Comparative study on liposomal amphotericin B and other therapies in the treatment of mucosal leishmaniasis: A 15-year retrospective cohort study

Carolina Rocio Santos1, Felipe Francisco Tuon2, Juliette Cieslinski2, Regina Maia de Souza3, Rui Imamura4, Valdir Sabbaga Amato1*  

1 Departamento de Molestias Infecciosas e Parasitarias, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, Sao Paulo, Brasil, 2 School of Medicine, Pontificia Universidade Catolica do Parana, Curitiba, Parana, Brasil, 3 Laboratório de Parasitologia LIM-46, Instituto de Medicina Tropical, Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, Sao Paulo, Brasil, 4 Departamento de Otorrinolaringologia, Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, Sao Paulo, Brasil  

* valdiramato@usp.br

Abstract

Background

Liposomal amphotericin B (L-AMB) has been used for mucosal leishmaniasis (ML), but comparative studies on L-AMB and other drugs used for the treatment of ML have not been conducted. The present study aimed to evaluate the outcome of patients with ML who were treated with L-AMB.

Methods

This is a 15-year retrospective study of Brazilian patients with a confirmed diagnosis of ML. The therapeutic options for the treatment of ML consisted of L-AMB, amphotericin B lipid complex (ABLC), deoxycholate amphotericin B (d-AMB), itraconazole, antimonial pentavalent, or pentamidine. Healing, cure rate and adverse effects (AEs) associated with the drugs used to treat this condition were analyzed.

Results

In 71 patients, a total of 105 treatments were evaluated. The outcome of the treatment with each drug was compared, and results showed that L-AMB was superior to other therapeutic regimens (P = 0.001; odds ratio [OR] = 4.84; 95% confidence interval [CI] = 1.78–13.17). d-AMB had worse AEs than other treatment regimens (P = 0.001, OR = 0.09; 95% CI = 0.09–0.43). Approximately 66% of the patients presented with AEs during ML treatment. Although L-AMB was less nephrotoxic than d-AMB, it was associated with acute kidney injury compared with other drugs (P <0.05).
Conclusion

L-AMB was more effective than other therapies for the treatment of ML. However, a high incidence of toxicity was associated with its use. Therapeutic choices should be reassessed, and the development of new drugs is necessary for the treatment of ML.

Introduction

The tegumentary form of the leishmaniasis can be classified in two main clinical forms: cutaneous and mucosal. Mucosal leishmaniasis (ML) is primarily caused by Leishmania braziliensis (L. braziliensis), and it occurs months or years after the cutaneous lesions have healed [1]. Mucosal leishmaniasis is a progressive disease that can destroy cartilages and the osseous structures of the face, pharynx, and larynx [2]. Most ML cases are treated with antimonial pentavalent. However, this drug has several adverse effects (AEs) and contraindications [3]. Liposomal amphotericin B (L-AMB) is a safe option. However, the cost of this drug can be a limitation in developing countries, and the ideal dose is not established.

L-AMB is the first-line treatment for visceral leishmaniasis [4], and it has been used for mucocutaneous leishmaniasis with controversial results. In some studies, the healing rate was similar to those who were not on therapy [5]. The variability of clinical response to L-AMB is associated with the Leishmania species. In Brazil, studies have found different cure rates [6, 7].

Considering the controversial benefits of L-AMB on the treatment of ML, the present study aimed to evaluate the treatment outcome of the largest cohort of patients with ML who were treated with liposomal amphotericin in comparison with other treatments.

Methods

This is a retrospective study that included a cohort of patients with a confirmed diagnosis of ML who received at least one treatment with any leishmanicidal drugs from January 2000 to July 2015. The study was approved by the local ethical committee (CAPpesq number: 0576/11). Informed consent was waived by the ethics committee considering the characteristics of this study. All data were fully anonymized before access. Patients from the different cities of Brazil were treated in a reference hospital in Sao Paulo, SP, Brazil.

The inclusion criteria were as follows: patients with confirmed ML who were older than 18 years and followed-up for at least 6 months. Exclusion criteria included patients without confirmation of ML and refuse to treatment. The diagnosis of ML consisted with the identification of Leishmania spp. in the tissue via molecular tests, culture, or the observation of the typical structure during histological examination or immunohistochemistry [8]. Otorhinolaryngological examinations were performed by the same physician throughout the study period.

The clinical variables included in the analysis were gender, age, Brazilian state of origin, race, previous cutaneous lesions suggesting cutaneous leishmaniasis, comorbidities, duration of the symptoms, site of the ML, symptoms, serum and cutaneous tests, and tomographic and clinical findings, as previously classified [9, 10].

The therapeutic options for the treatment of ML consisted of L-AMB (1–4 mg/kg/day), amphotericin B lipid complex (ABLC) (1–4 mg/kg/day), deoxycholate amphotericin B (d-AMB) (1 mg/kg/day), itraconazole (200 mg/day for 6 weeks), antimonial pentavalent (20 mg/Sb+5/kg/day for 30 days), or pentamidine (4 mg/kg for 10 days). All formulations of amphotericin B should achieve a cumulative dose of 2,500 mg. Antimonial pentavalent was considered
the drug of first choice. In the case of contraindications, the second choice drugs used were pentamidine and amphotericin B. For those cases where these therapeutic options were used without success or presented contraindication, lipid formulations of amphotericin B has been used. For refractory cases or contraindication to all therapeutic regimens, itraconazole was used.

Cure was defined as the total healing of mucosal lesion based on otorhinolaryngological and fiberoptic examinations until 1 year after the end of the therapy. Failure is defined as the absence of lesion improvement after therapy or the return of lesions before 1 year. Lesions that showed an improvement after 6 months of therapy but did not completely heal were considered failure. Recurrence is considered as new lesions or the return of the lesion after 1 year of therapy [11].

