LIPOSOMAL AMPHOTERICIN B FOR COMPLICATED VISCERAL LEISHMANIASIS (KALA-AZAR) IN EASTERN SUDAN: HOW EFFECTIVE IS TREATMENT FOR THIS NEGLECTED DISEASE?

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Abstract- objectives The aim of this study was to report the patient profile and treatment outcomes, including relapses, of patients with visceral leishmaniasis (VL) treated with liposomal amphotericin B (AmBisome) in Gedaref, Sudan.methods AmBisome was offered to two groups of patients: primary VL patients with specific criteria (age ≤2or ≥45 years, advanced clinical disease, pregnancy, HIV co-infection and contraindications for antimonials) and VL relapses. AmBisome was given at a total dose of 30 mg/kg, over 10 days. Slow responders received up to 50 mg/kg. Treatment failure was confirmed parasitologically. Standardised treatment outcomes were assessed. results Between March 2010 and June 2012, a total of 281 (74%) patients with primary VL and 98 (26%) patients with VL relapses received AmBisome (54% male, median age = 11 years, interquartile range 2–30). End-of-treatment outcomes for primary VL were 260 (92%) initial cure including three (1%) slow responders, three (1%) treatment failures, 14 (5%) deaths and four (1%) unknown outcomes. Outcomes for VL relapses were 92 (94%) initial cure with five (5%) slow responders, four (4%) treatment failures, one (1%) death and one (1%) unknown outcome. At 6 months, there were 19 (7%) relapses amongst primary VL and 10 (10%) VL relapses had a new relapse. Loss to follow-up in both groups was 38%. None of the deaths that occurred during the study period was attributed to AmBisome. conclusion AmBisome appears to be effective for initial cure of VL and the drug seems safe, but is expensive (400 USD/treatment). Sustained mechanisms to allow improved access of this expensive drug particularly in East Africa are urgently needed. Relapses and losses to follow-up require specific investigation.

Keywords- kala-azar, Sudan, amphotericin B, treatment, operational research

I. INTRODUCTION

Human VL or kala-azar is a life-threatening protozoan parasitic disease caused by the Leishmania donovani complex. VL is transmitted by an insect vector, the phle- botomus sandfly, predominantly found in warm tropical regions (Chappuis et al. 2007). VL is the second largest parasitic killer in the world after malaria and infects an estimated 200 000–400 000 people each year worldwide (Alvar et al. 2012). More than 90% of all VL cases occur in only six countries, namely India, Bangladesh, Nepal, Brazil, South Sudan and Ethiopia (Alvar et al. 2012). The parasite migrates to internal organs such as liver, spleen and bone marrow (hence visceral) and causes per- sisting fever, hepatosplenomegaly, severe anaemia and serious damage to the host's immune system (Chappuis et al. 2007). If untreated, this will almost always result in death, often due to secondary 'opportunistic infections' such as pneumonia, tuberculosis and dysentery.

As there are no known effective measures for vector control, the mainstay of disease control in Sudan is case finding and treatment (Elnaiem 2011). For the last several decades, treatment in Africa has relied on intramuscular injections of antimonials (Chappuis et al. 2007). Whilst these drugs are effective, they are characterised by high rates of toxicity and mortality, particularly for compli- cated cases (Collin et al. 2004), the need for long hospital stays and growing resistance in some areas like India (Chappuis et al. 2007).

Liposomal amphotericin B (AmBisome) – a relatively new drug that combines high efficacy with low toxicity – is currently the preferential treatment in high-income countries (Bern et al. 2006). This drug has also been included for first-line VL treatment in East Africa in the 2010 revised World Health Organization (WHO) recom- mendations (WHO 2010). However, only a small number of studies evaluating AmBisome in East Africa have been reported (Seaman et al. 1995; Berman et al. 1998; Muel- ler et al. 2007; Ritmeijer et al. 2011). Evidence from these studies is limited as they generally included few (<100) patients, and/or were conducted many years ago, and reported on few cases of VL relapse – VL cases with a history of VL – a patient population that generally responds less well to treatment. There is also a lack of data from this region on relapse rates after AmBisome treatment – an early indicator of inadequacies in dosing. Consequently, the optimal dosing of AmBisome in East Africa remains unknown.

Me ´decins Sans Frontie `res (MSF), Switzerland, started a vertical programme for diagnosing and treating VL from January 2010 in one of the most

endemic regions in Gedaref state of Sudan. Although antimonials – com- bined with paromomycin (PM) – are currently being used as first-line treatment for uncomplicated primary VL, AmBisome has been used for complicated primary VL cases, patients with VL relapse and patients with contra- indications to antimonials or PM. The aim of this study was to report on the effectiveness and safety of AmBisome in a routine programme setting in East Sudan. Amongst patients with VL treated with AmBisome between 2010 and 2012, we reported on the characteristics of patients who received AmBisome and treatment outcomes and the proportion of relapses.

