

Case Report

Combination therapy with liposomal amphotericin b (ambisome), n-methylglucamine antimoniate (glucantime), and pentamidine isethionate in a refractory visceral leishmaniasis case

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Abstract

Visceral leishmaniasis is a systemic disease that is potentially severe and endemic in Brazil. It clinically manifests as fever, weight loss, swelling, hepatosplenomegaly, paleness, and edema. In this study, we discuss a case of a 1-year-old child diagnosed with refractory visceral leishmaniasis after being treated with liposomal amphotericin B in two distinct occasions. Considering the persistent clinical features and weak response to conventional treatment, a combination therapy with liposomal amphotericin B (ambisome), n-methylglucamine antimoniate (glucantime), and pentamidine isethionate was initiated, and response to treatment was good.

Keywords: Visceral leishmaniasis. Refractory. Treatment.

INTRODUCTION

Visceral leishmaniasis (VL), also known as kala-azar, is a systemic disease, potentially severe and endemic in various countries^{1,2}. VL was previously considered to typically be a rural zoonosis; however, it is gradually expanding to urban areas and increasingly becoming an important public health problem³.

In Brazil, VL is caused by *trypanosomatid protozoans belonging to the genus Leishmania (Leishmania) infantum chagasi*, and dogs (*Canis familiaris*) are the primary reservoir of VL⁴. In the wild, main sources of infection are foxes (*Dusicyon vetulus* and *Cerdocyon thous*) and marsupials (*Didelphis albiventris*)³. Leishmaniasis is a vector-borne disease, transmitted via the bite of phlebotomine, genus *Lutzomyia*, and the species *Lutzomyia longipalpis* and *L. cruzi* are responsible for the disease transmission in Brazil⁴.

VL is a chronic disease with various clinical manifestations such as fever, paleness, weight loss, swelling, progressive

weakness, hepatosplenomegaly, and lymphadenopathy. Laboratory findings show leucopenia, sometimes together with *pancytopenia*, hypergammaglobulinemia, and hypoalbuminemia^{5,6}. Each year an estimated 50,000-90,000 new cases of VL occur worldwide. In 2015, >90% of new cases reported to the World Health Organization (WHO) occurred in seven countries, namely Brazil, Ethiopia, India, Kenya, Somalia, South Sudan, and Sudan⁷.

An epidemiological study conducted in the State of Rio Grande do Norte showed that the incidence of VL in children aged <15 years was higher than that in Brazil⁸. In the past 10 years, an average of 3,379 new cases were reported in Brazil each year, with an incidence of 1.9 cases per 100,000 inhabitants³. This study aimed to present and discuss a refractory VL case that was treated with a combination therapy of liposomal amphotericin B (LAB), n-methylglucamine antimoniate (NMG), and pentamidine isethionate (PI), with a good response to the treatment.

CASE REPORT

A 1-year-old male child from the Brazilian northeast region was admitted at the age of 5 months owing to persistent fever, weight loss, hepatosplenomegaly, hypoalbuminemia, and anemia with lymphopenia. His HIV test result was negative;

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K-39 test result was positive, and his bone marrow aspiration revealed *Leishmania spp.* He was diagnosed with VL and treated with 3mg/kg/day of LAB for 7 days.

Three months after being discharged, he was re-admitted to the hospital with persistent fever, substantial hepatosplenomegaly, and pancytopenia associated with pneumonia. At that time, there were erythematous-violaceous plaques with hemorrhagic vesicles in the upper right limb and feet (**Figure 1A**), characterizing severe sepsis. He was treated with 100mg/kg/day of oxacillin for 10 days and with 100mg/kg/day of ceftriaxone for 14 days. A new bone marrow aspiration revealed *Leishmania spp.*, and treatment was initiated with 3mg/kg/day of LAB for 7 days, resulting in fever dissipation, progressive improvement of the red cell indices, and weight gain.

At 9 months of age, he returned with the same clinical features. When a new bone marrow aspiration was performed, no *Leishmania spp.* were detected. After 3 weeks of continuing examinations to rule out other illnesses, such as *lymphoproliferative disorders and endocarditis*, a new bone marrow aspiration was performed, and at that time, parasites were found. Considering the short time between therapies and the persistence of the parasite, the case was diagnosed as refractory VL.

