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RESEARCH ARTICLE

Treatment of cutaneous leishmaniasis with a sequential scheme of pentamidine and tamoxifen in an area with a predominance of *Leishmania (Viannia) guyanensis*: A randomised, non-inferiority clinical trial

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Abstract

Objective: To determine whether a combination of a single intramuscular (IM) dose of pentamidine (7 mg/kg) followed by oral tamoxifen 40 mg/day for 20 days is non-inferior to three IM doses of pentamidine 7 mg/kg in the treatment of cutaneous leishmaniasis with a margin of 15%.

Methods: Phase II, randomised, controlled, open-label, non-inferiority clinical trial. Primary outcome was the complete healing of the lesions 6 months after starting treatment. Secondary outcomes were healing 3 months after starting treatment and determining the presence and severity of adverse effects (AE).

Results: The research was concluded with 49 patients; *Leishmania (Viannia) guyanensis* was the most frequent species isolated. In the primary outcome, 18 (72%) (95% CI: 52.4%–85.7%) of the 25 patients allocated to the intervention group and 24 (100%) (95% CI: 86.2%–100%) of the control group ($p = 0.015$) met the established criteria of cure. There was no AE with tamoxifen.

Conclusion: Although a 72% cure rate presented by the combination of tamoxifen and pentamidine was lower than in the control group that achieved a 100% cure, it is still a safe and is a clinically relevant result. It indicates that the therapeutic scheme evaluated may be a promising option for populations in remote areas, however it should be further studied, in order to include a larger number of patients.

KEYWORDS
clinical trial, cutaneous leishmaniasis, PCR, tamoxifen, telemedicine, treatment, Western Amazon

INTRODUCTION

Cutaneous leishmaniasis (CL) is a Neglected Tropical Disease with high morbidity, caused by the protozoan species of the genus *Leishmania*, which involves sandfly vectors and animal reservoirs in the transmission chain. Its clinical outcome is dependent upon the species of *Leishmania* and the immune response [1–3].

In Brazil, the main *Leishmania* species causing CL are: *Leishmania (Viannia) braziliensis* and *Leishmania (Viannia) guyanensis*, which both cause Localised-CL being the most common form of CL, and MCL. The *L. (Leishmania)*

amazonensis is also an important species that, in addition to causing Localised-CL, is related to the disseminated-CL and diffuse-CL forms. *L. (V.) guyanensis* is the etiological agent that predominates in some regions of Brazilian Amazon, within the state of Amazonas, unlike other regions of the country, where there is a predominance of infection by *L. (V.) braziliensis* in CL lesions [4–7].

Skin smear, parasite culture, histopathology and polymerase chain reaction (PCR) are the main diagnostic methods. PCR makes it possible to identify the *Leishmania* species by DNA sequencing analysis [8–10].

Pentavalent antimony (Sb^V), pentamidine isethionate, amphotericin B and miltefosine are the available drugs recommended in Brazil for CL [11–13]. In recent years, a decrease in cure rates has been observed with first-line therapeutic regimens as recommended by the Brazilian Health Ministry (BHM) [14]. That is, Sb^V 10–20 mg/kg of body weight/day, with 55.5% cure rate, and pentamidine, 4 mg/kg of body weight, with cure of 58.1% showed in a clinical trial that compared these drugs in CL by *L. (V.) guyanensis* in the state of Amazonas [11, 14]. Amphotericin B, recommended as a second-line therapy, has the disadvantage of being more toxic and needing to be administered by intravenous infusion in a hospital [11]. A better option in terms of efficacy would be pentamidine at the dose of 7 mg/kg body weight, intramuscular (IM), weekly, for 3 weeks, which has shown an efficacy of 96% in CL caused by *L. (V.) guyanensis* [15]. Miltefosine, the only oral medicine available, is currently the best option for rural areas, with observed efficacy varying from 66% to 71% for patients with *L (V.) guyanensis* and 75% to 76.6% for cases with *L. (V.) braziliensis*, compared with Sb^V, that showed cure rates of 52% to 53.6% and 44.4% to 53.3% for each species, respectively [16–19]. However, in Brazil, miltefosine is still restricted to reference centres, due to the care that must be taken with pregnant women [13].

