosis of primary myelofibrosis. Initially, no treatment was started, but due to constitutional symptoms and splenomegaly, the JAK inhibitor ruxolitinib was started one year after the visceral leishmaniasis and 21 months prior to the current episode. After a year his constitutional symptoms and splenomegaly got worse, and bone marrow examination showed progression of myelofibrosis. Treatment with peg-interferon alpha was initiated but was ineffective and an upfront alllo-SCT with a mismatched unrelated donor was performed. A reduced-intensity conditioning regimen with busulfan, fludarabine and anti-thymocyte globulin was used (Fig. 1B). Routine blood *Leishmania* PCRs were not performed in this period. Immunosuppression with cyclosporin and mycophenolate mofetil was started after allo-SCT as graft-versus-host disease prophylaxis. Cyclosporin was switched to tacrolimus because of nausea and increased bilirubin levels using prednisolone as a bridge to sufficient tacrolimus levels. Two months after the allo-SCT he was readmitted to the hospital because of fever and persistent pancytopenia (Table 1). At that time, he was treated with 50 mg prednisolone and 2 mg BID tacrolimus. Bone marrow and peripheral blood were PCR positive for *L. infantum* (Fig. 1C). L-amB treatment was started again aiming for a total dose 40 mg/kg (Fig. 1D). Because the patient was at risk for kidney function decline due to cotreatment with tacrolimus, we started with daily doses of 3 mg/kg (instead of the advised 4 mg/kg for immunocompromised patients) with daily assessment of kidney function. Unfortunately, glomerular filtrate declined after the first five days. Therefore L-amB was halted for two days until improvement and resumed in a daily dose of 2 mg/kg for an additional three days, up to a cumulative dose of 21 mg/kg. Then a weekly dose of 3 mg/kg was intended up to a total dose of 40 mg/kg. However, the *Leishmania* PCR cycle time did not increase, and the pancytopenia and constitutional symptoms persisted. Even though L-amB administrations were intensified and a cumulative dose of 97 mg/kg in a period of in total 3 months was given, we even observed an increase of the *Leishmania* blood load measured with a semi-quantitative rt-PCR. The immunosuppressants had been rapidly tapered to 10 mg of prednisone and 0.5 mg BID tacrolimus at that point. The patient had achieved full donor chimerism and had not experienced graft-versus-host disease but showed delayed immune reconstitution (Table 1).

As there was no response to L-amB, treatment with miltefosine TID 50 mg was started. Tacrolimus was stopped two weeks later, and prednisone was further tapered over several weeks. After six weeks of treatment there was a steep decrease of leishmania load on peripheral blood. Because of delivery problems miltefosine had to be interrupted for 1.5 weeks. After reinitiating the decline of parasite load continued. Due to severe nausea the dose of miltefosine was decreased to 50 mg BID for two weeks, after which the original dose could be resumed. No other side effects were observed. As there was still a positive PCR, additional immunotherapy with interferon gamma (IFNγ) was considered. However, the first day subcutaneously IFNγ was administered, the PCR turned out negative, so IFNγ was not continued and miltefosine was continued for another two weeks, for a total of 16 weeks of treatment.

