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Major Article

A randomized, open-label clinical trial comparing the long-term effects of miltefosine and meglumine antimoniate for mucosal leishmaniasis

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Abstract

Introduction: The treatment of mucosal leishmaniasis (ML) is difficult due to the toxicity and route of administration of standard drugs. Miltefosine is an oral agent used for leishmaniasis treatment; however, no data exist regarding its use for ML in Brazil. In this study, we aimed to evaluate the efficacy of miltefosine for ML treatment compared to that of pentavalent antimonial in a pilot study. **Methods:** We performed a randomized clinical trial with two parallel groups. The tested intervention consisted of miltefosine 1.3–2 mg/kg/day (two capsules) for 28 days or intravenous 20 mg SbV/kg/day of meglumine antimoniate (N-MA) for 30 days. The final endpoint was defined as complete healing of the lesion four years after treatment. We also analyzed an early endpoint at 90 days after treatment. **Results:** Forty patients were included in this study: each experimental group comprised 20 patients. Applying a multivariate model in an intention-to-treat analysis, we observed that patients treated with miltefosine had a cure probability 2.08 times greater (95% confidence interval [CI] = 1.03–4.18) than those treated with N-MA at 90 days after treatment. At the final endpoint, we observed no differences in cure probability between miltefosine and N-MA (relative risk = 0.66; 95% CI = 0.33–1.32). With respect to adverse reactions, significant differences between groups were related to gastrointestinal effects, which were more frequent in the miltefosine group. **Conclusions:** Miltefosine may be an interesting alternative for treating ML because of its oral administration and cure rate after long-term follow-up.

Keywords: Leishmaniasis. Mucosal Leishmaniasis. Controlled clinical trial. Pentavalent antimonial. Therapeutics. Miltefosine.

INTRODUCTION

American tegumentary leishmaniasis (ATL) is increasing alarmingly in incidence[1]. The mucosal form is characterized by the resulting facial disfiguration[2,3]. ATL is generally caused by *Leishmania (V.) braziliensis*, although it may also be caused by *Leishmania (V.) panamensis*, *Leishmania (V.) guyanensis* or, rarely, by *Leishmania (L.) amazonensis*[4-8]. Previous studies have indicated that mucosal lesions develop six to 24 months after cutaneous leishmaniasis (CL), beginning as septal infiltration and ultimately resulting in severe sequelae and morbidity[9]. Mucosal lesions may cause airway obstruction and, in rare cases (approximately 1%), lead to death due to malnutrition, respiratory infection, and sepsis[3,10,30]. The disease usually affects patients older than 60 years[11] and these patients are also more susceptible to the adverse effects of the drugs used to treat ATL[12,13].

The standard treatment for mucosal leishmaniasis (ML) defined by the World Health Organization (WHO), the Pan American Health Organization (PAHO), and the Brazilian Ministry of Health (MS) is a relatively toxic dose of N-methyl-glucamine (N-MA) at 20 mg SbV/kg/day for 30 days by parenteral route. Therapeutic failure is also a concern and occurs

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in 30%–42% of treated patients[1,11,14]. Antimony resistance is also possible and is a cause for concern, especially in India, although it has also been reported in other countries[15]. Second-line treatment options include drugs such as amphotericin B and pentamidine; however, they are also administered by injection and have numerous severe adverse reactions[16].

Miltefosine (MILT) is an oral drug option used for the treatment of leishmaniasis. Some studies have demonstrated its efficacy for visceral leishmaniasis (VL) and CL[17-20]. However, the efficacy of this drug in ML is unknown. A previously published systematic review of the literature did not include any relevant studies on the use of MILT in ML and confirmed the lack of randomized clinical trials for this form of the disease[21].