Tamoxifen, a selective oestrogen receptor modulator used to treat hormone receptor-positive breast cancer, has shown potential for the treatment of CL caused by. *L. (V.) braziliensis* and *L. (L.) amazonensis* in in vitro, in vivo studies and in a pilot clinical trial with patients diagnosed with localised-CL. Its antileishmaniasis effect is due to the induction of alkalinisation of parasitophores and to the extracellular action on promastigotes [20–23]. The use of tamoxifen in other diseases has also been studied [24–26].

These drug combinations have been discussed as an option to increase the treatment effectiveness and reduce adverse effects (AE) [17, 19, 27, 28]. In this present clinical trial, the aim is to determine whether a combination of a single dose of pentamidine and tamoxifen is non-inferior to three doses of pentamidine in the treatment of CL with a margin of 15%, in the state of Amazonas, Brazil.

METHODS

Ethics statement

This study was approved by the Research Ethics Committee of Alfredo da Matta Tropical Dermatology and Venereology Hospital Foundation (CEP-FUHAM): CAAE 36533620.3. 1001.0002, technical advice n. 4.663.792. Registered in the Brazilian Registry of Clinical Trials (ReBEC) RBR-7y2gbdf. Universal Trial Number (UTN): U1111-1259-2160.

Written informed consent forms and patient authorisations were obtained from all patients allocated to the clinical trial. The patients' photographs and the use of WhatsApp were authorised by all subjects, following the guidelines of the National Research Ethics Committee [29].

Study design

Phase II, randomised, controlled, open-label, non-inferiority clinical trial. The non-inferiority approach is justified by the fact that it offers the best therapeutic evidence for the control group, that is, a 96.2% chance of cure with three doses of 7 mg/kg of weight of pentamidine isethionate. This study was not blinded because the interventions were not similar.

Study population

Patients from the spontaneous and referenced demand of FUHAM and Dr Heitor Vieira Dourado Tropical Medicine Foundation (FMT-HVD), coming from the metropolitan region of Manaus (capital of the state of Amazonas) and neighbouring municipalities who presented ulcerated lesions suggestive of CL.

Inclusion criteria

Aged from 18 to 65 years, diagnosis of CL confirmed by direct parasitological examination, or PCR, with a maximum of six lesions without previous treatment. Having at least one lesion larger than 20 mm (in the largest diameter), with no maximum lesion size limit.

Non-inclusion criteria

Women of child-bearing age not using contraceptives, pregnant; lactating women, patients who had already had had leishmaniasis, presence of comorbidities (severe protein and/or caloric malnutrition, immunodeficiency, chronic kidney disease, heart disease, systemic arterial hypertension, liver disease, neoplasms, diabetes mellitus, infectious diseases). Furthermore, patients under treatment with immunosuppressants or drugs that prolong the QT interval and patients with difficulties in attending planned visits in the study (Figure 1).

Diagnosis

The diagnosis of CL was defined by the presence of ulcerated skin lesions and amastigotes in the microscopic

