

## Major Article

# Mucosal leishmaniasis: the experience of a Brazilian referral center

**Mariana Junqueira Pedras<sup>[1],[2]</sup>, Janaína de Pina Carvalho<sup>[1],[2]</sup>, Rosiana Estéfane da Silva<sup>[1]</sup>, Dario Brock Ramalho<sup>[3]</sup>, Maria Camilo Ribeiro de Senna<sup>[1]</sup>, Hugo Silva Assis Moreira<sup>[1]</sup>, Lorena Zaine Matos Martinho<sup>[3]</sup>, Ana Rabello<sup>[1],[2]</sup> and Gláucia Cota<sup>[1],[2]</sup>**

[1]. Pesquisa Clínica e Políticas Públicas em Doenças Infecciosas e Parasitárias, Instituto René Rachou, Fundação Oswaldo Cruz, Belo Horizonte, MG, Brasil.

[2]. Programa de Pós-Graduação Stricto Sensu em Saúde Coletiva, Fundação Oswaldo Cruz, Belo Horizonte, MG, Brasil.

[3]. Hospital Eduardo de Menezes, Fundação Hospitalar do Estado de Minas Gerais, Belo Horizonte, MG, Brasil.

### Abstract

**Introduction:** Pentavalent antimonials (Sb<sup>v</sup>) are the most commonly used drugs for the treatment of mucosal leishmaniasis (ML), despite their high toxicity and only moderate efficacy. The aim of this study was to report therapeutic responses with different available options for ML. **Methods:** This study was based on a review of clinical records of 35 patients (24 men and 11 women) treated between 2009 and 2015. **Results:** The median age of patients was 63 years, and the median duration of the disease was 24 months. Seventeen patients received Sb<sup>v</sup>, while nine patients were treated with liposomal amphotericin B (AmB), and another nine patients were treated with fluconazole. Patients treated with AmB received a total median accumulated dose of 2550mg. The mean duration of azole use was 120 days, and the daily dose ranged from 450 to 900mg. At the three-month follow-up visit, the cure rate was 35%, 67%, and 22% for Sb<sup>v</sup>, AmB, and azole groups, respectively. At the six-month follow-up visit, the cure rates for Sb<sup>v</sup>, AmB, and azole groups were 71%, 78%, and 33%, respectively. **Conclusions:** There is a scarcity of effective ML treatment alternatives, and based on our observations, fluconazole is not a valid treatment option.

**Keywords:** Mucosal leishmaniasis. Therapy. Meglumine antimoniate. Amphotericin B. Azole.

### INTRODUCTION

The World Health Organization<sup>1</sup> considers leishmaniasis as one of the six most important infectious diseases. Mucosal leishmaniasis (ML) is the most severe form of tegumentary leishmaniasis (TL), resulting from hematogenous and lymphatic dissemination of cutaneous tissue parasites into the nasal mucosa, oropharynx, palates, lips, tongue, larynx, and exceptionally, the trachea and upper respiratory tree<sup>2,3</sup>. The infection causes the onset of inflammatory reactions and sometimes deforming ulcers. The lesions may appear months or years after the onset of the cutaneous lesion, which in some cases may be absent. The mucosal form is related to functional alterations and facial deformations, which reflects much of the disease morbidity in the psychological and social dimensions of patients' lives.

According to a systematic review in America<sup>4</sup>, pentavalent antimonials (Sb<sup>v</sup>) are the most commonly used drugs for the treatment of ML, followed by amphotericin B. Other