The mechanism of action of MILT in *Leishmania* parasites is also unknown, although it is generally recognized that this medication affects lipid membranes by inhibiting cytochrome C oxidase, which is involved in mitochondrial function, as well as by inducing apoptosis, and producing immunomodulatory effects that improve phagocytosis by macrophages[22,23]. These mechanisms favor the use of MILT in the treatment of leishmaniasis because, in addition to its direct effects on parasites, it also modulates the body's immune response against *Leishmania*[22,23].

The main objective of this study was to evaluate the efficacy and adverse events of MILT in ML compared to those of pentavalent antimonials in a pilot study with early and long-term evaluation.

METHODS

The present study consists of a phase two, open-label, randomized clinical trial. Two parallel groups were compared.

Patient population

The study was conducted from January 2010 to December 2016 at the Hospital Universitário de Brasília (HUB) in Brazil, to which patients are referred by primary care facilities for diagnostic confirmation of suspected cases of ATL.

All patients with a confirmed diagnosis of ML were consecutively included after signing an informed consent form. The diagnosis of ML was made according to a previously described composite reference standard[6,7]. We included patients who had a clinical (the presence of any infiltration or ulceration in nasopharyngeal or oral structures) and an epidemiological history compatible with ML, in addition to parasite visualization (culture, direct examination, histopathology), or at least two of the following exams compatible with the diagnosis: Montenegro's skin test, compatible histopathological infiltrate, and indirect immunofluorescence. The subgenus of the detected parasite was identified using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)[6,7]. Patients who were over 70 years old or less than 18 years old, who underwent specific treatment for leishmaniasis less than six months before recruitment, who showed any evidence of immunosuppression (e.g., HIV, immunosuppressive drugs), or who had any clinical condition that contraindicated the use of medications (e.g., pregnancy, renal failure, cardiopathy) were excluded.

A sample size of 40 patients was expected. This population was defined based on the availability of the tested drug, which is not yet commercially available in Brazil.

Allocation and randomization

Patient allocation was made following a random assignment in fixed block sizes of four patients. A staff member, who was different from the principal investigator, randomly created a list containing ten groups of four patients. Two patients from each group were allocated to each block. The generated list was kept by an administrative employee who was not involved in either the intervention or the outcome measurements. Allocation started in June 2009 and finished in May 2012.

Miltefosine group

The tested intervention consisted of the use of 1.3 to 2 mg/kg/day (two capsules) of MILT for 28 days (Impavido® 50 mg capsules, donated by Æterna Zentaris GmbH, Frankfurt, Germany). This dose was successfully used in some of our ML patients who were unresponsive to pentavalent antimonials and other drugs in a previous pilot study of this drug at our center (unpublished data). Patient weight varied from 43 to 75 kg, with a mean value of 60.6 kg. Although there is no current consensus regarding the dose of MILT for the treatment of New World leishmaniasis, the WHO suggests a dose of 2 mg/kg/day, while the PAHO suggests 1.5–2 mg/kg/day[1,24]. This drug is not commercialized in Brazil.

Meglumine antimoniate group

The standard treatment was defined as the intravenous use of 20 mg SbV/kg/day N-MA for 30 days (Glucantime® 81 mg/mL of antimony, 5-mL ampoule, Sanofi-Aventis, São Paulo, Brazil)[1,9].

Outcomes

The main outcome was defined as complete re-epithelization and the absence of any inflammation of the lesion four years after the end of treatment. The patients were actively recruited at the hospital for clinical evaluation at 0, 30, 60, 90, and 180 days, as well as every six months up to four years after treatment. Laboratory exams, clinical evaluation, and an electrocardiogram were performed weekly during treatment and immediately after the end of the treatment to monitor for adverse effects or laboratory abnormalities that would prompt treatment interruption. The adverse reactions that would lead to treatment interruption included, a corrected QT (QTc) interval of more than 450 ms, T-wave inversion, the presence of any arrhythmia by electrocardiogram, any alterations in serum urea or creatinine, and a greater than 2-fold alteration in the level of amylase, aspartate transaminase, alanine transaminase, hemogram, or electrolytes in relation to the reference levels adopted by the HUB. We also tested for the presence of any comorbidity that did not require exclusion from the study, such as controlled diabetes mellitus and hypertension. These evaluations were performed by at least one dermatologist and one otolaryngologist. The otolaryngologist evaluation included clinical evaluation and nasal fiber-optic examination.

