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Visceral leishmaniasis in the Republic of North Macedonia: A Retrospective Cohort Study

Bosilkovski et al. Leishmaniasis in the Republic of North Macedonia

Mile Bosilkovski[1], Bachir Khezzani[2,3,*], Fadil Cana[4], Kostadin Poposki[1], Dejan Jakimovski[1,5], Jadranka Nikolic[6], Dajana Georgievska[4], Marija Dimzova[1]

[1]University Hospital for Infectious Diseases and Febrile Conditions, Faculty of Medicine, Ss Cyril and Methodius University, Skopje, Republic of North Macedonia
[2]Department of Biology, Faculty of Natural and Life Sciences, University of El Oued, El Oued, Algeria
[3]Laboratory of Biology, Environment, and Health (LBEH), Faculty of Natural and Life Sciences, University of El Oued, El Oued, Algeria
[4]University Hospital for Infectious Diseases and Febrile Conditions, Skopje, Republic of North Macedonia
[5]Clinical Medicine Task Force, Balkan Association for Vector-Borne Diseases, Novi Sad, Serbia
[6]University Clinical Hospital Mostar, School of Medicine, University of Mostar, Bosnia and Herzegovina

Bachir Khezzani, PhD, Department of Biology, Faculty of Natural and Life Sciences; Laboratory of Biology, Environment, and Health (LBEH), Faculty of Natural and Life Sciences, University of El Oued, El Oued, Algeria
bachirkhezzani05@gmail.com
0000-0003-3268-1325

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Abstract
Visceral leishmaniasis (VL) is a systemic protozoan vector-borne disease and represents the most severe clinical form of leishmaniasis, with fatal outcomes if left untreated. This study aimed to evaluate the key epidemiological, clinical, and laboratory findings, treatment options, and outcomes in patients with VL. A retrospective analysis was conducted on the epidemiological and clinical characteristics of 84 patients diagnosed and treated for VL at the University Hospital for Infectious Diseases in Skopje, Republic of North Macedonia (RNM), between 2001 and 2023. The median age of patients was 47 years (range 1-74), with 77.4% being male. Contact with dogs was reported in 41.7% of cases. Seven percent of patients were immunosuppressed, and all were HIV-negative. The median time from symptom onset to diagnosis was 30 days (range 4-330 days). The predominant clinical manifestations were splenomegaly (97.6%), fever (96.4%), hepatomegaly (90.5%), and weight loss (54.8%). On admission, anemia, leukopenia, thrombocytopenia, and hypergammaglobulinemia were detected in 75%, 73.8%, 70.2%, and 63.1% of patients, respectively. A favorable outcome was achieved in 91.7% of cases; therapeutic failure occurred in 1.2%, and 7.1% of patients died. VL should be considered a crucial differential diagnosis in patients from the RNM presenting with prolonged unexplained fever, splenomegaly, cytopenia, and hypergammaglobulinemia.
Keywords: Visceral leishmaniasis, fever, splenomegaly, cytopenia, treatment

Introduction

Visceral leishmaniasis (VL), also known as Kala-Azar, is the most severe clinical form of leishmaniasis, with a fatality rate exceeding 95% if left untreated[1,2]. It is a chronic systemic disease caused by flagellated protozoan parasites of the genus *Leishmania* (Trypanosomatida, Trypanosomatidae), most commonly *L. donovani* and *L. infantum* (syn. *L. chagasi*)[3,4]. The latter is almost the only autochthonous species in Europe[5,6].
Transmission occurs through the bite of infected female sandflies (Diptera, Psychodidae) during blood feeding, with vectors belonging to the genus *Lutzomyia* in the New World and *Phlebotomus* in the Old World[1,7]. In rare cases, transmission may occur via needle sharing, blood transfusion, or vertically from mother to child during pregnancy[8]. Dogs represent the major domestic reservoir of *L. infantum* and the most susceptible host species, while humans act as accidental hosts[5].
Globally, VL acts as the second deadliest parasitic disease[9]. Most cases occur in tropical and subtropical regions, particularly in India, Brazil, and East Africa[2]. Only 25-45% of VL cases are officially reported to the World Health Organization, yet statistics estimate 50,000-90,000 new cases annually[10]. Moreover, more than 350 million individuals across 98 countries remain at risk of infection[11].
Environmental and climatic changes affecting vector and reservoir distribution[12,13], inadequate control of competent reservoirs and vectors, population migration[14,15], and the rising number of immunocompromised individuals[16] present increasing challenges in the understanding and management of VL. In addition, disease characteristics such as the long incubation period, the potential for relapses, high mortality rate, long life pathogen persistence in infected humans, limited drug availability, and drug toxicity[2], as well as the lack of a commercially available vaccine, together contribute to VL being a serious global concern[2,12,17].
The southern countries of Europe, particularly those in the Mediterranean basin, are home to most of the VL vectors[18]. In this region, the high incidence of canine leishmaniasis, with an increase in the stray dog population, expansion of *Phlebotomus* sandfly populations, insufficient control measures, and an increasing number of immunocompromised patients, has made VL a growing public health concern[5,14,19]. The reported cumulative annual incidence in this region during 2005-2020 ranged from 0.02 to 2.1 per 100,000 population[5], equating to 700-875 reported[20] and 1,200-2,000 estimated new cases annually[21].