II. METHODS

This was a retrospective cohort study conducted between June 2012 and July 2013 using programme data collected between March 2010 and December 2012. The study was conducted in Tabarak Allah Hospital, in Gedaref State, eastern Sudan. This area is characterised by poverty and poor access to health care. Gedaref has the highest VL burden in Sudan. Since January 2010, MSF has been running a VL treatment centre in a remote area in Tabarak Allah in collaboration with the Ministry of Health. It is the major VL treatment centre in the area, with an estimated catch- ment population of 360 000. The study population included all patients diagnosed with VL who initiated treatment with AmBisome in the MSF VL treatment cen- tre between March 2010 and June 2012. All services were provided free of charge.

III. MANAGEMENT OF VL

Visceral leishmaniasis was diagnosed according to stan- dard MSF guidelines (MSF 2006). Individuals meeting the clinical cases definition of VL [history of fever (2 weeks or more) with splenomegaly or lymphadenopa- thy and/or wasting] underwent further diagnostic evalua- tion. For individuals without history of previous VL treatment (primary VL), diagnosis was carried out sero- logically by a positive rK39 rapid diagnostic test (Dia- Med-IT-Leish, DiaMed AG) (Ritmeijer et al. 2006). In case of a negative rK39 test, a direct agglutination test (DAT) was carried out, with a high titre (≥1:6400) indi- cating VL (Meredith et al. 1995). Patients with border- line DAT titre (1:800–1:3200) underwent lymph node aspiration to confirm VL parasitologically. Patients with suspected VL, but a negative rK39 test and a low DAT titre (<1:400), were evaluated for alternative illnesses and re-tested if their illness persisted. VL suspects with a his- tory of previous VL treatment (VL relapse) or HIV co- infection were all diagnosed parasitologically. Aspirates were graded using a logarithmic grading from 0 to 6 (Chulay & Bryceson 1983). No spleen or bone marrow aspirates were carried out in the centre. HIV testing was systematically offered to all confirmed VL cases. All VL suspects were tested for malaria as well using a rapid diagnostic test (Paracheck).

Complicated VL consisted of primary VL in severely ill patients with VL, patients with HIV infection, pregnant women, individuals with a contraindication for antimoni- als (e.g., renal failure) and those aged ≤2 years or ≥45 years and VL relapse. All complicated VL cases were treated with AmBisome (Gilead Sciences, USA) at a total dose of 30 mg/kg, divided into 10 intravenous infusions of 3 mg/kg on consecutive days. AmBisome was given as monotherapy. Treatment outcomes were assessed at the end of the 10 days of AmBisome treatment. Those with a slow treatment response received an extension of AmBi- some treatment for 7 days, up to a maximum dose of 50 mg/kg. For slow responders, treatment outcome was ascertained at the end of AmBisome treatment (day 17). Patients with primary and relapse VL failing AmBisome treatment received rescue treatment with sodium stibog- luconate (SSG)/PM combination therapy for 17 days. Treatment of non-complicated cases relied on antimonials (SSG monotherapy until July 2011 and then SSG/PM combination). Standard MSF protocols for the manage- ment of malnutrition were used (MSF 2011), and blood transfusion services were available. For HIV and TB treatment, patients were referred to a dedicated national treatment centre. Initial cure at the end of AmBisome treatment was established by assessing clinical improvement (fever reso- lution, spleen regression, haemoglobin increase and weight gain). A parasitological test of cure (TOC) per- formed by microscopic examination of lymph node aspi- rate was done in cases of poor or slow clinical response at the completion of the first-line treatment (day 11 for AmBisome); for patients with VL relapse; and for patients with HIV infection. At discharge, patients received a follow-up appoint- ment for 6 months later. At the same time, they were strongly encouraged to present to the health centre in case of recurrent symptoms of VL at any time. At the 6-month visit, patients were evaluated for VL relapse. All individuals clinically suspected of VL relapse underwent lymph node aspiration to determine the final treatment outcome. Those missing the 6-month appointment were contacted by phone or traced by the health educators. Patients were defined as lost to follow-up (LTFU) if no patient contact took place at the planned 6-month visit.