The patient was then treated with 14 doses of 3mg/kg/day of LAB on alternate days and with 20mg/kg/day of NMG daily for 35 days. After the eighth dose of LAB, it was suspended for 8 days, maintaining monotherapy with NMG. Therapy for treating urinary tract infection and febrile neutropenia was initiated with 100mg/kg/dose of amikacin for 8 days and

50mg/kg/dose of cefepime for 10 days, respectively. After discontinuing LAB, the patient's general condition worsened, with dyspnea, vomiting, and weight loss. The patient was re-evaluated by the infectious disease team; a combination therapy was established: 20mg/kg/day of NMG daily, 4mg/kg/day of PI daily, and 3mg/kg/day of LAB on alternate days. The patient was hospitalized for 66 days and received 15 doses of LAB and PI for 13 days and NMG for 35 days. At the end of this combination therapy, the patient's general condition improved significantly, with weight gain, reduction in spleen and liver size, and improvement in red cell indices (**Table 1**).

DISCUSSION

First, we attempted to treat the patient with LAB because he fulfilled LAB's use criteria established by the Brazilian Ministry of Health. Furthermore, the patient's age was under 1 year, and he presented a clinical and laboratory severity score of >6; however, the treatment failed. A second therapy was initiated with the same drug, as recommended in the literature³.

Because the therapy was still unsuccessful, we can imply that the disease is refractory. A relapse was not considered because of the short period between hospitalizations. This was confirmed by the patient's unfavorable progression (i.e., being readmitted after 3 months of the first LAB therapy and 1 month after the second therapy, recurrent intermittent fever, hepatosplenomegaly, and presence of *Leishmania spp.* in bone marrow aspirate even after adequate treatment). We believe that the disease was caused by the direct action of the parasite. In Brazil, the recurrence index of VL in children is around 2.3%, mainly occurring in



FIGURE 1: Images of the affected patient. **A.** Erythematous-violaceous plaques with hemorrhagic vesicles in the upper right limb and feet of the patient. **B.** The patient after the combination therapy.

TABLE 1: Progress of the patient's clinical and laboratory test results throughout the three hospitalizations.

	1 st Hospitalization		2 nd Hospitalization		3 rd Hospitalization		
	BT	AT	BT	AT	BT	BTC	ATC
Spleen	4cm from LCM	1cm from LCM	8cm from LCM	7cm from LCM	11cm from LCM	9.5cm from LCM	7cm from LCM
Liver	2cm from RCM	2cm from RCM	5cm from RCM	6cm from RCM	5.5cm from RCM	4.5cm from RCM	3cm from RCM
HB (g/dL)/HT (%)	6.1/19.7	9.5/30.1	4.4/13.4	7.6/23.9	7.3/22	7.9/25	9.9/30
Leukocytes (% × 10 ³ /mm ³)	4.5	7.9	3.7	5.0	2.1	5.9	7.4
Platelets (× 10 ⁹ /L)	211	230	114	162	85	110	296
NA ⁺ /K ⁺ (mmol/L)	130/5.4	136/5.5	128/5.3	135/5.3	137/4.9	134/4.9	132/5.5
UR/CR (mg/dL)	10/0.39	10/0.46	ND	10/0.41	24/0.57	13.6/0.32	49/0.47
TB/DB (mg/dL)	0.5/0.25	0.44/0.16	ND	ND	0.41/0.23	3.09/1.97	ND
ALKP/GGT (U/L)	ND	ND	ND	ND/271	ND	ND	ND
GOT/GPT (UI/L)	94/29	73/23	154/31	58.2/11	38.4/12	71.9/27	77/34
ALB (g/dL)	2.8	3.2	2.9	4.1	3.1	ND	4.6
GLOB (g/dL)	2.6	2.7	2.4	3.3	3.4	ND	2.7
LDH (U/L)	2,240	ND	1,990	ND	1,022	ND	ND