Statistical analysis

To estimate relative risks (RR) and 95% confidence intervals (CI) for each variable, a multiple analysis was conducted using Poisson regression with robust variance[25]. Significance level was set at 5%. All analyses were performed using SAS® 9.3 software (SAS Institute Inc., Cary, North Carolina, USA).

Losses

With the aim of measuring efficacy, we performed two analyses: a per-protocol analysis including only patients who concluded treatment and excluding patients who lost the analyzed outcome, and an intention-to-treat-analysis, performed at 90 days and four years after treatment, in which any patient that missed a follow-up visit was considered a therapeutic failure. Treatment suspension was determined when patients received medical recommendations to stop taking the medication due to adverse events. Treatment abandonment was defined as patients who individually decided to stop treatment or follow-up visits independently of medical recommendations.

Ethics

The study complied with the Declaration of Helsinki (1964 and subsequent revisions). The study was approved by the Ethics Committee of the Faculty of Medicine, University of Brasilia (076/2008) and is registered in the clinicaltrials.gov database (NCT01377974).

RESULTS

Basic characteristics and group comparisons

A total of 45 patients were screened for eligibility and of these, five patients did not meet the study criteria. Overall, 40 patients were included in this study: 20 in the MILT group and 20 in the N-MA group (**Figure 1**). The patients originated from a large area that included the Midwestern, Northwestern and Southwestern regions of Brazil; 46% were from the state of Goiás, 15% from Minas Gerais, 11% from Pará, and one patient from each of the following states: Bahia, Tocantins, Mato Grosso do Sul, Maranhão, Ceará, and the Federal District. Regarding clinical symptoms, 68% complained of nasal obstruction, 36% of nasal discharge, 23% of dyspnea, 13% of local pain, and 7% of dysphagia. Basic characteristics of the study population are described in **Table 1**.

Regarding the localization of the lesions, of the 38 patients analyzed, 35 patients had lesions limited to the nose, one presented only oral lesions, and two patients presented lesions on the nasal, oral, and pharyngeal mucosa. Clinical examination showed erythema and nasal infiltration in 68% of cases, while 60% revealed ulceration and septal perforation, 7% had soft palate infiltration and 2% had oropharyngeal fistulae or laryngeal infiltration. Four patients had concomitant mucosal and active skin lesions. Therefore, the majority of the patients had moderate to severe disease and were at least stage IV according to the clinical classification proposed by Lessa et al[26]. All patients resulted positive to the Leishmanin skin test (LST), and 55% of patients had positive indirect immunofluorescence titers at dilutions between 1:40 and 1:80. Amastigote forms were found in the histopathological examinations of 6% of patients. PCR using kinetoplastid DNA (kDNA) from nasal swabs was positive in 58.82% of patients, where *Leishmania (V.) braziliensis* was detected.

A difference between the two groups was found in relation to disease evolution time, which was 112.4 (standard deviation [SD] = 133.3) months in the MILT group and 141.5 (SD = 152.5) months in the N-MA group. Age also differed between the two groups: the mean age was 61.2 (SD = 11.3) years in the MILT group and 50.8 (SD =13.0) years in the N-MA group. In the N-MA group, three patients (16.7%) had comorbidities that did not result in exclusion (two patients with controlled hypertension and one with diabetes). In the MILT group, eight patients (42.1%) had comorbidities (three patients with controlled hypertension and five with diabetes).

Losses

Considering the main endpoint of four years after the end of treatment, we had two losses in the MILT group and six losses in the N-MA group (**Figure 1**). In the MILT group, one patient had their treatment suspended due to abdominal pain and elevation of serum amylase at 231 U/L (reference values: 20–160 U/L), while the second patient abandoned clinical follow-up after treatment completion (**Figure 1**). In the N-MA group, two

TABLE 1: Basic characteristics of the study population.