Outcome was ascertained at the end of treatment (ini- tial treatment response) and at the 6-month follow-up visit (final treatment response). Treatment outcomes were standardised (Table 1) and included: initial cure – resolution of signs and symptoms at the end of treatment (and a negative TOC if performed); treatment non- response (treatment failure) – a positive TOC (parasito- logical failure) and persisting clinical signs/symptoms; slow treatment response – a

partial clinical or para- sitological response; death – death from any reason; transfer-out – referral to another health structure; and LTFU. Final cure was defined as the absence of relapse by 6 months after achieving initial cure.

IV. DATA AND STATISTICAL ANALYSIS

Data related to the study objectives were sourced from patient cards and entered into an electronic database. Demographic, diagnostic, treatment (treatment history, drugs prescribed and treatment response), associated comorbidities and follow-up data were recorded using a standardised data collection tool. On a daily basis, these data were entered into the database, with regular data quality checks performed. For this study, data were extracted from this database. Medians [interquartile range (IQR)] and frequencies (%) were used to describe patients' characteristics and treatment outcomes.

V. ETHICS APPROVAL

The study met the MSF Ethics Review Board, Geneva, Switzerland, criteria for studies using routinely collected

Table 1 Definitions of treatment outcomes

Term	Definition
Initial outcomes (end of treatment)	
Initial cure	Improvement in signs and symptoms at the end of treatment (fever resolution, haemoglobin increase and weight gain)
Slow response	A partial clinical and/or parasitological response after receiving AmBisome 30 mg/kg total dose, achieving cure after AmBisome treatment extension
Non-response (treatment failure)	A positive TOC (parasitological failure) and persisting clinical signs and/or symptoms
Death	Patient who died for any reason during treatment
Transfer-out	Referral to another health structure
Unknown	No outcome data recorded in the patient file
Final outcomes (6-month follow-up visit)	
Final cure	Patient with initial cure showing no signs and symptoms of VL at the 6-month follow-up visit
Relapse	Parasitologically confirmed VL occurring after initial cure
Lost to follow-up	Failure to present at the planned 6-month follow-up visit
Death	Patient who died for any reason during treatment or during the 6-month follow-up period
Unknown	No outcome data recorded in the patient file

TOC, test of cure (lymph node aspiration); VL, visceral leishmaniasis.
data and of the Ethics Advisory Group of the International Union Against Tuberculosis and Lung Disease, Paris, France, and was approved by the Health Research Directorate of the Ministry of Health of Sudan.

VI. RESULTS

Characteristics of the study population Between March 2010 and June 2012, 2023 individuals were diagnosed with VL in the programme. This included 382 patients eligible for AmBisome treatment (Figure 1). Three died before treatment initiation. Of the remaining 379 patients included in the analysis, 54% were male. The median age was 11 years (IQR 2–30), with 59% children (<15 years). Characteristics of individuals receiving AmBisome and treatment history There were 281 (74%) cases of complicated primary VL and 98 (26%) were VL relapses. Table 2 shows the baseline characteristics of the two groups. Malnutrition was common in both groups. There were nine cases of VL–HIV co-infection.
Amongst the 281 patients with complicated primary VL, AmBisome was given to 98 (35%) children ≤2 years, 95 (34%) individuals with advanced clinical disease, 56 (20%) individuals aged ≥45 years, 23 (8%) pregnant women, seven (2.5%) with HIV co-infection and two (0.7%) with a contraindication for antimonials.

The vast majority (n = 95; 97%) of the VL relapse cases had only one single VL episode prior to the current

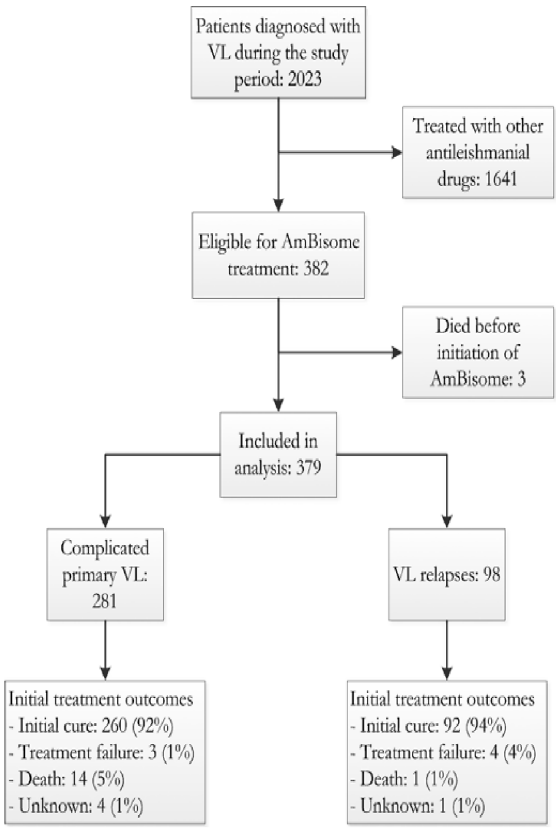


Figure 1 Flow chart of patients enrolled in the study

Table 2 Baseline characteristics of cases with complicated visceral leishmaniasis (VL) treated with AmBisome, Gedaref, Sudan (2010–2012)

