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ORIGINAL PAPER

Results of liposomal amphotericin B treatment in pediatric invasive fungal infections: a single-center experience

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ABSTRACT

Introduction: Invasive fungal infections (IFIs) are a common problem, and a major cause of morbidity and mortality in immunocompromised children. Most of them have at least one IFI during long and intensive treatment administered in oncology and hematology departments. Due to limited diagnostic options, final diagnosis of IFIs is often difficult and time-consuming, therefore empirical or prophylactic antifungal treatment is frequently initiated to avoid delays in therapy. The limited overall outcome led to the strategy of initiating either empirical or pre-emptive antifungal therapy before final diagnosis. One of the most effective antifungal drugs is liposomal amphotericin B (LAmB). The aim of the study was to retrospectively analyze patients treated using LAmB at the Department of Pediatric Oncology and Hematology UCH between 2015 and 2022.
Material and methods: Study group included 59 patients, aged 2–18 years (53% boys, 47% girls). Half of children were diagnosed with acute leukemia (acute lymphoblastic leukemia: 33%, acute myeloid leukemia: 17%), while the other half suffered from different cancers and immune disorders, such as Burkitt's lymphoma, T-cell non-Hodgkin lymphoma, Ewing's sarcoma, osteosarcoma, synovial sarcoma, neuroblastoma, central nervous system tumors, aplastic anemia, hemophagocytic histiocytosis, severe combined immunodeficiency, and gonadoblastoma. Data concerning patients status, therapy, and follow-up, including primary disease, chemo/radiotherapy, immune status, neutropenia duration, indications for antifungal therapy, response to therapy, supportive treatment, and complications, were analyzed.
Results: Eighty percent of patients received LAmB as empirical treatment. In 46% of patients, LAmB was applied as monotherapy, while in combination, micafungin, fluconazole, or voriconazole were used. Almost all patients presented an altered immune status due to pancytopenia and hypogammaglobulinemia. The median time of neutropenia before fungal infection was 19 days. Complications observed during LAmB therapy included liver and/or kidney dysfunctions and important electrolytes alteration. However, 76% of patients responded to the treatment.
Conclusions: The results indicated that adverse events were not linked to the cumulative dose of LAmB. There were no significant differences in cumulative doses between patients who experienced adverse events and those who did not. Kidney-related adverse events were weakly associated with fatal outcomes.

KEY WORDS:
pediatric oncology, amphotericin B, invasive fungal infections, pediatric hematology.

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INTRODUCTION

Liposomal amphotericin B (LAmB) has emerged as an important antifungal agent in the management of invasive fungal infections (IFIs), and attempts are being made to use it in prophylaxis. Children with cancer are at increased risk of developing IFIs due to their immunocompromised state, prolonged neutropenia, and application of broad-spectrum antibiotics. IFIs are associated with high morbidity and mortality, and the use of effective antifungal agents is essential for successful treatment [1].

LAmB has several advantages over conventional amphotericin B (AmB). Liposomal structure reduces the risk of nephrotoxicity and hepatotoxicity, which are common adverse effects of conventional AmB treatment. LAmB also has a higher AmB to lipid ratio, which allows for lower and less frequent doses, reducing the risk of toxicity. Pharmacokinetics and pharmacodynamics of LAmB in children are well-established, and studies have demonstrated its safety and efficacy in the treatment of IFIs in pediatric oncology patients, including patients undergoing allogeneic hematopoietic stem cell transplantation (HSCT) [2, 3].

Numerous studies have demonstrated the efficacy of LAmB in the treatment of various types of fungal infections, such as candidiasis, aspergillosis, cryptococcosis, and histoplasmosis. In a randomized clinical trial, LAmB was shown to be as effective as conventional AmB in the treatment of invasive aspergillosis, but with a lower incidence of nephrotoxicity and better tolerability. While LAmB was indicated an effective and safe alternative to conventional AmB, it is still associated with some degree of toxicity, particularly in patients with underlying kidney or liver diseases. The most common adverse effects of LAmB are infusion-related reactions, fever, and chills, but these side effects can be managed with pre-medication and close monitoring [4–7].

In a meta-analysis of randomized controlled trials regarding empirical antifungal therapy in high-risk patients with persistent febrile neutropenia, efficacy and safety of LAmB were evaluated in comparison with echinocandins. The analysis showed no significant difference in treatment success; however, mortality and adverse events in echinocandin-treated patients were significantly lower than in those treated with LAmB [8].

Therefore, LAmB is effective antifungal agent for the management of IFIs in pediatric oncology patients. Its use is associated with fewer adverse effects compared with conventional AmB, showing to improve clinical outcomes.

In this retrospective study, we described the efficacy and safety of LAmB for the treatment of IFIs in pediatric patients with oncological and hematological disorders.

MATERIAL AND METHODS

STUDY DESIGN

A retrospective analysis was conducted among patients treated with LAmB at the Department of Pediatric Oncology and Hematology of the University Children's Hospital (UCH) in Krakow, Poland. Between 2015 and 2022, 59 children were treated with LAmB for various indications, and the vast majority (85%) achieved a satisfactory response. However, some adverse effects were also observed.

The aim of the study was to evaluate the efficacy and safety of LAmB in the treatment of IFIs in pediatric oncology/hematology patients treated with conventional therapy and in children after HSCT. The drug was administered in accordance with the common clinical practice of clinicians, based on medical decisions. Patient data were obtained from medical records.