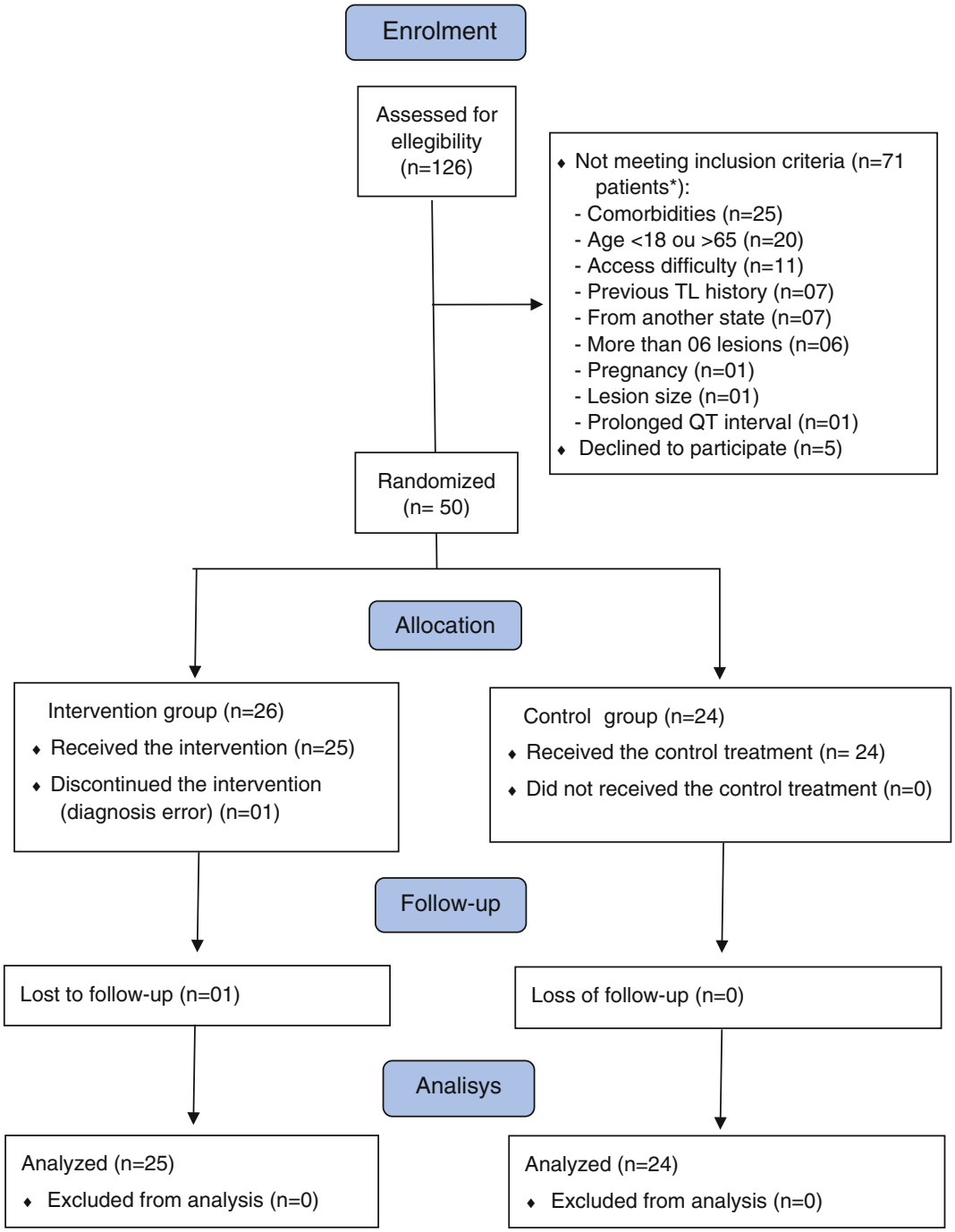

FIGURE 1 Trial flowchart. *08 patients with two reasons of non-inclusion.

examination of the skin smear obtained from the edge of the ulcer.

Regarding the species identification, the DNA was extracted from biopsies using DNeasy Blood & Fabric according to instructions by the manufacturer (QIAGEN) and quantified by the Nanodrop Lite Spectrophotometer (Thermo Fisher™). Amplification of the target fragment (heat shock protein 70 [hsp70]) was performed in the Veriti Thermal Cycler (Applied Biosystems™) using the PCR technique, with a final volume of 20 μL using the following reagents: 0.3 μM of each primer, Forward 5'-GGACGA GATCGAGCGCATGGT-3′ and Reverse 5'-TCCTTCGA CGCCTCCTGGTTG-3′, 1× Platinum Hot start PCR Mastermix™ (Thermo Fisher), 5 μL DNA (10–40 ng) and ultrapure H_2O. The fragments (234 pb) were visualised on a 2% agarose gel and purified with the Kit Wizard® SV and Gel PCR Clean-up System (Promega). The purified product was subjected to the reaction DNA sequencing using the BigDye™ Terminator Kit v3.1 (Thermo Fisher) and analysed in the Genetic Analyzer 3130 (Applied Biosystems™). The sequences were submitted to analysis in silico by Genbank's BLAST tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi) for analysis of homology

with Leishmania spp sequences deposited in the bank. The sequences were aligned with each other using MEGA v.11 software in order to identify possible polymorphisms [30].