	Group	
Variables	**Glucantime**	**Miltefosine**
Sex n (%)		
Female	9 (50.0)	11 (55.0)
Male	9 (50.0)	9 (45.0)
Comorbidities n (%)	3 (16.7)	8 (42.1)
Active cutaneous lesions n (%)	1 (5.6)	3 (15.0)
Age (years)	50.8 (SD=13.0)	61.2 (SD=11.3)
Disease time (months)	141.5 (SD=152.5)	112.4 (SD=133.3)

***SD:** Standard deviation.

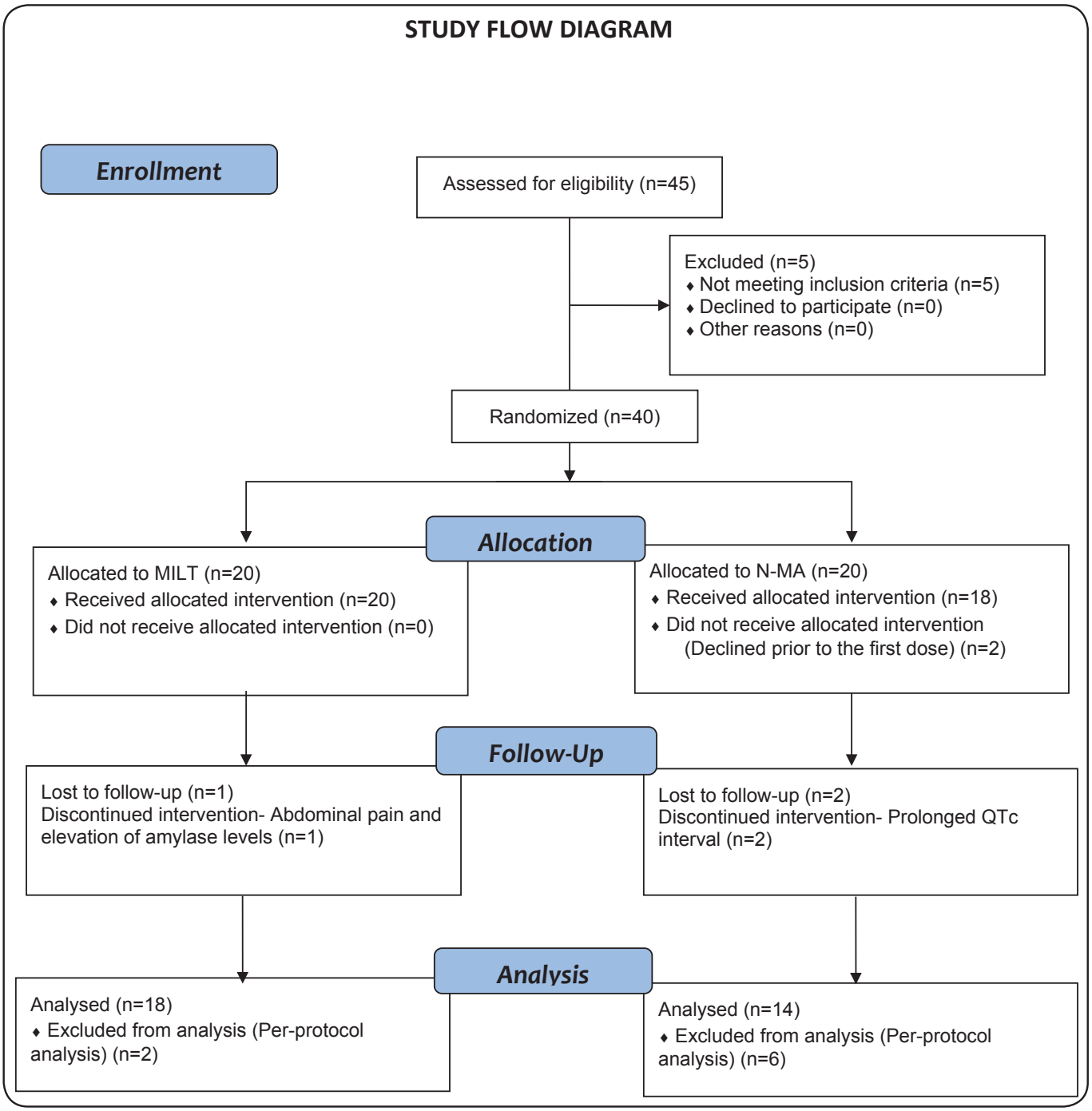

FIGURE 1: Flow diagram of progress through the phases of the study. **MILT**: miltefosine group; **N-MA:** meglumine antimoniate group.

patients did not complete the study due to treatment suspension related to an adverse prolonged corrected QTc interval. One of these patients had a QTc interval of 450 ms in the first week, while the other patient had a QTc interval of 500 ms at the end of the second week (RV: 350 to 450 ms). Furthermore, in the N-MA group, two patients declined to participate in the study after randomization prior to the first medication dose, alleging that the pentavalent antimonial side effects were too severe. Moreover, two additional patients also abandoned clinical follow-up after treatment completion. Upon analyzing the adverse reactions, the only significant differences found between the two groups were related to gastrointestinal effects (i.e., nausea, vomiting, and epigastric pain), which were more frequent in the MILT group (RR 2.97; 95% CI = 1.05–8.38). Among the cases of treatment suspension due to side effects, patients were treated with liposomal amphotericin B according to the Brazilian Ministry of Health protocols[27].

Per-protocol analysis

At the first endpoint 90 days after treatment, 11/12 and 7/10 patients in the MILT and N-MA groups, respectively, were considered cured; while at 180-day follow-up after treatment,

11/12 and 9/10 patients in the MILT and N-MA groups, respectively, were considered cured. The multivariate analysis showed no difference in the cure rate the early follow-up at 90 days after treatment (RR = 1.81; 95% CI = 0.88–3.74) (**Table 2**).