	Primary VL *n* (%)	VL relapse *n* (%)	Total *N*
Total	281	98	379
Age, years; median (IQR)	12 (2–30)	10 (6–16)	11 (2–30)
≤2 years	98 (35)	2 (2)	100
3–14 years	51 (18)	71 (72)	122
15–44 years	76 (27)	22 (23)	98
≥45 years	56 (20)	3 (3)	59
Sex			
Female	139 (50)	36 (37)	175
Male	142 (50)	62 (63)	204
Pregnancy	23 (8)	0 (0)	23
Spleen on admission (*n* = 349)			
Not palpable	103 (41)	17 (18)	120
Palpable	149 (59)	80 (82)	229
Size, cm; median (IQR)	6 (4–8)	6 (4–8)	6 (4–8)
Haemoglobin, g/dl; median (IQR); (*n* = 376)	8.1 (6.5–10.5)	8.7 (7.1–10.3)	8.2 (6.7–10.5)
<5 g/dl	25 (9)	8 (8)	33
MUAC, mm; median (IQR)*; (*n* = 121)	124 (115–134)	134 (120–142)	124 (116–136)
BMI, kg/m^2; median (IQR); (*n* = 250)*	18 (15–20)	14 (12–16)	16 (14–19)
Severe malnutrition (*n* = 371)†	77 (28)	69 (71)	146
Duration of symptoms (*n* = 354)			
>1 month	53 (20)	9 (10)	62
Co-infections			
HIV	7 (2)	2 (2)	9
TB	1 (0.3)	1 (1)	2

BMI, body mass index; HIV, human immunodeficiency virus; IQR, interquartile range; MUAC, mid upper arm circumference; TB, tuberculosis.

*MUAC used if age ≤5 years; BMI given if age >5 years.†Defined as MUAC <115 cm (0–5 years) or BMI <16 kg/m2(>5 years).

VL diagnosis. Similarly, for most cases (n = 91; 93%), antimonial therapy was used for the last episode prior to presentation; one case had been treated with AmBisome prior to the current VL diagnosis. In addition, five had received other drugs; treatment details were missing for one.

AmBisome treatment outcomes and proportion of relapses

Table 3 shows the treatment outcomes of all patients treated with AmBisome. Amongst individuals with com- plicated primary VL who started AmBisome treatment (n= 281), 260 (92%) achieved initial cure, including three (1%) slow responders requiring AmBisome treat- ment extension. There were three (1%) cases of AmBi- some treatment failure, and 14 (5%) individuals died during treatment. Case fatality was highest in patients with HIV infection (2/7; 29%), children ≤2 years (6/98; 6%) and those with severe disease (4/95; 4%). Of the 98 VL relapse cases who received treatment, 92 (94%) achieved initial cure, including five (5%) slow

Table 3 Treatment outcomes for complicated visceral leishmani-asis (VL) treated with AmBisome, Gedaref, Sudan (2010–2012)

	Primary VL *n* (%)	VL relapse *n* (%)
Total	281	98
Initial treatment response (at the end of AmBisome treatment)		
Initial cure	260 (92)	92 (94)
Slow responder	3 (1)	5 (5)
Treatment failure (non-response)	3 (1)	4 (4)
Death	14 (5)	1 (1)
Unknown	4 (1)	1 (1)
Final treatment response (at 6 months of follow-up visit)		
Final cure	121 (43)	34 (35)
Relapse	19 (7)	10 (10)
Death	18 (6)	3 (3)
Lost to follow-up	106 (38)	37 (38)
Unknown	17 (6)	14 (14)

responders. Treatment failed in four (4%) individuals and one (1%) died. One patient permanently discontinued AmBisome due to possible drug intolerance

(renal failure); no deaths were attributed to AmBisome treatment.

By the 6-month follow-up visit, there were 19 (7%) VL relapses amongst primary VL cases and 10 (10%) amongst VL relapse cases. LTFU was high (38%) in both groups. Another five cases of VL relapse (all following treatment as complicated primary VL) were documented after the 6 months of follow-up (range 233–676 days).