Sample size

Considering the cure rate of 90% in the control group and establishing a non-inferiority margin of −15%, power of 90%, and alpha of 2.5%, a sample size of 94 participants (47 per group) was defined. All calculations described here were performed in the R environment, version 4.0.2, with the functions implemented in the Sample Size 4 Clinical Trials, Trial Size and gsDesign package.

Randomisation

The allocation of patients into the intervention and control groups was performed by randomisation through a list generated by a computer, in the R software (package 'randomizerR'), with an initial seed (seed = 2020).

Intervention

Pentamidine isothionate 7 mg/kg in a single IM dose, followed by tamoxifen citrate, orally, at a dose of 40 mg daily (20 mg every 12 h), for 20 consecutive days.

The control group was treated with pentamidine isothionate, 7 mg/kg body weight, in three IM doses, with an interval of 7 days between doses. To prevent hypoglycaemia, which is relatively frequent after pentamidine administration, patients were instructed to eat properly before attending the outpatient clinic.

Clinical and laboratorial monitoring

Clinical-dermatological assessments and laboratory tests were performed before the intervention and during the follow-up of the patients. For the size of the lesion, the area (mm^2) was calculated from the two largest measurements, including the area of induration, around the ulcer.

The laboratory tests included a complete blood count, plasma levels of aspartate and alanine aminotransferase, amylase, urea, creatinine, glycaemia, glycated haemoglobin and Beta HCG for women of child-bearing age.

To assess the AE related to pentamidine administration, capillary blood glucose (postprandial), blood pressure and heart rate were measured before and 45–60 min after pentamidine administration.

Electrocardiography was performed in patients aged 40 years or older, before and after treatment. Whenever necessary, this examination was repeated.

Patients were reassessed at 30 days (D30), 60 days (D60), 90 days (D90) (±1 week) and 180 days (D180) (±2 weeks) after starting treatment. During the Covid pandemic in the years 2020 and 2021, the WhatsApp mobile application was used to assess absent patients.

Two dermatologists enrolled the participants, performed the physical examination and determined the outcomes. A clinician assessed AE. To assess adherence to treatment, patients were asked to return tamoxifen blister packs.

Outcomes

The primary endpoint is a clinical cure, defined as the complete healing of all ulcers (with flattening of the edges, disappearance of crust, scaling, infiltration or induration of the base, erythema and lymphangitis or adenitis, if any, and absence of new lesions) by the sixth month after the start of treatment (D180).

Healing 3 months after the start of treatment (D90) was evaluated, as a secondary outcome, and classified as follows:

- Partial cure—incomplete healing or incomplete regression of inflammatory induration of one or more lesions at follow-up.
- Initial cure—complete epithelialisation and regression of inflammatory indurations of all ulcers.
- Treatment failure was considered in the case of failure to completely epithelialise the lesions by 6 months after treatment (D180), or if the lesions worsened, or if a new lesion appeared at any time.

The rescue treatment was pentamidine isothionate, 7 mg/kg of body weight, in three weekly doses for patients under treatment with tamoxifen. In cases of AE associated with pentamidine, Sb^V was used, according to BHM guidelines.

Another secondary outcome was the presence of AE—being the presence of signs or symptoms related to the use of the medications. The AE was categorised as mild, when tolerated without medication; or moderate, when symptomatic treatment was needed; and severe AE, when symptoms were manageable only ceasing with the use of a specific medication.

Statistical analysis

Comparisons between groups were made by parametric and nonparametric analysis. For quantitative variables, the *t*-test was used when the data had a normal distribution (Shapiro–Wilk test), and the Mann–Whitney test when they were non-normally distributed. For qualitative variables, the Chi-square test or Fisher's exact tests were used.

To assess the treatment effectiveness in each group, the two-proportion comparison test was applied to obtain 95% confidence intervals (95% CI) and the *p* value as determined by the Fisher's exact test. Statistical significance was set at $\alpha = 0.05$.

For the survival analysis, the difference between the beginning day of treatment and the day of clinical-dermatological reexamination was used, with an assessment of cure or therapeutic failure. For this analysis, Kaplan–Meier curves were used and, subsequently, the logrank test.