Four years after the end of treatment, 16/18 and 12/14 patients in the MILT and N-MA groups, respectively, were considered cured. Multivariate analysis revealed that patients treated with N-MA had a better cure rate (RR = 1.43; 95% CI = 1.25–1.64) (**Table 2**). Patients with co-morbidity also had a greater probability of cure (RR = 1.49; 95% CI = 1.13–1.97), as did patients with active cutaneous lesions (RR = 1.47; 95% CI = 1.08–1.99).

Intention-to-treat analysis

Applying the multivariate model, 90 days after treatment, patients treated with MILT had a cure probability that was 2.08 times greater than patients treated with N-MA (95% CI = 1.03–4.18); furthermore, the probability of cure was slightly higher in younger patients (95% CI = 0.95–1.00) (**Table 2**).

Applying the proposed intention-to-treat analysis, four years after treatment, 16/20 and 12/20 patients in the MILT and N-MA groups, respectively, were considered cured. The MILT and N-MA groups had similar cure rates (RR = 0.66; 95% CI = 0.33–1.32) (**Table 2**).

DISCUSSION

The development of a new therapeutic regimen for the treatment of ATL is considered a key strategy for public health[1], since treatment represents the best modality to control the disease. The high profile of adverse events and the necessity of parenteral administration for all first- and second-line drugs used in the treatment of ATL present major limitations for disease control. MILT is the only effective oral drug for the treatment of leishmaniasis[17,28].

We performed a clinical trial in a center that is responsible for accepting referrals of ML patients in a broad area of Mid-western Brazil. The majority of patients (72%) came from states were *Leishmania (V.) braziliensis* is the predominant species, and we were able to confirm *Leishmania (V.) braziliensis* as the causative species in most patients. Regarding clinical signs and symptoms, the population characteristics were similar to what has been described in previous studies and reviews[29-31].