In addition, the AEs associated with the treatment of ML were analyzed during the hospitalization period: fever, headache, nausea or vomiting, tremors or chills, sweating, phlebitis on the infusion site, low back pain, chest pain, palpitation, myalgia, arthralgia, asthenia, and rashes. Systemic AEs included acute kidney injury (AKI) according to the AKIN criteria [12], electrolyte imbalance, hepatic enzyme alterations, pancreatic enzyme alterations, myelotoxicity, electrocardiographic changes, and hypoglycemia. Changes in hepatic and pancreatic enzymes were considered when their values exceeded three times the baseline value or, in the absence of baseline values, when they exceeded three times the normal limit. Myelotoxicity was considered when hemoglobin level decreases by 2 points from baseline, total leukocyte level is below 3,000/mm$^3$, and/or platelet count is below 150,000/mm$^3$.

Data of the patients were organized as general data, and the risk factors of the treatment were evaluated. New cases included patients who received more than one treatment during follow-up. Thus, in 71 patients, a total of 105 treatments were analyzed. The number of treatments exceeded the number of patients because one patient could be followed more than one treatment. A successful treatment was considered when achieved complete healing within 3 months. The secondary treatment followed a protocol using the different chemotherapy schedule.

Continuous data were expressed as mean or median with standard deviation (SD) or ranges. Frequencies were expressed as percentages. Categorical, continuous, and dichotomized independent variables were analyzed with forward conditional factorial binary logistic regression model to determine the statistical significance of the clinical and epidemiological findings, diagnosis, and treatment along with the outcome and recurrence of ML. A P-value <0.05 was considered statistically significant. All data were recorded in Microsoft Excel (Microsoft), and statistical analysis was performed using SPSS version 23.

**Results**

In 71 patients, a total of 105 treatments were evaluated. All patients have a confirmed diagnosis of ML. The mean age of the patients was 59.9 years, and approximately 61% of the study participants were men. Most patients came to our service with symptoms for more than 5 years (65.4%). The most common symptoms were epistaxis, nasal obstruction, and rhinorrhea. The nasal mucosa was the more commonly affected site in leishmaniasis (75.2%), followed by the pharynx and palate (Table 1). Additional data are available in the S1 File.

Most patients reported a previous cutaneous lesion (52.4%), which was located mainly in the lower limbs. Moreover, 9 patients reported previous lesions in the upper limbs. One patient reported a lesion in the back, and another patient had a lesion in the penis. In addition, one patient reported a lesion in the face.
| Data                              | N = 71 | %   |
|----------------------------------|--------|-----|
| **Brazilian Region**             |        |     |
| Northeast                        | 36     | 50.7|
| Southeast                        | 21     | 29.6|
| South                            | 6      | 8.4 |
| North                            | 2      | 2.9 |
| Midwest                          | 6      | 8.4 |
| **Sex**                          |        |     |
| Female                           | 27     | 38.0|
| Male                             | 44     | 62.0|
| **Age (years±SD)**               | 59.1±14.2|     |
| **Comorbidities**                |        |     |
| Systemic arterial hypertension   | 44     | 62.0|
| Diabetes mellitus                | 7      | 9.9 |
| **Previous cutaneous lesions**   |        |     |
| Inferior Limbs                   | 22     | 31.0|
| **Duration of symptoms**         |        |     |
| <1 year                          | 12     | 17.0|
| 1–5 years                        | 16     | 22.5|
| 5–10 years                       | 30     | 42.2|
| >10 years                        | 13     | 18.3|
| **Symptoms**                     |        |     |
| Epistaxis                        | 48     | 68.5|
| Nasal obstruction                | 50     | 71.4|
| Rhinorrhea                       | 38     | 54.2|
| Nasal crust                      | 26     | 36.6|
| Pruritus                         | 16     | 22.5|
| Cacosmia                         | 14     | 19.7|
| Sneeze                           | 4      | 5.6 |
| Odynophagia                      | 9      | 12.7|
| Hyposmia                         | 6      | 8.5 |
| Facial pain                      | 10     | 14.1|
| **Local of the mucosal leishmaniasis** |    |     |
| Nasal                            | 55     | 78.5|
| Palate                           | 14     | 19.7|
| Pharynx                          | 18     | 25.4|
| Larynx                           | 10     | 14.2|
| **Nasofibroscopy**               |        |     |
| Septal perfuration               | 47     | 66.2|
| Granulomatous activity           | 38     | 54.3|
| Crusts                           | 45     | 64.2|
| Edema                            | 12     | 17.1|
| Stenosis                         | 14     | 20.0|
| Bleeding                         | 4      | 5.7 |
| Mucosal atrophy                  | 5      | 7.1 |
| Hyperemia                        | 13     | 18.6|
| Ulceration                       | 4      | 5.7 |
| Purulent drainage                | 2      | 2.8 |

(Continued)
All patients were subjected to nasofibroscopy. Septal perforation was identified in 66.2% of the participants, followed by crusts and granulomatous inflammation of the mucosa. Computed tomography (CT) scan of the face has been included in the routine examination of ML since 2009. In 48 patients who were evaluated, 70.8% presented with mucosal thickening. Other findings are detailed in the Table 1.