RESULTS

The study started in October 2020 and ended in February 2022 with the evaluation of 126 positive cases of skin smear for LT. During this period, the demand for patients at both institutions was reduced due to a further worsening of the Covid-19 pandemic, which hampered the recruitment process. According to the eligibility criteria, 50 patients were included in the study, 26 in the intervention group and 24 in the control group. With this number of patients, the power of the study was recalculated maintaining the non-inferiority margin of 15% and alpha of 2.5%. The result obtained was 80% for a defined sample size of 48 patients (24 in each group).

The main reasons for non-inclusion were the presence of comorbidities in 25 (29.8%) patients as well as patients aged younger than 18 years or older than 65 years in 20 (23.8%) cases. Diabetes mellitus and systemic arterial hypertension were the most frequent comorbidities. The other causes of non-inclusion are listed in Figure 1. One patient allocated to the intervention group was withdrawn from the study due to an error in laboratory diagnosis. Another patient, from the intervention group, did not complete the post-treatment evaluation, being considered lost to follow-up.

The randomisation of patients allowed for the organisation of similar, comparable groups according to demographic

TABLE 1 Main clinical and baseline demographic characteristics of patients, according to treatment groups.

Features	Group		*p*-value
	Intervention $n = 25$	Control $n = 24$	
Gender			0.496[a]
Female	4 (16%)	6 (25%)	
Male	21 (84%)	18 (75%)	
Age (years)			0.575[b]
Median (interquartile range)	40 (27–43)	37 (28.2–48.5)	
Evolution time (weeks)			0.547[b]
Median (interquartile range)	6.5 (4–8)	6 (4–7.5)	
Number of lesions			0.316[b]
Median (interquartile range)	1 (1–3)	1 (1–2.2)	
Topography			0.897[a]
Face	1 (4%)	1 (0.2%)	
Trunk	4 (16%)	4 (16.7%)	
Upper extremity	10 (40%)	12 (50.0%)	
Lower extremity	10 (40%)	07 (29.2%)	
Area of the largest lesion (mm^2)			0.772[b]
Median (interquartile range)	9.1 (6.2–13.5)	8.7 (5.9–17.7)	
Lymphangitis			0.316[c]
Yes	14 (56%)	10 (41.7%)	
No	11 (44%)	14 (58.3%)	
Satellite lesions			0.684[c]
Yes	9 (36%)	10 (41.%7)	
No	16 (64%)	14 (58.%3)	
Species of *Leishmania* identified $n = 40$	$n = 23$	$n = 17$	0.218[a]
L. braziliensis	2 (8.7%)	0 (0)	
L. amazonensis	1 (4.3%)	0 (0)	
L. guyanensis	20 (87%)	15 (88.2%)	
L. lainsoni	0 (0)	2 (11.8%)	

[a]Fisher's exact test.
[b]Mann–Whitney test.
[c]Chi-square test.

and clinical characteristics. The species *L. (V.) guyanensis* was identified by PCR in 35 (87.5%) of the 40 (81.6%) evaluated cases (Table 1).

At the primary endpoint, through the intention-to-treat analysis, a definitive cure was verified in 18 (72%) (95% CI: 52.4–85.73) patients in the intervention group and 24 (100%) (95% CI: 86.2%–100%) in the control group. Therefore, six failures were observed in the intervention group and no failures in the three-dose pentamidine group. This difference was statistically significant ($p = 0.015$). All cases of failure were diagnosed at D90, that is, 70 days after the end of the treatment (Table 2).

According to the survival analysis data, there was no statistically significant difference at the time of healing of the skin lesions between the two groups. The median healing time in the intervention group was 133 days (95% CI, 112–205) and in the comparison group, 91 days (95% CI, 86–136) (Figure 2).

Patients considered to have clinically failed showed the presence of lymphangitis in common and all were infected by *L. (V.) guyanensis*. The clinical evidence of activity started from the edges of the lesions (Figure 3, Table 3).

There were no tamoxifen-associated AEs. Regarding pentamidine, immediate and late AE were observed. Among the main immediate clinical AEs after the pentamidine injection, patients reported 'bitter mouth', dyspepsia, abdominal pain, nausea, heartburn, sialorrhea and dry mouth. Asthenia, chills, 'heat', fever, 'cold', facial flushing, feeling of facial oedema, headache, paraesthesia, tremor in the extremities of the limbs and 'blurred vision' were the most frequent constitutional symptoms complaints. All these symptoms were classified as mild.