Differences between the two experimental groups relative to subject characteristics, such as age and disease time, likely occurred due to chance. In addition, the absence of blinding or sham intervention may have weakened the allocation concealment. However, allocation through randomization is suggested when dealing with small groups, since it allows for groups of the same size[32]. Moreover, we used a multivariate model for the analysis, which can partially correct the effects of this imbalance.

The presence of any co-morbidity that did not contraindicate the use of at least one of the interventions appeared to predict a better cure rate. Patients who had any type of incipient renal impairment, which is a frequent consequence of hypertension and diabetes, have a demonstrably slower metabolism of N-MA; accordingly, this metabolic impairment may also be true for MILT, as previous reports suggest[33].

TABLE 2: Multivariate analysis in the per-protocol and in the intention-to-treat environment 90 days after treatment and four years after treatment. The representation of the variables followed the positive association

90 days after treatment	**Per-protocol**	**Intention-to-treat**
	RR (CI 95 %)	**RR (CI 95 %)**
Male sex	1.93(0.77-4.84)	1.09(0.62-1.92)
No. comorbidities	0.97(0.36-2.63)	1.54(0.73-3.22)
No. active cutaneous lesions	1.63(0.91-2.94)	1.63(0.71-3.73)
Miltefosine group	1.81(0.88-3.74)	2.08(1.03-4.18)
No. treatment suspension	0.83(0.47-1.49)	3.67(0.8-16.66)
Age (years)	0.99(0.97-1.01)	0.98 (0.95-1.00)
Disease time (years)	1.00 (1.00–1.00)	1.00 (1.00–1.00)
4 years after treatment	**Per-protocol**	**Intention-to-treat**
	RR (CI 95 %)	**RR (CI 95 %)**
Female sex	1.01 (0.74–1.40)	1,01 (0,73–1,41)
Associated Comorbidities	1.49 (1.13–1.97)	1,01 (0,54–1,90)
Active cutaneous lesions	1.47 (1.08–1.99)	0,98 (0,54; 1,78)
Glucantime group	1.43 (1.25–1.64)	0,66 (0,33–1,32)
Treatment suspension	1.09 (0.90–1.32)	2,47 (0,86–7,04)
Age (years)	1.01 (1.00–1.01)	0,98 (0,96–1,00)
Disease time (years)	1.00 (1.00–1.00)	1,00 (1,00–1,00)

***RR:** Relative risk; **CI:** Confidence interval.

In the present study, the per-protocol analysis and the adjustment of the multivariate model showed that patients treated with N-MA had a probability of cure that was 1.43 times higher than that of those treated with MILT (95% CI = 1.25–1.64) (**Table 2**). Previously published data support N-MA as the gold standard for the treatment of ATL[21]. Additionally, in the per-protocol analysis, the presence of cutaneous lesions was associated with a better outcome. As ML is frequently the result of untreated cutaneous disease[34], we believe that a plausible explanation for these results relies on the fact that patients with cutaneous lesions may present with a shorter disease duration. Consistently, three patients had fewer than six months of symptoms. Early leishmaniasis infections are known to respond better to treatment compared to chronic lesions[33].

We believe that in the intention-to-treat analysis, in which no differences were identified between MILT and N-MA (RR = 0.66; 95% CI = 0.33–1.32) (**Table 2**), the most relevant factors were not related to the ability of medications to kill Leishmania. Two patients declined to use N-MA after being randomized due to fear of adverse reactions[35,36]. Additionally, MILT tends to have only minor adverse effects (i.e., gastrointestinal effects). Although the multivariate models did not show a significant influence of treatment suspension on the main endpoint, suspension may be considered a serious limitation to the use of N-MA. In our study, 10% of patients had to stop treatment due to prolongation of their QTc interval.