The treatment of all patients with ML was performed in the hospital with a mean hospitalization time of 22.9 days. In the treatment of these patients, L-AMB was the most commonly used drug (32), followed by pentavalent antimonial (25), d-AMB (14), ABLC (13), pentamidine (11), and itraconazole (10). The treatment outcome of each drug was compared, and results showed that L-AMB was superior than other therapeutic regimens (P = 0.001; odds ratio [OR] = 4.84; 95% confidence interval [CI]: 1.78–13.17). The risk factor associated with the healing was the absence of mucosal thickening on CT scan (Table 2). From 105 treatments, healing was achieved in 61, but cure rate was only 64.8%. In the multivariable analysis, L-AMB was an independent variable associated with healing (P = 0.008), and absence of mucosal thickness on CT scan too (P = 0.038). The group of patients that received L-AMB was clinically

Table 1. (Continued)

| Data                              | N = 71 | %     |
|-----------------------------------|--------|-------|
| **Diagnosis**                     |        |       |
| Histological exam                 | 70     | 98.5  |
| Positive immunohistochemistry     | 20     | 28.5  |
| Polymerase chain reaction (14 patients) | 10     | 71.4  |
| **Serum test**                    |        |       |
| ELISA                             |        |       |
| 1:40                              | 16     | 27.5  |
| 1:80                              | 5      | 7.0   |
| 1:160                             | 3      | 4.2   |
| 1:320                             | 9      | 12.6  |
| >1:320                            | 7      | 9.8   |
| Reagent without titre            | 4      | 5.6   |
| Non reagent                       | 5      | 7.0   |
| Positive Montenegro test          | 41     | 57.7  |
| **CT scan findings (n = 48)**     |        |       |
| Thickening of mucosa              | 18     | 37.5  |
| Sinusopathy                       | 10     | 20.8  |
| Mastoidopathy                     | 4      | 14.3  |
| **Hospitalization for treatment** | 57     | 80.3  |
| Duration of admission (days±SD)   | 22.7±10.5 |       |
| Treatment interrupted by side effect | 31     | 43.6  |
| **Follow up duration (months±SD)** | 55.6±46.6 |       |
| **Outcome**                       |        |       |
| Cure                              | 37     | 51.1  |
| Relapse                           | 12     | 16.9  |
| Interruption by side effect       | 31     | 43.6  |
| Final cure                        | 46     | 64.8  |

* ELISA (enzyme linked immunosorbent assay)
**IIF—indirect immunofluorescence

https://doi.org/10.1371/journal.pone.0218786.t001
Table 2. Comparative data of patients with mucosal leishmaniasis with complete healing after treatment and without healing.

| Data                              | Healing |      | No Healing |      | P Value | OR 95%CI |
|-----------------------------------|---------|------|------------|------|---------|----------|
|                                   | N (61)  | %    | N (44)     | %    |         |          |
| Sex                               |         |      |            |      |         |          |
| Female                            | 23      | 37.7 | 18         | 40.9 | 0.448   |          |
| Male                              | 38      | 62.3 | 26         | 59.1 |         |          |
| Age (years±SD)                    | 60.2±14.3| 60.0±12.3 | 0.935  |      |         |          |
| Comorbidities                     |         |      |            |      |         |          |
| Systemic arterial hypertension    | 38      | 62.3 | 26         | 59.1 | 0.448   |          |
| Diabetes mellitus                 | 5       | 8.2  | 6          | 13.6 | 0.534   |          |
| Previous cutaneous lesions        | 34      | 55.7 | 21         | 47.7 | 0.611   |          |
| Duration of symptoms              |         |      |            |      |         |          |
| <1 year                           | 7       | 15.9 | 5          | 13.5 | 0.835   |          |
| 1–5 years                         | 9       | 20.5 | 7          | 18.9 |         |          |
| 5–10 years                        | 16      | 36.4 | 14         | 37.8 |         |          |
| >10 years                         | 12      | 27.3 | 11         | 29.7 |         |          |
| Symptoms                          |         |      |            |      |         |          |
| Epistaxis                         | 41      | 65.6 | 33         | 75.0 | 0.283   |          |
| Nasal obstruction                 | 44      | 70.5 | 31         | 70.5 | 0.478   |          |
| Rhinorrhea                        | 33      | 54.1 | 21         | 47.7 | 0.266   |          |
| Nasal crust                       | 23      | 37.7 | 23         | 52.3 | 0.127   |          |
| Pruritus                          | 16      | 26.2 | 10         | 22.7 | 0.393   |          |
| Cacosmia                          | 12      | 19.7 | 9          | 20.5 | 0.589   |          |
| Sneezing                          | 6       | 9.8  | 3          | 6.8  | 0.410   |          |
| Odynophagia                       | 6       | 9.8  | 8          | 18.2 | 0.188   |          |
| Hyposmnia                         | 6       | 9.8  | 7          | 15.9 | 0.283   |          |
| Facial pain                       | 8       | 11.5 | 5          | 11.4 | 0.596   |          |
| Local of the mucosal leishmaniasis|         |      |            |      |         |          |
| Nasal                             | 46      | 75.4 | 33         | 75.0 | 0.569   |          |
| Palate                            | 9       | 14.8 | 13         | 29.5 | 0.061   |          |
| Pharynx                           | 14      | 23.0 | 16         | 36.4 | 0.110   |          |
| Larynx                            | 7       | 11.5 | 5          | 11.4 | 0.608   |          |
| Nasofibroscopy                    |         |      |            |      |         |          |
| Septal perforation                | 39      | 63.9 | 32         | 72.7 | 0.248   |          |
| Granulomatous activity            | 31      | 50.8 | 20         | 45.5 | 0.344   |          |
| Crusts                            | 39      | 63.9 | 26         | 59.1 | 0.353   |          |
| Edema                             | 8       | 13.1 | 8          | 18.2 | 0.336   |          |
| Stenosis                          | 10      | 16.4 | 11         | 25.0 | 0.372   |          |
| Bleeding                          | 2       | 3.3  | 2          | 4.5  | 0.564   |          |
| Mucosal atrophy                   | 3       | 4.9  | 6          | 13.6 | 0.115   |          |
| Hyperemia                         | 9       | 14.8 | 11         | 25.0 | 0.158   |          |
| Ulceration                        | 2       | 3.3  | 5          | 11.4 | 0.110   |          |
| Purulent drainage                 | 1       | 1.6  | 2          | 4.5  | 0.382   |          |
| CT scan findings (n = 48)         |         |      |            |      |         |          |
| Thickening of mucosa              | 16      | 26.2 | 18         | 40.9 | 0.044   | 0.24 [0.05–1.02] |
| Sinusopathy                       | 8       | 13.1 | 5          | 11.4 | 0.454   |          |
| Mastoideopathy                    | 2       | 3.3  | 3          | 6.8  | 0.379   |          |
| Therapy**                         |         |      |            |      |         |          |
| L-AMB                             | 26      | 81.3 | 6          | 18.7 | 0.001   | 4.84 [1.78–13.17] |