In the laboratory evaluation, 45–60 min after pentamidine administration, a decrease in glycaemia, without clinical manifestation, was observed in 24 (85.7%) out of the 28 evaluated patients.

TABLE 2 Outcomes in the intervention and control groups.

	Intervention group, *n* (%)			Control group, *n* (%)		
Outcome	**D30**	**D90**	**D180**	**D30**	**D90**	**D180**
Cure[a]	2 (8.0)	14 (56.0)	18 (72.0)	05 (20.8)	20 (83.3)	24 (100)
Partial cure	19 (76.0)	0	0	16 (66.7)	0	0
Failure	0	6 (24.0)	6 (24.0)	0	0	0
Lost to follow-up	4 (16.0)	5 (20.0)	1 (4.0)	3 (12.5)	4 (16.7)	0
Total	25 (100)	25 (100)	25 (100)	24 (100)	24 (100)	24 (100)

[a]Initial healing at D30 and D90, and cure at D180.

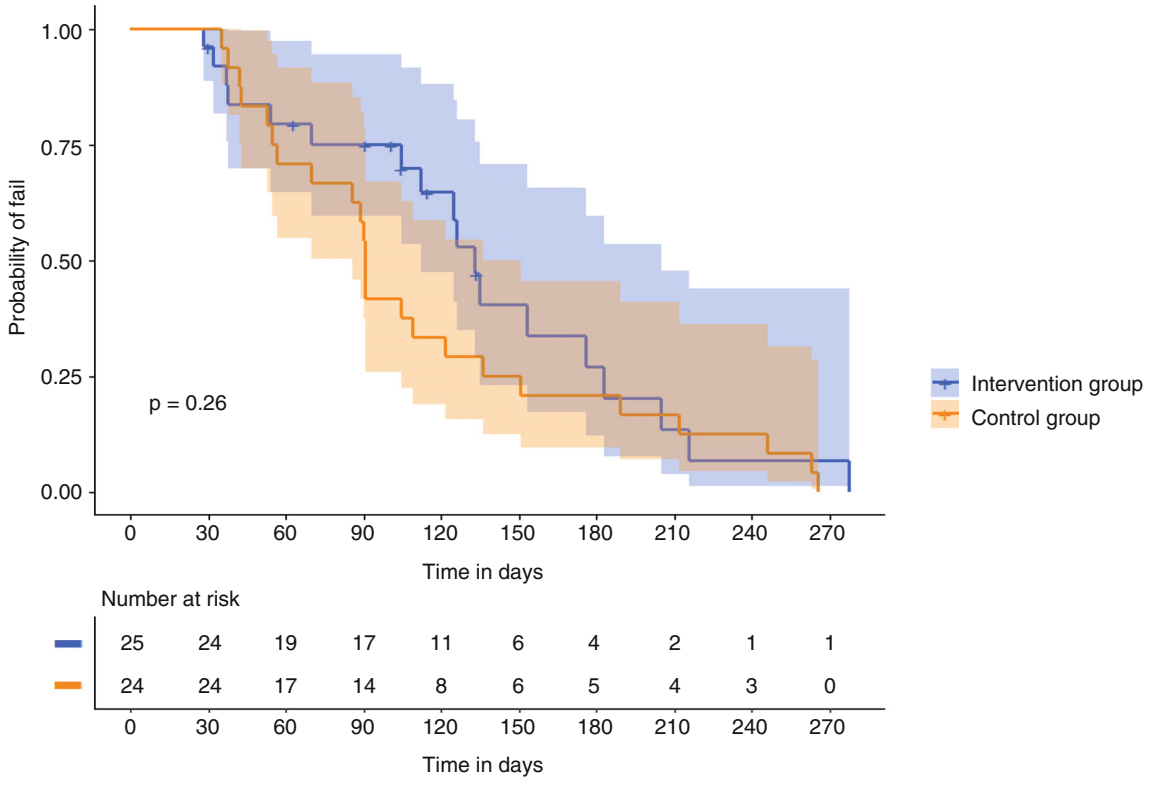

FIGURE 2 Survival analysis of the difference, in days, between the beginning of treatment and the outcome of cure or treatment failure.