ML is an uncommon form of the disease. In a study of 2,820 subjects in Brazil, approximately 5.3% of patients with CL had lesions on the nasal septum, palate or oropharynx[37]. Thus, ML studies designed to guide institutional therapeutic guidelines have been based on a limited number of patients [21]. In this regard, our study is comparable in sample sizes to previous studies evaluating different therapies for ML[31,38-40].

An important gap in the literature is related to the follow-up period. The primary endpoints of most ML studies vary between 150 days and 12 months[31,38-40]. However, different case series have shown a high relapse rate of ML, varying between 17% and 33.8%[41-44] and relapses of ML tend to occur one year after treatment completion[43]. Thus, we were able to evaluate these patients for an extended period of time after therapy, partially overcoming the limitations of previous studies. In contrast to CL, ML rarely resolves spontaneously[45,46]; therefore, it seems plausible that setting the primary endpoint after four years of treatment is indicative not only of relapse rates but also of initial cure rates.

According to the last WHO expert committee report on the control of leishmaniasis[1], MILT is an option for the treatment of ML; however, this recommendation was based on an open-label, non-randomized trial from Bolivia in which patients were followed for 12 months after the completion of therapy[46,47].

We believe that our study characteristics (i.e., allocation through randomization, inclusion and exclusion criteria, follow-up time, disease form, diagnostic methods, *Leishmania* species, and patient origin) were adequate to deal with the study question. In the last systematic review on leishmaniasis treatment, there were only four studies on ML in the Americas[21].

Previously published studies have reported that the management of clinical trials in ATL is extremely difficult[48]. The disease tends to affect people with economic difficulties, resulting in mobility issues and, ultimately results in poor adherence to treatment, which also occurred in the present trial. This fact makes the intention-to-treat analysis an important strategy for avoiding overly optimistic results taken from a per-protocol analysis. In a recent pilot study in Argentina, Bustos et al. did not find a difference in efficacy between patients treated with a higher dose of MILT (2.5 to 3.3 mg/kg/daily) and those treated with N-MA[49]. MILT dosing, especially in children, is frequently a subject of discussion and the pharmacokinetics of the drug are still being investigated[50]. Some evidence has suggested that MILT dosing should always be determined according to body weight[50]. In contrast to what we have observed regarding VL, regulatory institutions have no standardized MILT dose for ML treatment[1,24]. In this study, the MILT dosage used in some patients could also explain its lower performance when compared to N-MA in this preliminary analysis. Conversely, these doses have been already used with good results in clinical trials involving patients with VL and CL. Thus, we selected the 1.3–2 mg/kg/day dosage based on the good results of a previous pilot study from our institution that included patients switching to MILT after the failure of other conventional options.

Limitations

The cure rate was likely underestimated due to the number of patients lost to follow-up. As HUB is the main regional referral hospital for ATL in a region where medical support is scarce, it has been reported that many patients only return for consultations when relapse or treatment failure occurs[33]. Patients older than 70 years or younger than 18 years were not included in the study; additionally, patients with severe heart or kidney disease were also not included. Consequently, we are not able to provide data regarding patients with these characteristics[51]. Another limitation of this study is that no comparison between body weight and applied dosage was performed. It is also important to stress that the study protocol was approved before the Brazilian Ministry of Health's new guidelines were published, which recommend that patients aged 50 years or more be treated with liposomal amphotericin B[9].

Conclusions

This study represents a significant advancement in the field, since studies for ML are frequently limited by a small number of patients, absence of a control group, or low quality of evidence[52]. Considering that the intention-to-treat analysis is better suited to the effects of interventions in daily practice[53], we consider MILT an important option for the treatment of ML, in line with other indications in other forms of leishmaniasis. The range of adverse effects seems to be more easily managed than the effects of classical treatments, resulting in improved patient safety and well-being. Nevertheless, comparison of MILT to N-MA in ML should be evaluated in a larger patient population.

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Conflict of Interest: The authors declare no conflicts of interest.

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