(Continued)
similar with other treatments, except by stenosis in nasopharynx on nasofibroscopy exam (Table 3).

The AEs associated with the treatment of this condition were analyzed. Approximately 66% of the patients presented with AEs during ML treatment. The most frequent symptoms were infusion-related AEs (fever, chills, sweating, and palpitations) in 48.1% of the participants, followed by phlebitis (20.2%) and nausea/vomiting (18.3%). The most common systemic AEs were electrolyte imbalance (28.8%) and AKI (19.2%) (Table 4). d-AMB had worse AEs than other regimens (P = 0.001; OR = 0.09; 95% CI = 0.09–0.43). ABLC was associated with infusion-related AEs (P < 0.05). Meanwhile, antimonial pentavalent was associated with metabolic disturbances, such as hyperamylasemia and increased liver enzymes. However, fewer electrolyte disorders were observed when amphotericin is used (P < 0.05). Although L-AMB was less nephrotoxic than d-AMB, it was associated with AKI compared with other drugs (P < 0.05).

**Discussion**

Data on L-AMB have been published in the literature since 1990, and in 1997, the FDA approved its use for visceral leishmaniasis. With regard to the efficacy of the drug in the treatment of cutaneous leishmaniasis, the efficacy rate ranged from 84% to 85% [13–15]. The few published studies on the treatment of ML with L-AMB presented a small number of patients and reported a high efficacy rate between 83.3% and 100% [6, 7, 11]. This study enrolled 32 patients treated with L-AMB and was the first to compare L-AMB with other drugs used in the treatment of ML. The cure rate of the group that used L-AMB was 5 times higher than that of the other groups (OR = 4.89, 95%CI [1.78–13.17], p = 0.001). The group “healing” was different from “non-healing” considering only the thickness of mucosa on CT, a finding that probably cannot influence therapeutic outcome according with different drugs. The group of patients treated with L-AMB was similar with the group of patients treated with other drugs. The number of patients treated with each drug was too small to compare with those treated with L-AMB, so, we grouped the patients treated with itraconazole, pentamidine, d-AMB, antimonial pentavalent and ABLC. L-AMB is the first choice treatment for visceral leishmaniasis [4], and the current study suggest that L-AMB should be considered in the group of first line options.

Our study revealed a therapeutic success rate of 58.3% with antimonial use. Some studies have shown a clinical cure rate between 71.0% and 77.0% of the cases [16, 17]. Recurrence with the use of antimonials is around 22% [2]; our study showed a rate of 15.3%. Pentamidine has an efficacy rate of approximately 90%, and recurrence was observed in 25% of the treated cases [3]. Moreover, pentamidine (72.7%, 8/11 patients) had a greater efficacy than pentavalent antimonial (58.3%, 14/24 patients). However, no statistically significant difference was
| Data | Other | | L-AMB | |
|---|---|---|---|---|
| N (73) | % | N (32) | % | P Value |
| **Sex** | | | | |
| Female | 31 | 42.5 | 10 | 31.3 | 0.194 |
| Male | 42 | 57.5 | 22 | 68.7 | |
| **Age (years±SD)** | 61.8±13.7 | 59.1±13.5 | 0.366 | |
| **Comorbidities** | | | | |
| Systemic arterial hypertension | 46 | 63.0 | 18 | 56.3 | 0.330 |
| Diabetes mellitus | 8 | 11.0 | 3 | 9.4 | 0.555 |
| **Previous cutaneous lesions** | 37 | 82.2 | 18 | 78.3 | 0.464 |
| **Duration of symptoms** | | | | |
| <1 year | 8 | 14.5 | 4 | 15.4 | 0.877 |
| 1–5 years | 11 | 20.0 | 5 | 19.2 | |
| 5–10 years | 19 | 34.5 | 11 | 42.3 | |
| >10 years | 17 | 31.0 | 6 | 23.1 | |
| **Symptoms** | | | | |
| Epistaxis | 51 | 69.9 | 23 | 74.2 | 0.422 |
| Nasal obstruction | 51 | 69.9 | 24 | 77.4 | 0.296 |
| Rhinorrhea | 39 | 53.4 | 15 | 48.4 | 0.399 |
| Nasal crust | 30 | 41.1 | 16 | 52.4 | 0.220 |
| Pruritus | 18 | 24.7 | 8 | 25.8 | 0.542 |
| Rhinorhea | 12 | 16.4 | 9 | 28.8 | 0.117 |
| Sneeze | 5 | 5.5 | 5 | 16.1 | 0.087 |
| Odynophagia | 10 | 13.7 | 4 | 12.9 | 0.593 |
| Hyposmia | 8 | 11.1 | 5 | 16.1 | 0.342 |
| Facial pain | 10 | 13.7 | 3 | 9.7 | 0.417 |
| **Local of the mucosal leishmaniasis** | | | | |
| Nasal | 54 | 69.0 | 25 | 68.8 | 0.576 |
| Palate | 16 | 21.9 | 6 | 18.8 | 0.466 |
| Pharynx | 20 | 27.4 | 10 | 31.3 | 0.428 |
| Larynx | 9 | 12.3 | 3 | 9.4 | 0.472 |
| **Nasofibroscopy** | | | | |
| Septal perfuration | 49 | 63.9 | 22 | 72.7 | 0.248 |
| Granulomatous activity | 35 | 49.3 | 17 | 53.1 | 0.442 |
| Crusts | 48 | 67.6 | 17 | 53.1 | 0.118 |
| Edema | 10 | 14.1 | 7 | 21.9 | 0.239 |
| Stenosis | 10 | 14.1 | 11 | 34.4 | 0.020 |
| Bleeding | 0 | 0.0 | 4 | 12.5 | 0.002 |
| Mucosal atrophy | 7 | 10.0 | 2 | 6.3 | 0.420 |
| Hyperemia | 15 | 21.4 | 6 | 18.8 | 0.489 |
| Ulceration | 5 | 7.0 | 2 | 6.3 | 0.624 |
| Purulent drainage | 1 | 1.4 | 2 | 6.3 | 0.227 |
| **CT scan findings (n = 48)** | | | | |
| Thickening of mucosa | 22 | 71.0 | 12 | 70.6 | 0.614 |
| Sinusopathy | 8 | 25.8 | 5 | 29.4 | 0.522 |
| Mastoidopathy | 3 | 9.7 | 2 | 11.8 | 0.588 |