drugs described are aminosidine, azoles, allopurinol, and immunotherapy, in combination with pentoxifylline. According to this review, the efficacy of Sb<sup>v</sup> in ML was estimated to be 67%. In Brazil, meglumine antimoniate is the drug of choice<sup>5</sup>, despite its recognized toxicity, especially in pancreatic, hepatic and cardiac systems. Clinical experience of ML treatment with lipid formulations of amphotericin B has been increasing in recent years, but it is still scarce, although a quite promising<sup>6,7</sup>. On the other hand, azole drugs have been also identified as a treatment option for *Leishmania major* and *Leishmania mexicana*<sup>1</sup>. There are few studies addressing the cure rate of patients infected with other *Leishmania* species and additional evidence is necessary to draw conclusions on the azole applicability. In addition to the existing therapeutic limitations, there is a paucity of evidence to support disease management. Moreover, although there is a panel of available options, patients that cannot undergo any of the recommended therapies, mainly due to the risk of renal deterioration, are a reality in reference centers. Even with a renal toxicity rate lower than that observed with amphotericin B deoxycholate, the use of liposomal amphotericin B, at doses necessary for ML treatment, may represent a real risk of renal deterioration at the dialysis level in elderly patients already suffering from renal dysfunction. Another complicating factor for the use of therapeutic parenteral alternatives

**Corresponding author:** Dra. Gláucia Fernandes Cota.

**e-mail:** cota@minas.fiocruz.br

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is the hospitalization required for treatment, which adds a disproportionate effort for families, compared with the chronic and often moderate symptoms caused by the disease. In the context of therapeutic challenges, the aim of this study was to report the therapeutic responses and the adverse events related to some of the available ML treatment options.

## METHODS

This is a retrospective study based on review of clinical records of patients attended at the Leishmaniasis Referral Centre of *Instituto René Rachou* (IRR), *Fundação Oswaldo Cruz* (FIOCRUZ) unit in Belo Horizonte, Minas Gerais, Brazil.

### Ethical considerations

The study protocol was reviewed and approved by the two institutional ethical review boards (ERB) involved: IRR-ERB number 1,578,874 and HEM-ERB number 2,001,556.

In the present analysis, patients diagnosed with ML from 2009 to 2015 were included. According to local routine, ML diagnosis was established in all patients based on mucosal involvement defined by nasofibroscope, associated with one or more of the following confirmatory tests: Montenegro skin test, serology (indirect immunofluorescence), polymerase chain reaction (PCR) targeting *Leishmania* kinetoplast deoxyribonucleic acid (kDNA) and a compatible histopathological exam. Although *Leishmania* culture is not performed during routine ML investigation, based on previous surveillance studies, the main circulating species in the Southeast region of Brazil is *Leishmania braziliensis*<sup>5</sup> Mucosal biopsies were routinely conducted under local anesthesia and tissue fragments were submitted for histological examination. The PCR test for *Leishmania* became routinely available only after 2013. A few patients refused biopsies or had a contraindication for the procedure, mainly due to chronic use of anticoagulants or platelet disturbance.

Symptom severity was considered as: mild, symptoms confined to the nose; moderate, odynophagia, dysphonia, and/or mild respiratory distress; and severe, odynophagia, dysphonia, and severe respiratory distress<sup>6</sup>. The response to treatment was assessed according to the otorhinolaryngological examination at three different time points:  $90 \pm 15$  days, six months and one year from the first day of treatment. Cure was defined as the total healing of the mucosal lesion. In this study, any condition other than cure was considered as therapeutic failure. Relapse was defined as a new lesion or return of lesion activity after a complete healing at any moment. The cure rate at six months was chosen as the primary endpoint.

During therapy, all patients were monitored with laboratory tests and electrocardiograms. For the assessment of adverse events, all records and laboratory test results present in the medical charts were evaluated.

According to the Brazilian guidelines for TL management in force at the time of patients' treatment, Sb<sup>v</sup> was the first drug therapy in all cases except for patients with contraindications to the use of Sb<sup>v</sup>, namely, patients with renal, cardiac or hepatic insufficiency or chronic users of medications that extend the QT

interval (the measure of the time between the start of the Q wave and the end of the T wave in the heart's electrical cycle). The available Sb<sup>v</sup> in Brazil is N-methyl-glucamine (Glucantime<sup>®</sup>), which is produced by Aventis Pharma, Brazil.