BT: before therapy; **AT:** after therapy; **BTC:** before triple therapy; **ATC:** after triple therapy; **HB:** hemoglobin; **HT:** hematocrit; **NA⁺:** sodium; **K⁺:** potassium; **UR:** urea; **CR:** creatinine; **TB:** total bilirubin; **DB:** direct bilirubin; **ALKP:** alkaline phosphatase; **GGT:** gamma-glutamyl transpeptidase; **GOT:** glutamic oxaloacetic transaminase; **GPT:** glutamic pyruvic transaminase; **ALB:** albumin; **GLOB:** globulin; **LDH:** lactate dehydrogenase; **LCM:** left costal margin; **RCM:** right costal margin; **ND:** not done.

male patients aged 3-6 years who are primarily black or mulato; VL is mostly treated with meglumine antimoniate⁹. Nevertheless, NMG is the first-line drug for leishmaniosis treatment^{9,10}, leaving amphotericin B for cases resistant to pentavalent antimonial⁹.

The national literature on refractory cases recommends using two drug lines, i.e., LAB and NMG on alternate days³, which was done during the third treatment of this patient. A therapeutic option would be splenectomy¹¹; however, this was not the team's option because the patient was aged 1 year, and it could possibly have several systemic effects. Treatment with immunotherapy and immunochemotherapy¹² could also be useful; however, such therapies were unavailable.

Laboratory results shown in **Table 1**, together with the absence of clinical response, led the authors to believe that a new combination therapy is needed based on the severe and refractory features. Considering the severity of the case, the authors' choice was not to pursue treatment with NMG alone.

Thus, this case presented leads to a new perspective for

treatment: what should we do when we encounter a case of drug resistance to Amphotericin B, the most powerful commercially available drug for VL treatment³?

A combination therapy was initiated, considering the possibility of the parasite being resistant to one of the drugs. Although amphotericin is a very potent drug for treating leishmaniasis, its use in veterinary medicine may have generated a resistant strain.

Using the same principle as the one used for treating tuberculosis – that it is very unlikely for an infectious agent to be resistant to four drugs – we could, in this case, discuss the possibility of a successful therapy using a combination of three drugs.

This combined therapy is an empiric therapy, which is not yet published. However, one author had a similar experience 15 years ago with another patient. The combination of these drugs resulted in successful treatment. Before the therapy, the disease was very active with patient's hemoglobin level at 4.4g/dL by the second hospitalization, increasing to 9.9g/dL after the therapy. Leukocyte cell count that was 3,700/mm³ initially

increased to 7,400/mm³, platelets increased from 114,000/mm³ to 296,000/mm³ after therapy; the spleen had a 2.5-cm reduction (still indicating the disease presence, which confirms its severity); and the liver size reduced by 1.5cm. Forty-one days after the treatment ended, the patient was re-evaluated, and no clinical features of VL were observed (**Figure 1B**).

However, considering the possible serious side effects, this therapy is limited to patients who are not cured by the treatment recommended by the Ministry of Health. In our case, laboratory test results for renal function were slightly altered, such as increases in urea (13.6mg/dL before therapy and 49mg/dL afterward) and creatinine (from 0.32mg/dL to 0.4mg/dL), as well as minimal alterations for hepatic function (glutamic oxaloacetic transaminase from 71.9 to 77UI/L and glutamic pyruvic transaminase from 27 to 34UI/L). Thus, there were no serious side effects in our case.

To prescribe such a therapy, ensuring that a failed previous therapy, which was not abandoned, is necessary. It is crucial that patients are treated by a multidisciplinary team in a facility where an ICU is available throughout the treatment. It is also necessary to have biochemical profiles to assess renal, hepatic, and pancreatic functions and glucose and hematological parameters and perform electrocardiographic monitoring.

We believe that the combination therapy was successful because of the possible synergistic effect of the drugs.

Finally, the patient should be followed up 3, 6, and 12 months after the treatment ends. If the patient's condition is stable during the last evaluation, he is considered to be cured. It is worth mentioning that the occurrence of eosinophilia at the treatment end or along the segments is a good prognosis. We should also state that serological testing is not indicated for the patient's follow-up¹³.

We suggest that the combination therapy is a viable therapeutic option for difficult to treat VL cases, as well as immunodeficiency cases.

Conflict of interest

The authors declare that there is no conflict of interest.

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