L-AMB—liposomal amphotericin B

* cell with value = 0

https://doi.org/10.1371/journal.pone.0218786.t003
observed. In this study, pentamidine had fewer adverse events than other drugs. The use of amphotericin B deoxycholate should be limited, although it produces a small number of recurrences and has better action on mucosal lesions compared to antimonials [18]. The number of adverse events was high, and in this study, the actual recurrence rate with the use of amphotericin B deoxycholate cannot be evaluated due to the large number of patients who were not able to complete the treatment.

Studies that compared lipid formulations and the use of ABCL in the treatment of ML are not available. Previous studies on the use of amphotericin B colloidal dispersion in the treatment of ML showed an efficacy rate between 88% and 100% [14]. This is the first study that used ABCL in the treatment of ML, and results showed a success rate limited to 46.2% (6/13) and a relapse rate of 7.7% in patients who were treated with a high rate of permanent interruption (61.5%).

Despite the fact that itraconazole is a safe therapeutic option for the treatment of ML, its use had a low efficacy (44.4%). A recent review of the use of azoles in the treatment of ML

| Table 4. Adverse effects of drugs used in the treatment of mucosal leishmaniasis. |
|---------------------------------|-----------------|-----------------|-----------------|-------|-------|-------|-------|-------|-------|-------|-------|
| Data                            | Global          | Antimonial       | Pentamidine     | ABCL  | d-AMB | Itraconazole | L-AMB | P value* | P value |
| Total Adverse events (AE)       | 79              | 76.0            |                 |       |       |               |       |         |         |
| Clinical                        |                 |                 |                 |       |       |               |       |         |         |
| Infusion related AE             | 50              | 48.1            | 11              | 44.0  | 5     | 45.5           | 10    | 76.9     | 7       | 50.0   | 0     | 0.0   | 17    | 54.8  | 0.026 |
| Phlebitis                       | 21              | 20.2            | 2               | 8.0   | 4     | 36.4           | 6     | 46.2     | 3       | 21.4   | 0     | 0.0   | 6     | 19.4  | 0.023 |
| Nausea/vomits                   | 19              | 18.3            | 3               | 12.0  | 3     | 27.3           | 5     | 38.5     | 4       | 28.6   | 0     | 0.0   | 0     | 12.9  |
| Fever                           | 9               | 8.7             | 2               | 8.0   | 0     | 0.0            | 3     | 23.1     | 1       | 7.1    | 0     | 0.0   | 0     | 3     | 9.7   |
| Headache                        | 9               | 8.6             | 2               | 8.0   | 1     | 9.1            | 1     | 7.7      | 3       | 21.4   | 0     | 0.0   | 0     | 2     | 6.5   |
| Chest pain                      | 8               | 7.7             | 1               | 4.0   | 0     | 0.0            | 3     | 23.1     | 0       | 0.0    | 0     | 0.0   | 0     | 4     | 12.9  |
| Tremor                          | 7               | 6.7             | 0               | 0.0   | 0     | 0.0            | 0     | 0.0      | 4       | 30.8   | 0     | 0.0   | 0     | 0     | 3     | 9.7   | 0.004 0.031 |
| Chills                          | 6               | 5.8             | 0               | 0.0   | 0     | 0.0            | 2     | 15.4     | 3       | 21.4   | 0     | 0.0   | 0     | 1     | 3.2   |
| Muscle pain                     | 6               | 5.7             | 3               | 12.0  | 0     | 0.0            | 1     | 7.7      | 0       | 0.0    | 0     | 0.0   | 0     | 0     | 2     | 6.5   |
| Lumbar pain                     | 5               | 4.8             | 0               | 0.0   | 0     | 0.0            | 1     | 7.7      | 0       | 0.0    | 0     | 0.0   | 0     | 0     | 4     | 12.9  | 0.027 |
| Palpitations                    | 5               | 4.8             | 1               | 4.0   | 0     | 0.0            | 1     | 7.7      | 0       | 0.0    | 0     | 0.0   | 0     | 0.0   | 3     | 9.7   |
| Arthritis                       | 4               | 3.8             | 4               | 16.0  | 0     | 0.0            | 0     | 0.0      | 0       | 0.0    | 0     | 0.0   | 0     | 0     | 0     | 0.003 |
| Sweat                           | 3               | 2.9             | 0               | 0.0   | 0     | 0.0            | 2     | 15.4     | 0       | 0.0    | 0     | 0.0   | 0     | 1     | 3.2   | 0.041 |