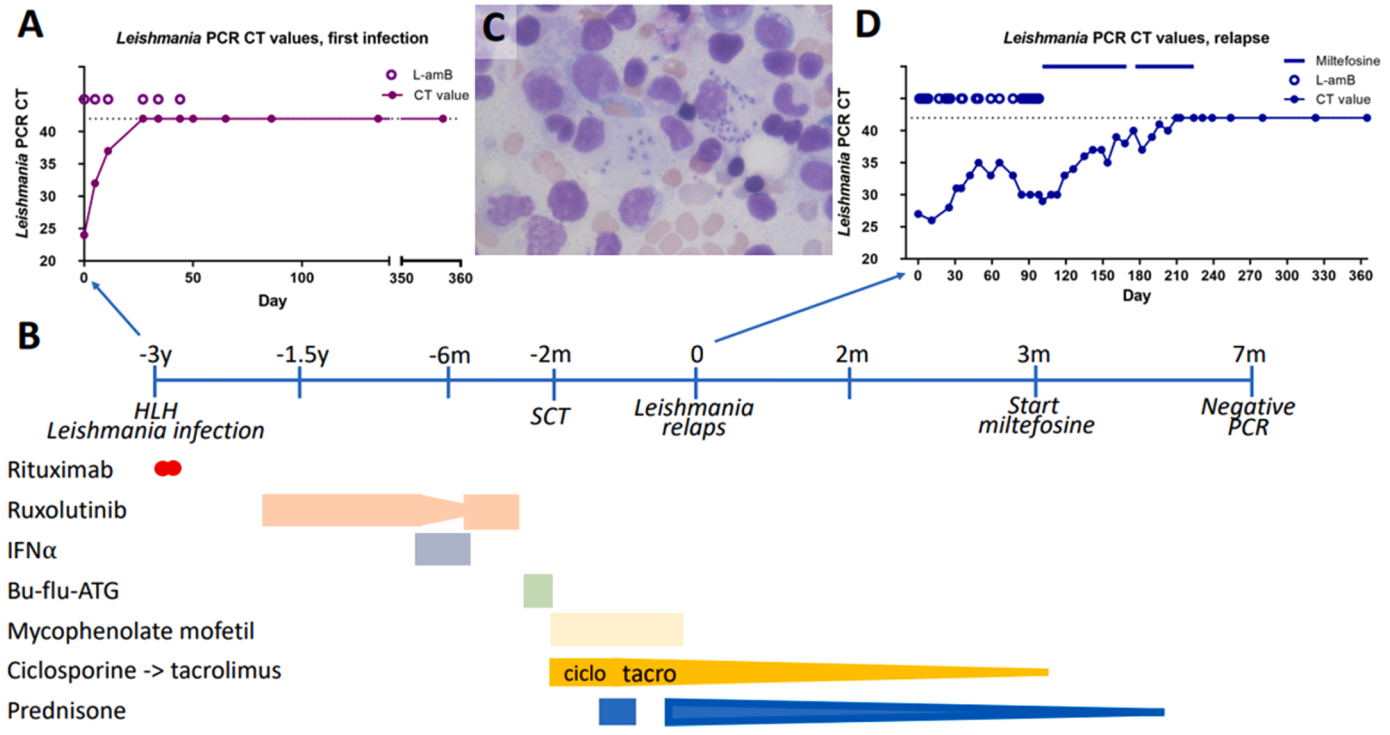


Fig. 1. Overview of timeline. A. L-amB treatment and PCR CT values over time during the first infection. B. Timeline and immunosuppression use. C. Bone marrow aspirate showing Leishmania parasites at relapse. D. L-amB and miltefosine treatment and PCR CT values over time during relapse infection. Abbreviations: Hemophagocytic lymphohistiocytosis (HLH), hematopoietic cell transplantation (SCT), interferon (IFN), busulfan (Bu), fludarabine (flu), antithymocyte globulin (ATG), polymerase chain reaction (PCR), year (y), months (m).

Table 1
Overview of laboratory results over time.

	First diagnosis VL − 3 years	After L-amB treatment − 2,7 years	Before SCT − 2 months	Diagnosis VL relapse 0	At peak creatinine + 2 months	Start miltefosine + 3 months	Stop miltefosine + 7 months	Recent + 10 months
Hemoglobin (mmol/L)	**7.0**	**6.8**	**8.5**	**7.5**	**5.6**	**7.0**	**8.0**	**8.1**
Leukocytes (10^9/L)	**3.8**	**7.9**	**5.2**	**5.7**	**1.7**	**2.6**	**3.0**	**3.1**
Lymphocytes (10^9/L)			**0.90**	**0.23**	**0.38**	**0.41**	**0.58**	**0.53**
Thrombocytes (10^9/L)	**148**	**303**	**243**	**73**	**61**	**48**	**124**	**102**
Absolute CD4 + T-cell count *(% of T-cells)*	**N/A**	**N/A**	**0.47 (*51.3*)**	**0.02 (*8.1*)**	**0,05 (11.7)**	**0.05 (*18.4*)**	**0.15 (*33.9*)**	**0.13 (*23.8*)**
B-cells (10^9/L)	**N/A**	**N/A**	**0.09**	**0.01**	**0.01**	**0.01**	**0.10**	**0.16**
Creatinine (μmol/L)	**61**	**111**	**80**	**100**	**159**	**135**	**120**	**99**

Abbreviations: hematopoietic stem cell transplantation (SCT), visceral leishmaniasis (VL), N/A not applicable (test not performed at specific time point).

The patient did not present with clinical symptoms of visceral leishmaniasis and frequent PCRs on peripheral blood were performed which remained negative, now ten months after the end of miltefosine treatment. He remains in complete remission of his primary myelofibrosis and there are no signs of chronic graft-versus-host disease.

Discussion

This case report describes a patient that suffered from a relapse *L. infantum* infection after an allo-SCT, after he had been treated for visceral leishmaniasis, which was complicated by an HLH, almost three years earlier. Due to the allo-SCT and the immunosuppressive drugs a severe immunodeficiency was induced, causing the relapse of *L. infantum* visceral leishmaniasis with fever and a persistent pancytopenia.