The Balkans represent a hotspot region for leishmaniasis. The challenging conditions of recent decades have resulted in a limited understanding of the epidemiology of this disease and other zoonoses[18]. The Republic of North Macedonia (RNM), as a part of the broader Mediterranean region, is no exception when it comes to favorable environmental conditions for local disease transmission. *L. infantum* is the causative agent, with *P. tobbi, P. neglectus*, and *P. perfiliewi* serving as vectors[22], and domestic dogs act as proven or suspected reservoirs[5]. Over the past two decades, the annual incidence has ranged from 0.1 to 1.0 per 100,000 inhabitants, with a median annual incidence of 0.371 per 100,000 population[23]. Despite its low incidence in the country, VL possesses a considerable public health burden due to diagnostic difficulties, the requirement for targeted therapy, and the risk of lethality. Additionally, RNM has been identified as a source of infection for travelers[22,24,25].

In this study, we aimed to evaluate the demographic, epidemiological, clinical, and laboratory findings, diagnostic tools, treatment options, and the outcome of VL among patients treated in a tertiary care hospital in Skopje, RNM, during 2001-2023.

Materials and Methods

Study Design and Patients

The RNM is a country in the Balkan Peninsula with an area of 25,700 km^2 and a total population of approximately 2 million. This single-center, descriptive, retrospective analysis was conducted on hospital records from 84 consecutive patients with confirmed VL, who were diagnosed and treated at the university hospital for infectious diseases and febrile conditions in Skopje during 2001-2023. This tertiary hospital serves as the sole referral center for diagnosing and treating VL in adults and some pediatric patients worldwide. The study analyzed the patients' demographic, epidemiological, clinical, and laboratory characteristics as well as the diagnostic methods, treatment regimens, and outcomes. Patients who withdrew from treatment or had incomplete medical records were excluded from the study.

Diagnosis

VL diagnosis was based on the clinical and laboratory characteristics (such as prolonged fever, fatigue affecting daily functions, splenomegaly, hepatomegaly, significant weight loss, cytopenia, and hypergammaglobulinemia) alongside laboratory confirmation. Laboratory confirmation involved detection of serum antibody titers by using the indirect immunofluorescence antibody test [(IFAT), *Leishmania* Spot-IF; bioMérieux, Marcy l'Etoile, France] at a threshold of 1:80 or higher and/or identifying *Leishmania* amastigotes through microscopic examination of Giemsa-stained smears from sternal punctures or iliac crest biopsies by experienced laboratory personnel. Although the parasite species was not definitively identified, clinical and epidemiological data strongly indicated *L. infantum* as the infection agent.

Data Collection

Data on the demographic, epidemiological, clinical, and baseline laboratory parameters, as well as the treatment regimens and patient outcomes, were collected retrospectively from medical records by using a standardized protocol chart. The extracted data included information on age, sex, exposure history, such as the presence of dogs near the patients' residence, family history of VL, travel to endemic areas within the year before the symptom onset, human immunodeficiency virus (HIV) status, other immunodeficiencies, and previous VL episodes. Clinical data included duration of illness before diagnosis, fever, weight loss, cough, vomiting, diarrhea, abdominal pain, hepatomegaly, splenomegaly, bleeding, jaundice, peripheral edema, and ascites. Laboratory investigations comprised kidney and liver function tests, complete blood count, erythrocyte sedimentation rate (ESR), and protein electrophoresis. Diagnostic confirmation was established through parasitological and serological methods. Treatment regimen, time to defervescence, and outcomes, including cure, relapse, therapeutic failure, or death, were also recorded. Notably, the study did not investigate the vector and the disease reservoir.