| Gastrintestinal other           | 3               | 2.9             | 3               | 12.0  | 0     | 0.0            | 0     | 0.0      | 0       | 0.0    | 0     | 0.0   | 0     | 0     | 0     | 0.0   |
| Malaise                         | 2               | 1.9             | 1               | 4.0   | 1     | 9.1            | 0     | 0.0      | 0       | 0.0    | 0     | 0.0   | 0     | 0     | 0     | 0.00 |
| Rash                            | 1               | 1.9             | 1               | 4.0   | 0     | 0.0            | 0     | 0.0      | 0       | 0.0    | 0     | 0.0   | 0     | 0     | 0     | 0.0   |
| Laboratorial                    |                 |                 |                 |       |       |               |       |          |         |        |       |       |       |       |       |
| Electrolyte disturbance         | 30              | 28.8            | 1               | 4.0   | 4     | 36.4           | 6     | 46.2     | 7       | 50.0   | 0     | 0.0   | 12    | 38.7  | 0.001 0.001 |
| Acute kidney injury             | 20              | 19.2            | 0               | 0.0   | 0     | 0.0            | 0     | 7.7      | 8       | 57.1   | 0     | 0.0   | 11    | 35.5  | 0.001 0.008 |
| Liver enzyme alteration         | 7               | 6.7             | 3               | 12.0  | 0     | 0.0            | 0     | 0.0      | 0       | 0.0    | 0     | 0.0   | 0     | 0.0   | 0     | 0.0   | 0.042 |
| Hyperamylasemia                 | 7               | 6.7             | 7               | 28.0  | 0     | 0.0            | 0     | 0.0      | 0       | 0.0    | 0     | 0.0   | 0     | 0.0   | 0     | 0.0   | 0.001 |
| ECG alterations                 | 5               | 4.8             | 5               | 20.0  | 0     | 0.0            | 0     | 0.0      | 0       | 0.0    | 0     | 0.0   | 0     | 0.0   | 0     | 0.0   | 0.001 |
| Any mielotoxicity               | 3               | 2.9             | 1               | 4.0   | 1     | 9.1            | 1     | 7.7      | 0       | 0.0    | 0     | 0.0   | 0     | 0.0   | 0     | 0.0   |
| Hypoglicemia                    | 1               | 1.0             | 0               | 0.0   | 1     | 9.1            | 0     | 0.0      | 0       | 0.0    | 0     | 0.0   | 0     | 0.0   | 0     | 0.0   |

* P value for gray box in the line. The first p value is reference to the first gray box from left to right. The second P value is reference to the second gray box from left to right.

https://doi.org/10.1371/journal.pone.0218786.t004
revealed a 50% efficacy rate: 37% for ketoconazole and 61% for fluconazole and itraconazole, which varied according to the species of Leishmania, with the worst responses to L. braziliensis, which is the most important species in Brazil [19]. In total, 38 patients with ML were treated with azoles, showing an efficacy rate of 52.6%, ranging from 23% to 73.3% [20, 21].

In Brazil, ML is considered a public health problem due to its morbidity. Most ML cases involved men due to increased work-related exposure to sandflies [22]. In addition, a high level of testosterone increases the production of IL-4 and TGF-β, leading to more severe lesions [23]. The most frequent symptoms of ML were nasal obstruction (72.9%), epistaxis (70.0%), and rhinorrhea (52.9%), as observed in the literature [24]. According to Marsden (1986), these are among the earliest symptoms of ML. However, late diagnosis was an important factor in our study because only 7 out of 50 patients (14%) were diagnosed within the first year of symptom onset and 32 (64%) after 5 years or longer. In the examination of the mucosa, erythema, infiltration, erosion, ulceration, crusts, and mucopurulent exudate can be observed. The most common findings on rhinoscopy and/or nasofibroscopy were septal perforation, crusts, and granulomatous process in 66.2%, 65.2%, and 58.0% of the cases, respectively. Different results were recorded in the literature, and septal perforation was observed in 41.4–77.0% of the patients [11, 25].

The sinus CT of the face revealed alterations in 86.2% of the patients. Mucosal thickening of some paranasal sinuses occurred in 62.1% (18/29) of the cases, and sinusopathy was observed in 27.2% of the participants. Camargo et al. have revealed the expressive value of 96.0% for paranasal sinus mucosal thickening on CT scan, suggesting that inflammation in patients with ML is not restricted to the nasal mucosa and may extend to the paranasal sinuses and other structures of the respiratory tract [10].