The descriptive analysis included simple frequencies and the median and its respective interquartile range of 25-75% (IQR 25-75%) or the mean and standard deviation, whenever appropriate. Continuous variables were analyzed with unpaired Student's t tests for normally distributed variables and Wilcoxon tests for variables with skewed distributions. Chi-square tests were used to compare categorical variables. Cure rates with different treatment options are presented with the 95% confidence interval (95% CI). For comparisons in a bivariate model, we assumed the cure with antimony treatment as the reference standard. Statistical significance was set at the 0.05 level. All analyses were performed using Statistical Product and Service Solutions, Statistical Package for the Social Sciences [(IBM - SPSS<sup>®</sup>); version 23, California, USA].

## RESULTS

From 2009 to 2015, a total of 35 patients were diagnosed with ML at IRR: 24 (70%) were men and 11 (31%) women. The nasal mucosa was affected in 33 (94%) out of 35 patients, and 30 had a septum ulcer or perforation. Following the nasal mucosa, the most affected sites were the mouth/oral cavity and pharynx (11% each). The median age was 63 years (from 16 to 85 years), and the median duration of the disease was 24 months, with an IQR 25-75% ranging from 7 to 48 months. ML diagnosis was parasitologically confirmed in 18 (51%) patients. Qualitative PCR based on k-DNA was performed in mucosal fragments from 20 patients, and the test was positive in 16 (80% positivity). One patient had the parasitological confirmation by the direct exam and another by the histological examination (presence of *Leishmania* bodies). In the remaining seventeen (49%) patients, diagnosis was defined on a clinical-epidemiology basis: a positive *Leishmania* intradermal skin test or serological test plus a compatible inflammatory pattern on histological examination. Concerning therapy, half of the ML patients (17 patients) received parenteral pentavalent antimony, while the rest had one or more contraindications or had experienced severe side effects with antimony derivatives, thus requiring alternative therapies: nine were treated with liposomal amphotericin B, and another nine were treated with fluconazole. In these nine cases, antimony was contraindicated, and liposomal amphotericin B was considered as an alternative with a high risk of causing irreversible renal damage. The relevant patient clinical data are summarized in **Table 1**.

Antimony therapy was administered according to the Brazilian treatment recommendation of 20mg/kg/day of Sb<sup>v</sup> for 30 days. However, due to the upper daily limit of three ampoules, four patients weighing more than 90kg received less than 15mg Sb<sup>v</sup>/kg/day. Patients treated with liposomal amphotericin B (AmB) received a total median accumulated dose of 2,550mg (minimum 2,100 to a maximum 3,000mg), corresponding to a dose of three mg/kg/day that could be achieved in a variable number of days (median 14, ranging from 11 to 20 days).

**TABLE 1:** Clinical characteristics of ML patients treated in IRR according to the course of therapy, 2009-2015.

Characteristic	Antimony (17 patients)	Amphotericin B (9 patients)	Azole (9 patients)	P value*
Sex				0.78
male	11	7	6	
female	6	2	3	
Age in years, median (min-max)	39 (16-64)	68 (63-80)	74 (51-85)	0.00
Median lesion duration before treatment, months (min-max)	12 (6-168)	36 (2-420)	24 (3-240)	0.36
Nasal mucosal perforation	13/17	8/9	9/9	0.25
ML classification				
mild	14	6	9	0.34
moderate	1	2	0	
severe	2	1	0	
Previous ML therapy with Sb <sup>v</sup>	2/17	3/9	1/9	0.32
Alternative therapy indication				
systemic antimony contraindication	-	9	8	0.54
previous systemic antimony treatment failure	-	0	1	
Previous serious adverse event with Sb <sup>v</sup>	-	3	4	
Comorbidities				
hypertension	1/17	5/9	9/9	0.00
diabetes mellitus	0/17	1/9	2/9	0.15
chronic heart disease	0/17	3/9	0/9	0.05
chronic renal disease	0/17	2/9	3/9	0.05
alcohol abuse	0/17	1/9	1/9	0.36
Systemic antimony contra-indications**				
heart disease	0	3	0	0.05
renal disease	0	2	3	0.05
continuous use of medications that extend QTc interval	0	2	1	0.46
liver disease	0	0	0	-
>50 years	3/17	9/9	9/9	0.00

**ML:** mucosal leishmaniasis; **IRR:** Instituto René Rachou; **Sb<sup>v</sup>:** pentavalent antimonial; **QTc:** the measure of the time between the start of the Q wave and the end of the T wave in the heart's electrical cycle. \*Chi-square and Wilcoxon tests. \*\*Some patients presented more than one contraindication condition.