In this patient we did not perform monitoring of *Leishmania* PCR after allo-SCT. There is no clear evidence whether routine monitoring after SCT, next to clinical monitoring, is efficient, but given high relapse percentages monitoring it has been advised by some authors [4,5] and is also included as a strong suggestion with weak evidence in the guideline of the Infectious Diseases Society of America [6]. In retrospect, routine monitoring could have led to an earlier diagnosis in this patient.

First-line treatment for visceral leishmaniasis in the Mediterranean region is L-amB [7]. In immunocompromised patients, a higher cumulative dose of 40 mg/kg of L-amB is suggested instead of 20 mg/kg. A higher dose, however, is associated with more renal toxicity, especially in combination with other nephrotoxic drugs such as calcineurin inhibitors. In the presented case the daily dose was adjusted and by extending the L-amB treatment, we were able to give a high cumulative dose.

The question remains why L-amB treatment was not effective. It has been stated that there is no amB resistance known in vivo in *L. infantum* [8–10]. However, routine laboratories do not perform susceptibility tests. Yet, in experimental conditions it has been shown that in *L. donovani* in clinically amB-resistant cases the in vitro lethal doses of amB are up to 8-fold higher, which suggests a dose effect [11]. For several leishmania species amB-resistance can be induced in vitro, mainly in the promastigote forms (reviewed in [8]). Apart from the parasite, host factors also play an important role in treatment success. It has been shown that patients with an immunodeficiency are particularly hard to treat, and that therapy failure is mostly due to immunologic failure and not to drug failure [6,8,10,12]. Relapses of *Leishmania* infection are for example frequently seen in HIV patients with a CD4 count below 200 cells/μl [13] and a chronic *Leishmania* infection in an HIV patient that was suppressed (but not cured) with up to 65 g of L-amB over a time period of 6 years has also been reported [14].

In treatment of fungal infections, amB has been shown to be only fungistatic. If the same were to account for Leishmania, an effective immune response would be essential for curing the disease, which was obviously lacking in the described patient. This combined with a high parasite burden could also explain the therapy failure of L-amB.

Other potential treatment options are antimonials, but they have a high risk of side effects and were at that moment not available in The Netherlands, or intravenous pentamidine, which is proven less effective [6]. A final option is oral miltefosine, which is for most of the Leishmania subspecies an effective treatment. However, data on its use in visceral leishmania caused by *L. infantum* are conflicting. In a Brazilian trial it was shown that only 6 out of 14 patients were cured from visceral leishmania after 28 days of treatment and only 19 out of 28 after 42 days of treatment, which was claimed due to natural resistance to miltefosine [15], however no control group with e.g. L-amB treatment was included in this study. A genetic marker has been identified, called the miltefosine sensitivity locus (MSL), which was highly predictive for miltefosine treatment response [16]. Interestingly, MSL was present in almost all the *L. infantum* isolates from the Old World, whereas it was almost completely absent in all isolates from certain parts of Brazil, which could explain treatment failure in the former trial [16]. Since no commercial MSL test is available, this was not tested in our patient. Apart from MSL several other resistance gene markers (for both amB and miltefosine) are being studied, although for most of them the clinical application remains to be proven (reviewed in [9]).

Given potential drug resistance and the inability to routinely test for drug sensitivity, several authors suggest treating visceral leishmaniasis with combination therapy, also to prevent the induction of resistance, especially in immunocompromised hosts [6,8,9]. The World Health Organization recommends a combination therapy with L-amB and miltefosine in East Africa and South-East Asia for visceral leishmaniasis caused by *L. donovani* in HIV patients [3], which is mainly based on two trials [17,18]. In our patient, we initially started with L-amB monotherapy given the prior response to this therapeutic regimen. In the absence of a response to L-amB therapy and considering previous renal side effects and the slow elimination of L-amB after high cumulative doses, we decided to give miltefosine as monotherapy.

The dose and duration of miltefosine are also subject to debate. Generally, a target dose of 2,5 mg/kg, with a maximum daily dose of 150 mg is recommended [6]. However in combination therapy with L-amB a fixed dose of 100 mg is used [3,17,18], whereas others suggest even a higher daily dose than 150 mg in monotherapy [19]. In our patient, given his initial weight of more than 100 kg, a higher dose than 150 mg could have been considered, however, already at a dose of 150 mg per day severe gastro-intestinal side effects occurred, which necessitated a temporary dose reduction to 100 mg, to prevent from treatment withdrawal. In the end, we decided to continue treatment until two weeks after the PCR on peripheral blood became negative, which resulted in a treatment duration of 16 weeks in total. Although generally treatments longer than six weeks are not performed for visceral leishmaniasis, there is experience with treatment of up to 16 weeks for post kala azar dermatitis [20] and in this case we decided, given the severely impaired host response, for an extended treatment.