Definitions

Fever was defined as an axillary temperature ≥37.5˚C on different occasions. Hematological abnormalities were defined as the presence of anemia (hemoglobin <11 g/L), leukopenia (leukocyte count $<4.0 \times 10^9$/L), and thrombocytopenia (platelet count $<150{,}000 \times 10^9$/L). Elevated ESR, hypoalbuminemia, and hypergammaglobulinemia were considered with values >20 mm Hg, <35 g/L, and >35 g/L, respectively. The illness duration (or diagnostic delay) was defined as the number of days between the onset of symptoms and the establishment of a VL diagnosis. Defervescence was defined as the period from the initiation of specific treatment to the normalization of temperature. Patients who exhibited an improvement in their general condition, weight gain, resolution of fever, regression of splenomegaly, and restoration of laboratory parameters by the end of treatment, with no recurrence of symptoms during the follow-up, were considered to have been cured. Therapeutic failure was defined as the lack of initial improvement, with the persistence or worsening of laboratory and clinical findings at the end of treatment. Relapse was defined as the recurrence of symptoms and signs after an initial successful treatment course.

Treatment

The therapeutic regimen was selected based on the year in which VL was diagnosed and drug availability. Meglumine antimonate was administered intramuscularly at a dosage of 20 mg/kg of pentavalent antimony per day, starting with a gradually increasing daily dose during the first 3 days of therapy. It was administered for 14 days, stopped for 14 days, and resumed for another 14 days. Conventional amphotericin B deoxycholate was administered at a dosage of 0.75-1 mg/kg/day, either daily or every alternate day, for 15-20 doses. Liposomal amphotericin B (L-AmB) was administered at 3 mg/kg daily on days 1-5, 14, and 21. Amphotericin B lipid complex was administered at a 3 mg/kg daily dose for 7-10 consecutive days. In addition, antipyretics, blood transfusions for severe anemia, antimicrobials for coinfection, and appropriate hydration and electrolyte replacement were provided, along with therapy for chronic diseases.

Follow-Up

The patients were hospitalized for the entire duration of antileishmanial therapy. During this period, physical examination was conducted daily, and their temperature was monitored at least four times a day. Standard laboratory tests were repeated every 3-5 days. Electrocardiograms and checks of diastases were routinely performed every 7 days during the treatment with meglumine antimonate and additionally, as clinically indicated. After discharge, clinical evaluations and laboratory analyses were repeated monthly for the first 3 months and then at 3-6-month intervals. Abdominal ultrasonography was performed upon hospital admission and on days 15, 30, 90, and 180 after the initiation of therapy. The IFAT titers were recorded at 3-6 months during the follow-up period.

Data Analysis

Data were analyzed using SPSS version 15.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics included frequencies and percentages for categorical variables and medians with ranges for continuous variables.

Ethics Statement

Clinical samples were collected during the routine diagnosis, treatment, and follow-up. Patients' identities were anonymized before conducting the analysis. The study was approved by the SS Cyril and Methodius University, Faculty of Medicine Ethics Committee in Skopje. (decision number: N.03-96/9, dated: 17.01.2025) Further, all participants provided their informed consent.

Results

Out of 171 reported cases of VL in the RNM between 2001 and 2023, 96 patients were treated at the University Hospital for Infectious Diseases and Febrile Conditions in Skopje. Twelve cases were excluded due to incomplete data or inadequate follow-up, leaving 84 patients eligible for analysis. Of these, 65 (77.4%) were male. The median age was 47 years (range: 1-74 years), with 5 patients (6.0%) younger than 14 years and 11 (13.1%) older than 64 years (Table 1). Symptom onset occurred most frequently in winter (30 patients, 35.7%), followed by spring (24, 28.6%), summer (18, 21.4%), and autumn (12, 14.3%). A history of close contact with dogs, mostly stray, was reported in 35 patients (41.7%), while 7 (8.3%) had traveled to neighboring countries within the past year. None reported a family history or prior VL infection. Six patients (7.1%) were immunosuppressed, although all were HIV-negative.