In our study, more than 70% of the patients presented with some AEs. The drugs with more AEs were d-AMB and ABCL. Pentavalent antimonials had presented electrocardiographic and pancreatic enzyme changes occurred in 20% and 28% of the patients who used the drug, respectively (p = 0.005; p <0.001). In addition, these AEs were the primarily cause for the permanent interruption in the use of this drug (8 out of 11 interruptions). The most frequent pentamidine AE was phlebitis (36.4%), followed by nausea and vomiting (27.3%). Despite the high frequency rate of the general AEs in this study, the use of pentamidine is considered safe, as previously described [11]. The use of ABCL led to a significantly high incidence rate of phlebitis (46.2%) and tremors (30.8%) compared to other leishmanicidal drugs (p = 0.029 and p = 0.005). The AEs of ABLC are more common than those of L-AMB [26]. These observations have important clinical relevance because the infusion in 8 of the 13 patients who used ABLC was interrupted. Nevertheless, the liposome of amphotericin B attenuates the release of cytokines, which decreases infusion-related AEs [27]. In contrast, the rapid removal of ABCL from the circulation by reticuloendothelial tissues, particularly in the liver [28], may result in the release of proinflammatory cytokines from the surrounding macrophages and contribute to the AEs associated with the administration of ABCL.

The major limitations of this study are the retrospective analysis, few patients for sub-analysis, different duration of the disease. Unfortunately these biases cannot be controlled. The doses of drugs were different among patients with the same therapeutic group, considering that this was not a clinical trial.

This retrospective study suggests that L-AMB is an effective drug in the treatment of ML. Approach of patients with ML should be reconsidered and L-AMB could be considered as a first line of therapy. A controlled study is need in this context, but at this moment, this is the best evidence we have to treat ML. The treatment of ML treatment is still far from ideal because the best-acting drugs for this condition have numerous AEs, and drugs that are safer to use have a low efficacy. L-AMB presented with better results than other drugs used for the
treatment of ML. However, the incidence rate of nephrotoxicity associated with this drug is high.

**Supporting information**

S1 File. Clinica data of patients. (XLSX)

**Author Contributions**

**Conceptualization:** Valdir Sabbaga Amato.

**Formal analysis:** Felipe Francisco Tuon, Juliette Cieslinski.

**Investigation:** Carolina Rocio Santos, Regina Maia de Souza, Rui Imamura.

**Methodology:** Valdir Sabbaga Amato.

**Project administration:** Juliette Cieslinski.

**Resources:** Regina Maia de Souza.

**Supervision:** Carolina Rocio Santos.

**Visualization:** Rui Imamura.

**Writing – original draft:** Felipe Francisco Tuon.

**Writing – review & editing:** Carolina Rocio Santos, Felipe Francisco Tuon, Regina Maia de Souza, Valdir Sabbaga Amato.

**References**

1. Boaventura VS, Cafe V, Costa J, Oliveira F, Bafica A, Rosato A, et al. Concomitant early mucosal and cutaneous leishmaniasis in Brazil. *Am J Trop Med Hyg* 2006, 75(2):267–269. PMID: 16896130

2. Amato VS, Tuon FF, Bacha HA, Neto VA, Nicodemo AC. Mucosal leishmaniasis. Current scenario and prospects for treatment. *Acta Trop* 2008, 105(1):1–9. https://doi.org/10.1016/j.actatropica.2007.08.003 PMID: 17884002

3. Amato VS, Tuon FF, Siqueira AM, Nicodemo AC, Neto VA. Treatment of mucosal leishmaniasis in Latin America: systematic review. *Am J Trop Med Hyg* 2007, 77(2):266–274. PMID: 17690398

4. 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(12):e202–e264. https://doi.org/10.1093/cid/ciw670 PMID: 27941151

5. Guery R, Henry B, Martin-Blondel G, Rouzaud C, Cordoliani F, Harms G, et al. Liposomal amphotericin B in travelers with cutaneous and muco-cutaneous leishmaniasis: Not a panacea. *PLoS Negl Trop Dis* 2017, 11(11):e0006094. https://doi.org/10.1371/journal.pntd.0006094 PMID: 29155816

6. Nonata R, Sampaio R, Marsden PD. Mucosal leishmaniasis unresponsive to glucantime therapy successfully treated with AmBisome. *Trans R Soc Trop Med Hyg* 1997, 91(1):77. https://doi.org/10.1016/s0035-9203(97)90404-1 PMID: 9093636

7. Amato VS, Tuon FF, Camargo RA, Souza RM, Santos CR, Nicodemo AC. Can we use a lower dose of liposomal amphotericin B for the treatment of mucosal American leishmaniasis? *Am J Trop Med Hyg* 2011, 85(5):818–819. https://doi.org/10.4269/ajtmh.2011.11-0287 PMID: 22049033

8. Amato VS, Tuon FF, de Andrade HF Jr., Bacha H, Pagliari C, Fernandes ER, et al: Immunohistochemistry and polymerase chain reaction on paraffin-embedded material improve the diagnosis of cutaneous leishmaniasis in the Amazon region. *Int J Dermatol* 2009, 48(10):1091–1095. https://doi.org/10.1111/j.1365-4632.2009.04099.x PMID: 19775402

9. Camargo RA, Tuon FF, Sumi DV, Gebrim EM, Imamura R, Nicodemo AC, et al. Mucosal leishmaniasis and abnormalities on computed tomographic scans of paranasal sinuses. *Am J Trop Med Hyg* 2010, 83(3):515–518. https://doi.org/10.4269/ajtmh.2010.10-0081 PMID: 20810813
10. de Camargo RA, Nicodemo AC, Sumi DV, Gebrim EM, Tuon FF, de Camargo LM, et al. Facial structure alterations and abnormalities of the paranasal sinuses on multidetector computed tomography scans of patients with treated mucosal leishmaniasis. *PLoS Negl Trop Dis* 2014, 8(7):e3001. https://doi.org/10.1371/journal.pntd.0003001 PMID: 25080261