Besides the role of antiparasitic drugs in the treatment of visceral leishmaniasis, the host immune system also plays an essential role, which is a likely explanation for the L-amB treatment failure in our

patient. We initially decreased the immunosuppressants as quickly as possible, but we were limited because of the recent allo-SCT with a mismatched unrelated donor and the risk for graft-versus-host disease. Nevertheless, due to the use of ATG the immune reconstitution was delayed with profound $CD4^+$ T cell and B cell lymphopenia (Table 1), which is also in HIV patients a known risk factor for treatment failure [10,18]. Although B-cells are not the main immune cells of importance in the defense against leishmania, their role has been established in leishmaniasis in mice [21], which therefore was another risk factor in this patient. This shows therefore a severe combined deficiency of the host immune system.

To induce a more effective immune response, immunostimulatory treatments have also been suggested as additional treatment in visceral leishmaniasis [22]. The leishmania parasites mainly reside in macrophages, which is also the major effector cell in clearing the infection [2]. In the induction of activation of macrophages and killing of the leishmania parasite, IFNγ plays a key role [22]. Therefore, in patients in whom antiparasitic drugs seem to be insufficient to treat the infection, especially if there is a known defect in the host immune system, such as in our patient, IFNγ supplementation could be effective. Addition of IFNγ to antimony therapy resulted in an effective treatment in patients that had been unresponsive to several courses of antimony therapy before. Six of the eight treated patients showed a sustained response [23]. In our patient we were initially reluctant to start IFNγ in fear of induction of graft versus host disease in this patient with a recent allo-SCT that had already had an accelerated tapering of the immunosuppressants. At the point when we decided to start IFNγ supplementation the leishmania PCR turned out negative and the IFNγ therapy was halted.

We decided not to start secondary prophylaxis for several reasons. Most importantly a better host response was to be expected due to immune reconstitution after allo-SCT and withdrawal of all immunosuppressive agents and the effective treatment of *Leishmania* itself. Furthermore, we did not know whether L-amB could be used as prophylaxis as it was unknown whether the earlier treatment failure was due to resistance. The only other available option in The Netherlands is miltefosine, which we did not want to use in a lower prophylactic dose, given the risk of resistance induction, and thereby losing the only proven effective drug in this patient, and the still existing gastrointestinal side effects even at a lower dose of miltefosine. We decided to follow up with frequent (initially weekly) PCRs on peripheral blood, which remained negative until now, more than ten months since discontinuation of miltefosine. This is of course no proof of complete elimination of the parasite, but given the reconstitution of the immune system the current chance of relapse is very small.

In conclusion, here we presented a complicated case of a *L. infantum* relapse infection in a patient two months after SCT, which resulted in a severe combined immune deficiency. Initial treatment with L-amB was infective, either due to induced resistance or due to the defective host response. We now show that long-term treatment with miltefosine, despite reported inherent resistance in literature, can be effective, but that reconstitution of the immune system is essential too. Future research should mainly focus on prediction of treatment failure, resistance assays, and the effect of immunotherapy especially in the immunocompromised host.

Funding

Not applicable.

CRediT authorship contribution statement

R. Arts, G. Ector, P. Bosch-Nicolau, I. Molina, M McCall, W vd Velden, A. van Laarhoven, Q. de Mast and S van Dorp wrote the paper. R. Arts, G. Ector, M McCall, A. van Laarhoven, S van Dorp collected the data and analysed the data.

Ethical approval

Not applicable.

Consent

Patient has given written informed consent.

Declaration of Competing Interest

None.