The median time from symptom onset to diagnosis was 30 days (range: 4-330 days). Forty-six patients (54.8%) were diagnosed within the first month, and an additional 14 (16.7%) by the end of the second month.

As shown in Figure 1, the most common clinical manifestations included splenomegaly in 82 patients (97.6%), fever in 81 (96.4%), hepatomegaly in 76 (90.5%), malaise in 55 (66.0%), and weight loss in 46 (54.8%). Less frequent symptoms were respiratory involvement in 17 patients (20.2%), gastrointestinal symptoms in 14 (16.7%), peripheral edema in 6 (7.1%), and jaundice, petechiae, or ascites in 2 (2.4%) each. Epistaxis was observed in 4 patients (4.8%).

Laboratory findings at admission revealed elevated ESR in 76 patients (90.5%), anemia in 63 (75.0%), leukopenia in 62 (73.8%), thrombocytopenia in 59 (70.2%), pancytopenia in 45 (53.6%), hypergammaglobulinemia in 53 (63.1%), and hypoalbuminemia in 59 (70.2%) (Figure 2). Positive serology for VL by IIF was obtained in 72 of 79 patients (91.1%), while amastigotes were identified in 25 of 39 patients (64.1%) who underwent sternal puncture or iliac crest biopsy. Both serology and myelogram were positive in 13 patients. In 14 cases, serology was positive but the myelogram was negative, while in 7 cases serology was negative despite a positive myelogram. Diagnosis was also confirmed in 45 patients based on serology alone and in 5 patients based solely on myelogram findings.

Therapeutic regimens are summarized in Table 2. The median defervescence period was 4 days (range: 1-21 days), and 77 patients (91.7%) achieved full recovery. No relapses were observed. One patient (1.2%) experienced therapeutic failure, while 6 patients (7.1%) died. Fatal outcomes were linked to advanced disease in 4 cases (due to severe bacterial superinfections and hemorrhagic diathesis) and to meglumine antimonate cardiotoxicity in 2 cases. Survivors were followed for a median of 4 months (range: 3-63 months).

Discussion

This study represents the first report on a cohort of patients with VL from the RNM. In this country, VL is a sporadic, endemic, and primarily autochthonous disease. However, the possibility of a small number of imported cases cannot be excluded, as many Macedonian citizens frequently travel to and reside in neighboring countries for business and holidays. Some of these countries also report a proportion of imported VL cases[20,22,26]. Distinguishing between autochthonous and imported cases remains challenging, as molecular techniques such as multilocus enzyme electrophoresis or species-specific identification[26] are not widely available.

Two-thirds of patients experienced the onset of illness during the winter or spring season, which may support the theory of the seasonal activity of sandflies and the long incubation period reaching up to 1 year[8]. A report from Greece found that 67% of cases had a symptom onset in the seasons of spring and summer[27], although an Italian study showed that 81% of symptoms occurred in the seasons of autumn and winter[28]. However, two other studies from Greece reported no seasonal difference in the disease onset[3,29].

Our cohort was primarily composed of immunocompetent male adults. In addition to the nature of activities practiced by males (such as work in agriculture, herding, etc.), this group tended to reside in open areas and was more frequently exposed to phlebotomine bites. According to some religious and societal traditions, females tend to cover most of their body area, which reduces the possibility of phlebotomine bites compared to males[13,30]. The dominance of male sex among VL patients ranges from 45%[31] to 100%[11], with several studies showing similar findings to ours[15,28,32,33]. The age distribution in our study was comparable to that reported from neighboring countries[3,20,22,34,35]. Only five patients were aged <14 years, and not all were malnourished. The remaining children with VL in the country were treated at the University Hospital for Pediatric Diseases. Pediatric VL is typically associated with malnutrition, and the incidence of VL in children in European countries has decreased with the improvement in living standards[5].