All patients treated with liposomal amphotericin B received Ambisome<sup>®</sup> produced by Gilead, USA, during hospitalization in the Hospital Eduardo de Menezes, a partner institution. Treatment with fluconazole has been indicated in our center in recent years as an alternative for select patients with severe restrictions to the use of antimoniate and liposomal amphotericin B. This alternative approach is based on few reports<sup>9,10</sup> and on the convenience of using this oral medication with satisfactory safety profile. Regarding the fluconazole treatment duration, there was no pre-established protocol. Routinely, an otorhinolaryngological evaluation was performed every 90

days to assess the patient's response and the need to continue treatment. The absence of any improvement over the previous physical evaluation triggered discontinuation of treatment. The median duration of azole use was 120 days (ranging from 49 to 396 days), and the daily dose varied from 450 to 900mg (median 600mg). Antimony, liposomal amphotericin B and fluconazole therapy schedules are presented in **Table 2**.

Patients' outcomes in response to different treatments are presented in **Figure 1**. In this retrospective analysis, therapeutic responses were assessed with the most conservative approach; therefore, miss of follow-up visits was considered as treatment





of patients treated with antimony daily doses lower and higher than 15mg/kg. Three out of four patients, who had received less than 15mg/kg/day of antimony, were considered as therapeutic failure at the six-month visit ( $p=0.05$ ). Among patients treated with fluconazole, at the one-year follow-up, four (44%) patients had complete resolution of symptoms, but only two of them had a complete cure according to the otorhinolaryngological exam (a final cure rate of 22%). It should be emphasized that the loss of information in this retrospective study is not negligible. Thus, of the nine patients who started using fluconazole, three had no record of follow-up evaluations for outcome assessment. In a protocol-by-protocol analysis, i.e., including only those patients who received the prescribed treatment and who could be evaluated, the clinical response rate was 67%, and complete cure with fluconazole upon physical exam was 33%.

## DISCUSSION

The main contribution of this study is to emphasize on a real challenge in the management of ML: the high frequency of patients with advanced age or comorbidities that have limited therapeutic options. This study also demonstrates that some experience with alternative therapies for ML, particularly with azole derivatives, has been accumulated in recent years. Despite the lack of a validated protocol, in few and selected cases, we have used a prolonged therapeutic regimen with high daily fluconazole doses. Using a rigorous analysis, intention-to-treat principle and complete cure concept according to otorhinolaryngological examination results, we observed a modest overall cure rate at six months of approximately 30% and an adverse events rate that is not negligible, reinforcing caution in using this approach.

The observations presented here are based on a small but representative patient set: a predominantly male group, aged >50 years, presenting with several chronic comorbidities. In addition, many patients had already undergone prior treatment with first-line drugs without achieving therapeutic success, as reported in **Table 1**. This study confirms the existence of a symptomatic group of patients requiring therapy, for whom there is no safe therapeutic option available.

The cure rates observed for ML in our center were similar to those reported by other authors in Brazil<sup>7,11</sup>. However, the small number of patients and the retrospective design of this study do not allow us to extrapolate the effectiveness and toxicity data. Significant differences were observed between patients undergoing different treatments, confirming that the therapeutic choice is mainly influenced by the clinical conditions of patients, especially the age, presence of comorbidities, and dysfunctions of various organs. Therefore, considering all the determinants involved in the cure rates, effectiveness cannot be directly compared between treatments, alternatively, only one exploratory approach was carried out. However, some points can be made: the cure rate, defined as complete healing of mucosal lesions in association with the absence of any infiltration, is an infrequent event at 90 days of treatment, regardless of the treatment used. Although the rate of improvement (a partial response) is higher, complete resolution at six months of treatment is achieved in fewer than 80% of cases, at best,

revealing that ML is a slow-response condition. Even with the first-line therapeutic option, antimonial derivatives, the cure rate reported in the ML literature can be considered only sub-optimal, reaching approximately 70% efficacy<sup>4,12,13</sup>. It is important to emphasize the lack of a universal criterion for ML cure establishment, as well as when this evaluation should be performed, in addition to other equally important factors, such as the genetic variety of *L. braziliensis*, which could explain some differences observed between studies.