11. Amato VS, Tuon FF, Imamura R, Abegao de Camargo R, Duarte MI, Neto VA. Mucosal leishmaniasis: description of case management approaches and analysis of risk factors for treatment failure in a cohort of 140 patients in Brazil. *J Eur Acad Dermatol Venereol* 2009, 23(9):1026–1034. https://doi.org/10.1111/j.1468-3083.2009.03238.x PMID: 19453817

12. Tuon FF, Koenig F, Jacometto D, Rocha JL. Are there risk factors for acute renal failure in adult patients using deoxycholate amphotericin B? *Rev Iberoam Micol* 2013, 30(1):21–24. https://doi.org/10.1016/j.rim.2012.09.003 PMID: 22995903

13. Solomon M, Pavlotzky F, Barzilai A, Schwartz E. Liposomal amphotericin B in comparison to sodium stiboglucanate for Leishmania braziliensis cutaneous leishmaniasis in travelers. *J Am Acad Dermatol* 2013, 68(2):284–289. https://doi.org/10.1016/j.jaad.2012.06.014 PMID: 22858005

14. Amato VS, Tuon FF, Campos A, Bacha HA, Nicodemo AC, Amato Neto V, et al. Treatment of mucosal leishmaniasis with a lipid formulation of amphotericin B. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America* 2007, 44(2):311–312.

15. Wortmann G, Zapor M, Ressner R, Fraser S, Hartzell J, Pierson J, et al. Liposomal amphotericin B for treatment of cutaneous leishmaniasis. *Am J Trop Med Hyg* 2010, 83(5):1028–1033. https://doi.org/10.4269/ajtmh.2010.10-0171 PMID: 21036832

16. Bermudez H, Rojas E, Garcia L, Desjeux P, Dujardin JC, Boelaert M, et al. Generic sodium stibogluconate is as safe and effective as branded meglumine antimonate, for the treatment of tegumentary leishmaniasis in Isibo Secure Park, Bolivia. *Ann Trop Med Parasitol* 2006, 100(7):591–600. https://doi.org/10.1179/136867906X118495 PMID: 16989686

17. Scope A, Trau H, Anders G, Barzilai A, Confino Y, Schwartz E. Experience with New World cutaneous leishmaniasis in travelers. *J Am Acad Dermatol* 2003, 49(4):672–678. PMID: 14512915

18. Castro RM. Tratamento da leishmaniose tegumentar americana pela anfotericina B–A propósito de 70 casos. *An Bras Dermatol* 1972, 47(3).

19. Galvao EL, Rabello A, Cota GF. Efficacy ofazole therapy for tegumentary leishmaniasis: A systematic review and meta-analysis. *PLoS One* 2017, 12(10):e0186117. https://doi.org/10.1371/journal.pone.0186117 PMID: 29016694

20. Amato VS, Padilha AR, Nicodemo AC, Duarte MI, Valentini M, Uip DE, et al. Use of itraconazole in the treatment of mucocutaneous leishmaniasis: a pilot study. *Int J Infect Dis* 2000, 4(3):153–157. PMID: 11179919

21. Calvopina M, Guevara AG, Armijos RX, Hashiguchi Y, Davidson RN, Cooper PJ. Itraconazole in the treatment of New World mucocutaneous leishmaniasis. *Int J Dermatol* 2004, 43(9):659–663. https://doi.org/10.1093/ijid/156.1.73 PMID: 15357745

22. Jones TC, Johnson WD Jr., Barretto AC, Lago E, Badaró R, Cerf B, et al. Epidemiology of American cutaneous leishmaniasis due to Leishmania braziliensis braziliensis. *J Infect Dis* 1987, 156(1):73–83. https://doi.org/10.1093/infdis/156.1.73 PMID: 3598227

23. Snider H, Lezama-Davila C, Alexander J, Satoskar AR. Sex hormones and modulation of immunity against leishmaniasis. *Neuroimmunomodulation* 2009, 16(2):106–113. https://doi.org/10.1159/000180265 PMID: 19212130

24. Saenz RE, Paz HM, de Rodriguez GC, de Vasquez AM, Mata RE, Johnson CM. [Mucocutaneous leishmaniasis in Panama. Etiologic agent, epidemiologic and clinical aspects]. *Rev Med Panama* 1989, 14(1):6–15. PMID: 2727332

25. Cunha MA, Leao AC, de Cassia Soler R, Lindoso JA. Efficacy and Safety of Liposomal Amphotericin B for the Treatment of Mucosal Leishmaniasis from the New World: A Retrospective Study. *Am J Trop Med Hyg* 2015, 93(6):1214–1218. https://doi.org/10.4269/ajtmh.15-0033 PMID: 26483120

26. Wingard JR, White MH, Anaissie E, Raffalli J, Goodman J, Arrieta A, et al. A randomized, double-blind comparative trial evaluating the safety of liposomal amphotericin B versus amphotericin B lipid complex in the empirical treatment of febrile neutropenia. *L Amphot BLC Collaborative Study Group. Clin Infect Dis* 2000, 31(5):1155–1163. https://doi.org/10.1086/317451 PMID: 11073745

27. Arning M, Klische KO, Heer-Sonderhoff AH, Wehmeier A. Infusion-related toxicity of three different amphotericin B formulations and its relation to cytokine plasma levels. *Mycoses* 1995, 38(11–12):459–465. PMID: 8720196

28. Kan VL, Bennett JE, Amantea MA, Smolak MC, McManus E, Grasela DM, et al. Comparative safety, tolerance, and pharmacokinetics of amphotericin B lipid complex and amphotericin B deoxycholate in healthy male volunteers. *J Infect Dis* 1991, 164(2):418–421. https://doi.org/10.1093/infdis/164.2.418 PMID: 1856491