The high prevalence of canine leishmaniasis and the absence of an effective prevention strategy contribute significantly to the persistence of human disease in RNM. As highlighted by Khezzani et al.[36], the negligence of some dog owners in providing appropriate health care for their dogs represents an important risk factor for various zoonoses, including VL. The reported seroprevalence rate of canine leishmaniasis in endemic regions ranges from 2% to 86%[3,19,20,29,35,37]. In RNM, a study documented a 28% canine seroprevalence, with only 6% of infected dogs displaying clinical symptoms[38]. Interestingly, we did not identify HIV-positive patients with VL, and the incidence among immunosuppressed individuals was low, which contrasts with the well-established knowledge that *L. infantum* causes diseases in such populations. A similarly low or absent frequency of HIV co-infection has been reported in neighboring Balkan countries[17,20,22,34,35]. Nevertheless, other studies describe higher VL incidence among immunosuppressed patients due to non-HIV-related causes[3,7,16,17,29,39]. The prolonged diagnostic delay observed in our cohort may be attributed to the long incubation period, the non-specific nature of the initial clinical presentation, the broad differential diagnosis (including hematological malignancies and infectious and autoimmune diseases), as well as the lack of familiarity with VL among healthcare providers in the region. Similar diagnostic delays to ours have been reported in other studies[16,33,40].

The clinical manifestations of VL depend on the *Leishmania* species and the host's immune response[4]. Our findings regarding clinical features and laboratory parameters are consistent with those reported across the world. For example, fever was reported in 64% of cases[17] to 100%[1,11,41-43], malaise in 49%[32] to 93%[43], weight loss in 18%[27] to 100%[41], gastrointestinal and respiratory manifestations in 12%[17] to 81%[42], and 6%[44] to 76%[42], respectively. In addition, splenomegaly was reported in 58%[17] to 100% of cases[1,11,16,41,43], and hepatomegaly in 36%[1] to 100%[11,16,45]. Jaundice prevalence ranged from 3%[16,44] to 78%[11], peripheral edema from 3%[17,42] to 24%[46], and bleeding and ascites ranged from 2%[44] to 51% and 2%[42] to 32%[15], respectively. Our present laboratory findings were comparable to those reported elsewhere: elevated ESR in 78%[41] to 100%[45], anemia in 69%[7] to 100%[11,41,45], leucopenia in 33%[11] to 100%[45,47], thrombocytopenia in 33%[41] to 100%[11], pancytopenia in 33%[11,17] to 85%[47], hypergammaglobulinemia in 60%[7,17] to 100% [27], and hypoalbuminemia in 14% [48] to 100% [39]. These discrepancies in the frequency of clinical and laboratory manifestations reported by different studies may be attributed to factors such as population characteristics (e.g., socio-demographics, comorbidities, nutritional status, HIV, and other immune status), the nature of the causative agent, geographic variations, illness duration, diagnostic and therapeutic procedures, the country's economic conditions, and even the study design[9,40].

Serological testing and direct microscopic identification of *Leishmania* amastigotes in bone marrow aspirates are the most commonly employed diagnostic methods for VL [2,28,49]. The reported utility of serology in diagnosis ranges from 41%[17,45] to 100%[16,33], whereas that of direct microscopy ranges from 27%[28,33] to 97%[16]. On the other hand, VL misdiagnosis is common, which leads to dangerous delays in proper treatment and causes death in some cases[18].

Treatment depends on the *Leishmania* species, geographic region, and host immune status[32]. Between 2001 and 2017, our patients were mainly treated with meglumine antimonate, guided by drug availability, clinical experience, and literature recommendations[5,12,46,50]. Our protocol, involving gradual dose escalation and two treatment intervals, differed from standard regimens. Although rarely used elsewhere[27,51], this approach was acceptable in our setting, particularly as relapses in immunocompetent patients were infrequent (as confirmed in our results). The 14-day break also allowed resolution of injection site complications from high-volume intramuscular therapy. After 2017, amphotericin B derivatives became the treatment of choice, with formulation determined by availability. While antimonials and amphotericin formulations are similarly effective in [50] immunocompetent patients, liposomal amphotericin B (L-AmB) should be preferred for Mediterranean VL due to shorter treatment duration and reduced toxicity[16,17,52]. The complete recovery rate in our series aligns with previous studies, which reported recovery rates ranging from 61%[53] to 100%[35,45]. Similar to other reports[35,45], we did not observe any relapses, although the relapse rate could range from 6%-7%[7,16,27,53] to 17%-19%[28,31,40]. The occurrence of fatal outcomes in our study was similar to that reported from previous studies[17,28,34], with mortality rates ranging from 0%[35,45] to 22%[40,41]. VL-related mortality can often be attributed to bacterial superinfections, acute bleeding, severe anemia, heart or liver failure, or drug toxicity[15,48].