In recent years, based on a few retrospective studies<sup>6,14</sup>, lipid formulations of amphotericin B have been considered as the most attractive treatment modalities for ML, due to better safety profile. In our experience, consistent with a previous systematic review<sup>4</sup>, liposomal amphotericin B was as efficacious as Sb<sup>v</sup>. It is important to note that there are two main challenges in using amphotericin B: the requirement for hospitalization and its potential renal toxicity. In contrast, *in vitro* azole activity against *Leishmania* has been recognized for several years<sup>15</sup>, but clinical results with this therapeutic modality, mainly in cutaneous leishmaniasis, are limited and the results are divergent<sup>16-19</sup>. To the best of our knowledge, there are only three studies evaluating imidazole drugs for ML treatment<sup>11,17,20</sup>. All used itraconazole and were uncontrolled studies. The cure rates observed were 33%, 60% and 23% with a daily dosage of itraconazole between 200 and 400mg for 2-3 months. This low efficiency and wide variation in cure rates confirms that the efficacy of the approach is not yet clearly defined. In contrast to our experience, no adverse events related to the azole therapy have been reported by these authors. However, the absence of a protocol for systematic monitoring of symptoms and laboratory tests may have underestimated the rate of adverse events reported by all available series. Nevertheless, the long treatment period for most patients (median, 4 months) should be emphasized, as it would suggest a good acceptance of the treatment by the patients. The relatively low frequency of liver enzyme elevation in our study (only one patient) is consistent with the safety profile previously described for fluconazole. At the same time, the modest elevation of glutamic-oxaloacetic transaminases (approximately six times the upper normal limit), which was accompanied by nausea, required fluconazole interruption and emphasizes the need for monitoring liver function during treatment. In addition, the high frequency of adverse events during antimonial therapy is clearly demonstrated in **Table 3**.

In conclusion, the context of few therapeutic options for *Leishmania* and the unsatisfactory safety and efficacy profiles of the current first-line therapies, our observations confirm that fluconazole is not a valid treatment option for ML. However, the partial clinical impact observed in this study, i.e., resolution of symptoms in 44% of the azole treatment group, even without resolution of inflammatory activity could represent a possible improvement in the quality of life and should be explored in the future.

Some patients who did not improve with fluconazole chose not to receive any other curative treatment at the risk of deteriorating their fragile clinical condition. This fact confirms the current therapeutic limitations, and the disease may be intractable for some patients even for just controlling their symptoms.

**TABLE 3:** Adverse events reported by patients with ML according to the treatment, IRR 2009-2015.

Symptoms	Antimony	Amphotericin B	Azole	P value*
Abdominal pain	1/17	1/9	0/9	0.59
Diarrhea	1/17	0/9	0/9	0.58
Nausea	4/17	1/9	1/9	0.62
Vomiting	2/17	0/9	0/9	0.32
Myalgia	13/17	0/9	0/9	0.00
Arthralgia	9/17	0/9	0/9	0.00
Hyporexia	3/17	0/9	1/9	0.40
Dizziness	1/17	0/9	0/9	0.58
Fever	1/17	0/9	0/9	0.59
Rash	1/17	0/9	0/9	0.59
Liver enzymes elevation	5/17	1/9	1/9	0.10
Creatinine level elevation	1/17	2/9	0/9	0.34
Lipase level elevation	9/17	0/9	0/9	0.00
ECG abnormalities	6/17	0/9	0/9	0.03

**ML:** mucosal leishmaniasis; **IRR:** Instituto René Rachou; **ECG:** electrocardiogram. \*Chi-square test.

#### Conflict of interest

The authors declare that there is no conflict of interest.

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