DISCUSSION

This is one of the largest studies reporting on the effec- tiveness of AmBisome treatment in East Africa. The treat- ment was effective, with nine of 10 patients with complicated primary VL or VL relapse having had an ini- tial cure with AmBisome. There were also no reported AmBisome-related deaths, suggesting that the drug is safe. This is particularly encouraging, as use of antimoni- als for such complicated cases had been associated with high mortality and toxicity – both of which constitute serious operational limitations for its continued use (Chappuis et al. 2007). These data thus advocate for wider availability and accessibility of AmBisome in VL-endemic low-income countries.

The strengths of this study included the large sample size, and the findings came from an operational setting and therefore likely to represent the operational reality on the ground. Moreover, standardised treatment protocols and data collection tools were in place. There were also a num- ber of limitations. As parasitological testing was not sys- tematically carried out to assess treatment response, misclassification might have occurred. There might also have been unascertained treatment failures amongst the early deaths. Loss to follow-up was high, and this may have included unascertained deaths and relapses, which limits our ability to assess treatment effectiveness at 6 months. However, these observations are of key interest, as they highlight operational gaps that need urgent attention. Our study population mainly consisted of HIV-negative patients and showed good treatment response in immune-competent individuals, but poor outcomes in the few HIV-positive individuals. This is similar to what was reported in Ethiopia (Ritmeijer et al. 2011). Of particular note is that, amongst cases of complicated VL, case fatal- ity rates of up to 30% have been reported with the use of antimonials in several East African countries (Veeken et al. 2000; Ritmeijer et al. 2001, 2011; Collin et al. 2004; Chappuis et al. 2011). Our study showed a much lower mortality with AmBisome, which supports the advo- cacy for making availability and access a priority. Although antimonials remain in use for uncomplicated VL, their practical limitations include a relatively lengthy, painful and toxic treatment. AmBisome has currently become the first-line choice for treating VL in the West, and it would seem necessary that it be similarly considered for use in low-income countries. To achieve this, national programmes should endorse its use within their national guidelines, and the international community should sup- port all efforts to achieve cost reductions of AmBisome or produce cheaper alternatives (e.g. through the develop- ment of quality-assured generic versions of liposomal for- mulations). Even at the current preferential pricing, a course of AmBisome (30 mg/kg) still costs around 350– 450 USD for an adult patient (Meheus et al. 2010). Whilst helpful to fill the urgent gap, the recent one-time donation of AmBisome to low-income countries by the manufactur- ing company is far from what is needed to ensure sustain- ability of the drug pipeline in the longer run (Balasegaram et al. 2012). There are other issues besides cost that require consideration. AmBisome needs to be stored below 25 °C, requires careful preparation (reconstitution, filtra- tion and dilution) before use and needs slow intravenous administration. Formulations that are less demanding in these respects would facilitate decentralisation of AmBi- some treatment and scaling-up at country level.

There were a number of important issues that require attention. First, the 7–10%observed relapse rate is sub- stantial, and a proportion of patients only achieved cure after AmBisome treatment extension, which suggests the possible need for higher drug dosing. Based on several small studies in East Africa generally studying lower AmBisome dosing, the currentWHO recommendation is to use 30 mg/kg, which is what is included in the 2010WHO revised guidelines for this region (WHO 2010). One won- ders if this dosing is adequate and randomised, controlled trials are warranted to answer this question. In particular, for VL relapses, more potent regimens might be required; this also merits further investigations. Combination therapy might be another way to achieve higher effectiveness whilst also lowering the risk of emergence of drug resistance (Van Griensven et al. 2010). The high proportion of patients lost to follow-up is a serious operational concern. Specific qualitative research is needed to correctly address this issue. Finally, five patients presented with relapse after the 6-month follow-up visit, raising the question whether further later visits need to be scheduled (Rijal et al. 2013). However, the high losses to follow-up even at the 6-month visit limit the operational feasibility. We also do not know whether these are true relapses or new infections; parasite genotyping would be required to answer this question. In conclusion, AmBisome was found to be effective and safe for the treatment of complicated VL in Sudan, suggesting the urgent need for improved access to this drug in the East African region and beyond.

ACKNOWLEDGEMENTS

This research was supported through an operational research course, which was jointly developed and run by the Centre for Operational Research, International Union Against Tuberculosis and Lung Disease, France, and the Operational Research Unit, Me ´decins sans Frontieres, Brussels-Luxembourg, and Me ´decins sans Frontieres, Switzerland. Additional support for running the course was provided by the Institute for Tropical Medicine, Antwerp, Belgium, the Center for International Health, University of Bergen. We thank Me ´decins sans Frontieres,

Switzerland, staff and patients at the Tabarak Allah Kala-Azar project in East Sudan. We are also grateful for the support of the Kala-Azar programme and Health Research Unit at the Ministry of Health of Sudan.

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