Of the 23 patients aged over 40 years of age, 22 had an ECG before treatment and 17 of these patients (10 in the intervention group and 7 in the control group) also had an electrocardiogram during follow-up. No changes were observed.

The serious AEs observed were transient hyperglycaemia occurring in two patients, one out of each group, and insulin-dependent diabetes mellitus occurred in another patient from the control group. Induration at the injection site was observed in 19 (38.8%) patients, 16 in the control group and 3 from the intervention group. Furthermore, lipoatrophy at the site of the injection occurred in four (8.2%) patients, two from each group.

The use of WhatsApp Messenger to monitor the clinical evolution and therapeutic response, allowed us to carried out 35 (71.4%) evaluations using images sent to a researcher at some point during the follow-up. Among these patients, 18 were in the experimental group and 17 from the control group.

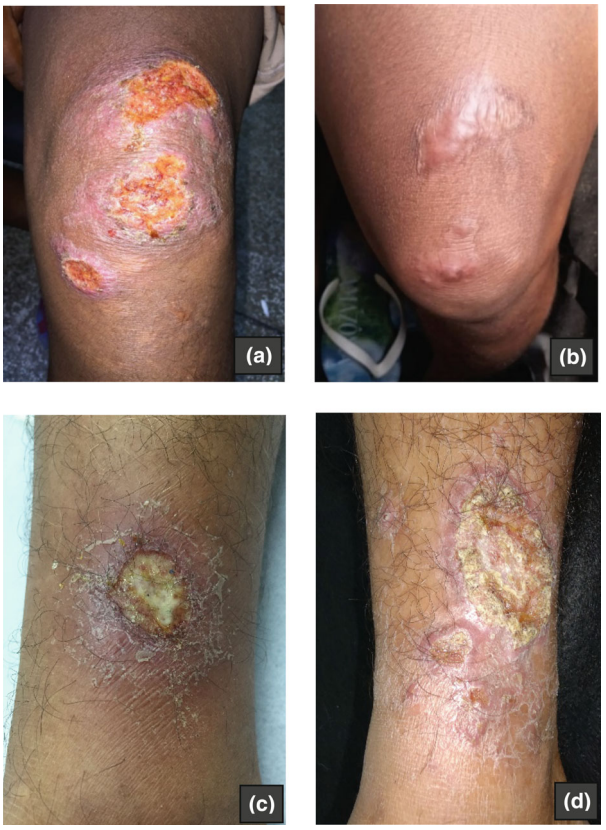

FIGURE 3 Ulcer healing after 210 days of treatment with the pentamidine–tamoxifen combination (a and b). The photo b was sent by the patient. Treatment failure after 120 days with the same scheme (c and d).

DISCUSSION

As for new ways to approach these classic problems, the scientific community has proposed strategies such as drug repositioning, which can be summarised as 'old drugs for new therapies' [31]. An important aspect related to CL, which does not exist yet, is the availability of effective and easy-to-operate therapeutic regimens for the treatment of patients residing in areas of difficult access, such as the Amazon region.

In the search for a more effective treatment, with a lower risk of resistance and easy administration, a combination of drugs is the best option. Single-drug therapeutic regimens have shown to decrease in effectiveness over the years.

The cure rate for CL, obtained with the drug combination of one injectable dose (pentamidine) at the moment of diagnosis and 20 days using an oral medication (tamoxifen), is promising for the treatment of patients living in rural areas, with difficult access to health centres. Although the effectiveness of three doses of pentamidine has the best efficacy, the patient had needed to return for administration of the other doses and therefore was more prone to AEs, particularly those observed at the drug application sites, and diabetes, which has been related to a cumulative effect [32, 33]. Furthermore, both transient hyperglycaemia and a case of diabetes mellitus occurred within the control group.

TABLE 3 Clinical characteristics of patients with treatment failure allocated to the intervention group.