References

[1] Pasquier G, Demar M, Lami P, Zribi A, Marty P, Buffet P, et al. Leishmaniasis epidemiology in endemic areas of metropolitan France and its overseas territories from 1998 to 2020. PLoS Negl Trop Dis 2022;16:e0010745.
[2] Burza S, Croft SL, Boelaert M. Leishmaniasis. Lancet 2018;392:951–70.
[3] WHO guideline for the treatment of visceral leishmaniasis in HIV co-infected patients in East Africa and South-East Asia; 2022.
[4] Tatarelli P, Fornaro G, Del Bono V, Nicolini LA, Raiola AM, Gualandi F, et al. Visceral leishmaniasis in hematopoietic cell transplantation: case report and review of the literature. J Infect Chemother 2018;24:990–4.
[5] Gajurel K, Dhakal R, Deresinski S. Leishmaniasis in solid organ and hematopoietic stem cell transplant recipients. Clin Transpl 2017:31.
[6] Aronson N, Herwaldt BL, Libman M, Pearson R, Lopez-Velez R, Weina P, et al. 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:e202–64.
[7] Gradoni L, López-Vélez R, Mokni M. Manual on case management and surveillance of the leishmaniases in the WHO European Region; 2017.
[8] Ponte-Sucre A, Gamarro F, Dujardin JC, Barrett MP, López-Vélez R, García-Hernández R, et al. Drug resistance and treatment failure in leishmaniasis: a 21st century challenge. PLoS Negl Trop Dis 2017;11:e0006052.
[9] Salari S, Bamorovat M, Sharifi I, Almani PGN. Global distribution of treatment resistance gene markers for leishmaniasis. J Clin Lab Anal 2022;36:e24599.
[10] Lachaud L, Bourgeois N, Plourde M, Leprohon P, Bastien P, Ouellette M. Parasite susceptibility to amphotericin B in failures of treatment for visceral leishmaniasis in patients coinfected with HIV type 1 and Leishmania infantum. Clin Infect Dis 2009;48:e16–22.
[11] Purkait B, Kumar A, Nandi N, Sardar AH, Das S, Kumar S, et al. Mechanism of amphotericin B resistance in clinical isolates of Leishmania donovani. Antimicrob Agents Chemother 2012;56:1031–41.
[12] Vase M, Hellberg YK, Larsen CS, Petersen E, Schaumburg H, Bendix K, et al. Development of splenic marginal zone lymphoma in a HIV-negative patient with visceral leishmaniasis. Acta Haematol 2012;128:20–2.
[13] Bourgeois N, Bastien P, Reynes J, Makinson A, Rouanet I, Lachaud L. 'Active chronic visceral leishmaniasis' in HIV-1-infected patients demonstrated by biological and clinical long-term follow-up of 10 patients. HIV Med 2010;11:670–3.
[14] Al Balushi A, Khamis F, Klaassen CHW, Gangneux JP, Van Hellemond JJ, Petersen E. Double infection with. Open Forum Infect Dis 2018;5:ofy323.
[15] Carnielli JBT, Monti-Rocha R, Costa DL, Molina Sesana A, Pansini LNN, Segatto M, et al. Natural resistance of. Am J Trop Med Hyg 2019;101:789–94.
[16] Carnielli JBT, Crouch K, Forrester S, Silva VC, Carvalho SFG, Damasceno JD, et al. A Leishmania infantum genetic marker associated with miltefosine treatment failure for visceral leishmaniasis. eBioMedicine 2018;36:83–91.
[17] Diro E, Blesson S, Edwards T, Ritmeijer K, Fikre H, Admassu H, et al. A randomized trial of AmBisome monotherapy and AmBisome and miltefosine combination to treat visceral leishmaniasis in HIV co-infected patients in Ethiopia. PLoS Negl Trop Dis 2019;13:e0006988.
[18] Burza S, Mahajan R, Kazmi S, Alexander N, Kumar D, Kumar V, et al. AmBisome monotherapy and combination AmBisome-miltefosine therapy for the treatment of visceral leishmaniasis in patients coinfected with human immunodeficiency virus in India: a randomized open-label, parallel-arm, phase 3 trial. Clin Infect Dis 2022;75:1423–32.
[19] Dorlo TP, Balasegaram M, Beijnen JH, de Vries PJ. Miltefosine: a review of its pharmacology and therapeutic efficacy in the treatment of leishmaniasis. J Antimicrob Chemother 2012;67:2576–97.
[20] Ghosh S, Das NK, Mukherjee S, Mukhopadhyay D, Barbhuiya JN, Hazra A, et al. Inadequacy of 12-week miltefosine treatment for Indian post-kala-azar dermal leishmaniasis. Am J Trop Med Hyg 2015;93:767–9.
[21] Firmino-Cruz L, Decote-Ricardo D, Gomes DCO, Morrot A, Freire-de-Lima CG, de Matos Guedes HL. How to B(e)-1 important cell during. Front Cell Infect Microbiol 2019;9:424.
[22] Mazire PH, Saha B, Roy A. Immunotherapy for visceral leishmaniasis: a trapeze of balancing counteractive forces. Int Immunopharmacol 2022;110:108969.
[23] Badaro R, Falcoff E, Badaro FS, Carvalho EM, Pedral-Sampaio D, Barral A, et al. Treatment of visceral leishmaniasis with pentavalent antimony and interferon gamma. N Engl J Med 1990;322:16–21.