Several challenges have been reported in association with the VL outbreak in the Balkans and Mediterranean areas. In the context of the host, a recent study by Alcover et al.[54] suggested expanding the list of *L. infantum* potential hosts to include new species of small wild mammals, such as *Mus spretus*, *Erinaceus europaeus*, and *Sciurus vulgaris*. In addition to *L. infantum*, the only autochthonous species in Europe, some studies report new species, such as *L. donovani* sensu stricto in Cyprus and *L. tropica* in Greece[5]. On the other hand, most recent studies and reports agree that climate change has exacerbated the problem related to mosquito-borne diseases[13].

The migration and refugee crisis is another dilemma surrounding Eastern European countries. Because conflict and terror can contribute to leishmaniasis incidence or coincide with it through processes of population displacement and health system deterioration[55], the situation in the Balkan countries is believed to worsen as they are a transit area for migrants and refugees. In this regard, *Phlebotomus* spp. sandflies collected in Greece from refugee camps have displayed significant infection rates of *L. tropica* and *L. donovani*[56]. Cumulatively, all these factors complicate the situation and add to the challenges against VL control.

Currently, there are no registered vaccines against human leishmaniasis[4]. Although preventive measures to combat leishmaniasis may vary from one region to another[57][57]. Reference to delete, most studies agree that animal and human reservoir control, vector population control, and personal protection are the main axes that can reduce the rate of human leishmaniasis incidence [22]. [22] Reference to the added (already cited), with a high emphasis on the development of a human vaccine.

Study Limitations

The main limitations of this study are its retrospective design and the exclusion of pediatric cases managed at the University Hospital for Pediatric Diseases, which limits the generalizability of our findings to children.

Conclusion

VL is a sporadic endemic disease in RNM, primarily attributable to insufficient surveillance and control of the competent reservoir and vectors. Despite its low incidence, the severe course and potential for fatal outcomes make VL a significant public health concern. As such, VL should be considered in the diagnostic workup for patients presenting with prolonged fever, splenomegaly, hepatomegaly, hypergammaglobulinemia, and/or persistent pancytopenia. Early diagnosis and treatment are thus considered crucial for improving patient outcomes.

Ethics

Ethics Committee Approval: The study was approved by the SS Cyril and Methodius University, Faculty of Medicine Ethics Committee in Skopje. (decision number: N.03-96/9, dated: 17.01.2025)

Informed Consent: Informed consent was obtained.

Footnotes

Authorship Contributions

Surgical and Medical Practices: M.B., F.C., K.P., D.J., D.G., M.D., Concept: M.B., B.K., J.N., D.G., M.D., Design: M.B., B.K., F.C., K.P., D.J., J.N., D.G., M.D., Data Collection or Processing: M.B., F.C., K.P., J.N., D.G., Analysis or Interpretation: M.B., B.K., F.C., K.P., D.J., J.N., D.G., M.D., Literature Search: M.B., B.K., D.J., M.D., Writing: M.B., B.K., D.J., M.D.

Conflict of Interest: No conflict of interest was declared by the authors.

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References

Table 1. Demographic and epidemiological data in 84 patients with VL

Parameter		Patients data
Age in years - median (range)		47 (1-74)
Male gender		65 (77.4%)
Symptom onset	Spring	24 (28.6%)
	Summer	18 (21.4%)
	Autumn	12 (14.3%)
	Winter	30 (35.7%)
Contact with dogs		35 (41.7%)
Travel abroad		7 (8.3%)
Immunosuppression		6 (7.1%)
HIV positive		0
Diagnostic delay in days - median (range)		30 (4-330)

VL: Visceral leishmaniasis, HIV: Human immunodeficiency virus

Table 2. Therapeutical regimens employed in patients with VL	
Therapeutical regimen	**n (%) of the patients**
Meglumine antimonite	64 (76.2)
Conventional amphotericin B deoxycholate	8 (9.5)
Liposomal amphotericin B (L-AmB)	7 (8.3)
Amphotericin B lipid complex	4 (4.8)
No treatment	1 (1.2)