Number	Evolution time (weeks)	Number of lesions	Topography of lesions	Size of the largest lesion (mm^2)	Presence of satellite lesions and/or lymphangitis	Dermatological aspects of lesions in patients with therapeutic failure
1	4	1	Lower limb	20.2	Lymphangitis	Increased lesion size, with ulceration and satellite lesions
2	3	1	Lower limb	2.0	Lymphangitis	New lesions, satellite lesions and papules at the edge of the initial ulceration
3	8	5	Lower limbs	12.6	Lymphangitis	Partial regression, with papules and ulceration at the edges of the initial lesion
4	7	1	Upper limb	4.4	Lymphangitis	Partial regression, with active edges
5	8	2	Upper limbs	14.7	Lymphangitis	Partial regression with the presence of papular and scaly lesions on the edges of the previous lesion
6	7	5	Cervical and upper limbs	8.7	Lymphangitis	Infiltration, exulceration and increase of the lesion size

Tamoxifen is an oral drug with an antileishmanial effect already demonstrated in preclinical studies and in a pilot clinical trial with patients infected by *L. (V.) braziliensis* [20–22, 24]. In this trial, a combination of tamoxifen and Sb^V showed a 58% (7/12) cure in comparison with 40% (6/15) cure with Sb^V alone ($p = 0.89$). The authors suggest further investigations with this drug [24]. So far, there are no clinical trials that indicate the effectiveness of tamoxifen with other species of *Leishmania* and in monotherapy schemes or associated with other drugs already used in leishmaniasis. The association of drugs has been investigated as an option to increase the effectiveness and operationalisation in the treatment of leishmaniasis. Several drugs have been used in association with Sb^V, pentamidine or amphotericin B, but there are currently no results that allow these therapeutic regimens to be used in public health programs [17, 19, 27, 28]. The efficacy obtained with the combination of tamoxifen and pentamidine in this study was 72%. However, the results obtained indicate the need for further studies with a larger number of patients.

The pentamidine dose of 4 mg/kg of body weight was recommended for pentamidine mesylate, already withdrawn from the market. Currently, pentamidine isethionate is the available salt. According to studies carried out with this drug, the corresponding dose with the exchange of salts is 7 mg/kg of weight [34]. Clinical trials performed with this dose in *L. (V.) guyanensis* showed a cure rate of 45% with a single dose, 81% with two doses, and 96.2% with three doses [15].

In this study, the observed AEs were with pentamidine. Known serious AEs that have being related to tamoxifen are pulmonary embolism, stroke and endometrial cancer [35]. Other AEs are hot flushes, vaginal dryness, night sweats, irregular periods, vaginal discharge, mood changes, nausea and vomiting, weakness, arthralgia, hypertension and peripheral oedema [26]. These effects are also reported with prolonged use of tamoxifen in breast cancer. There are few studies that refer to AEs in a short-term use. Fatigue occurred more frequently with tamoxifen (80 mg/day) as an adjunct to lithium, in a 6-week treatment for acute bipolar mania than with lithium alone [36].

Comparatively, miltefosine, the oral medication used for LC having a good efficacy for *L. (V.) guyanensis* (71.4%) and *L. (V.) braziliensis* (75%), has presented AE mainly related to the gastrointestinal tract such as vomiting (45%), nausea (up to 85%) and diarrhoea (6.7%) and abdominal pain (23%) in addition to other transient effects [16–19]. All these drugs, whether taken orally (tamoxifen and miltefosine) or injected (pentamidine and Sb^V), are contraindicated during pregnancy [37–39].

According to the survival analysis data, there was no statistically significant difference in the time from the beginning of treatment to the healing of skin lesions. The cure in D90 was like the one observed in the D180 group. The median healing time in the intervention group was 133 days (95% CI, 112–205) and in the comparison group, 91 days (95% CI, 86–136). We emphasise that all the therapeutic failures occurred up to D90. For the evaluation of the healing time, in addition to the medication or therapeutic regimen, it is also relevant to consider the size and location of the lesions [19, 40, 41]. This is a topic that also needs to be further investigated.

The main limitation of the study was the sample size. Another limitation is the irregularity of the clinical reexamination of patients, due to the outbreak of the Covid pandemic during the development of the project, with mobility difficulties of patients and the reduction in outpatient clinical activities. Sending photos and interviews through WhatsApp made it possible to contact patients who would be considered absent on the day scheduled for reexamination.

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