DOSAGE

Product characteristics recommend using LAmB at the same dose per kilogram for children aged 1 month to 18 years, but no recommendations for newborns and neonates (up to 1 month old) are provided. Typically, therapy starts with a daily dose of 1.0 mg/kg body weight, which can be gradually increased to 3.0 mg/kg body weight. Currently available information recommend a dosage of 3–5 mg/kg body weight for IFIs [9].

Standard procedure for treatment of fever of unknown origin in patients with neutropenia involves using a daily LAmB dose of 3 mg/kg body weight, and this regimen should continue until body temperature remains within normal range for 3 consecutive days. In LAmB treatment, dose and duration of should be individually adjusted; however, optimal pharmacokinetics and stable concentration are achieved only after 4 days of treatment. Moreover, it is not recommended to use the drug for longer than 42 days [10, 11].

PATIENTS

The study evaluated patients treated from January 1, 2015, to December 31, 2022. Age ranged between 2 and 18 years, with median age of 8.76 years (Table 1). Inclusion criteria included a confirmed, probable, or possible IFI diagnosed by an experienced pediatric oncologist. Additionally, patients had to be receiving LAmB treatment according to appropriate guidelines [1, 12].

Patients were divided into groups based on their invasive fungal disease (IFD) status, defined based on the latest standards from the revision and update of the consensus definitions of invasive fungal disease developed by the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium (EORTC/MSG) [13–16].

Detailed characteristics of the study group included gender, underlying disease, patient immunological status, adverse effects of LAmB treatment, neutropenia duration, therapy duration, material from which the microorganism was isolated, confirmation status of IFI, fungal species responsible for IFD, use of antifungal prophylaxis,

TABLE 1. Characteristics of the study group

Factor	
Median age (years)	8.76 (8/12–19)
Boys, *n* (%)	31 (53)
Girls, *n* (%)	28 (47)
Diagnosis, *n*	
Acute lymphoblastic leukemia	21
Acute myeloid leukemia	11
Neuroblastoma	6
Medulloblastoma	3
Aplastic anemia	3
Non-Hodgkin T cell lymphoma	2
Other	13
Adverse reactions (AEs) during treatment with LAmB, *n*	
Liver AE	16
Kidney AE	20
Electrolytes AE	16
Death on observation	21
Immune status of patients, *n*	
Pancytopenia	52
Pancytopenia + hypogammaglobulinemia	4
Pancytopenia + CMV	1
Pancytopenia + EBV	1
IgG deficiency, leukopenia	1

CMV – cytomegalovirus, EBV – Epstein-Barr virus, IgG – immunoglobulin G

use of antifungal polytherapy, response to antifungal therapy, cumulative dose, and patient outcomes.

DISEASE CHARACTERISTICS

In the study group, only confirmed and probable IFD cases were included. Patients were also classified according to their underlying disease. Among patients with a confirmed diagnosis, a further classification was made based on the species causing IFD, with duration of therapy and treatment outcomes examined. Data from medical records were assessed by an experienced attending physician [1, 14].

OUTCOME MEASURES

Effectiveness

Primary outcome was a positive response to treatment. Percentage of patients, for whom treatment failed (disease progression or treatment resistance) and that of death occurrence were calculated. Sub-group analysis was conducted according to dose used (below 4 mg/kg and above 4 mg/kg of LAmB). Cure criteria were evaluated with clinical indicators, such as imaging abnormalities, inflammatory markers (erythrocyte sedimentation rate, C-reactive protein, procalcitonin, white blood cell count, and differential). Patients with positive fungal tests had confirmation of cure based on negative control of fungal cultures or serological tests. Neutropenia was defined as neutrophil count below 1,000 cells/mm^3, and its duration was a basic criterion in the analysis.

Tolerability and safety

Adverse events (AEs) were defined as events that, in the doctor's opinion, could or were likely related to LAmB treatment, with main variables including complications during treatment and their frequency. Complications directly caused by administration of the drug (acute infusion-related reactions), such as nausea, vomiting, hypotension, dyspnea, bronchospasm, fever, or shock, were omitted due to prophylactic steroid therapy aimed at minimizing these effects. Instead, more delayed complications were assessed and categorized into three groups: renal, hepatic, and electrolyte disturbances. The laboratory criteria for their assessment are presented in Table 2.

To assess drug tolerance, reasons for treatment discontinuation were also considered; only one case, i.e., anaphylactic shock during LAmB therapy, was observed.

To enhance treatment safety and reduce the risk of immediate complications, a pre-treatment safety qualification before starting LAmB therapy was introduced, with a review of clinical data, laboratory test results, and electrocardiogram on treatment days 1 and 3. Any clinically significant changes in these parameters were documented for further study. AEs or any toxicity identified before LAmB treatment were attributed to previous therapy or the underlying condi-

TABLE 2. Laboratory criteria for assessment of liposomal amphotericin B (LAmB)-associated hepatic, renal, and electrolyte disturbances

Liver	Kidneys	Electrolytes
ALAT increased: > 5.0–20.0 × ULN if baseline was normal; > 5.0–20.0 x baseline if baseline was abnormal AspAT increased: > 5.0–20.0 × ULN if baseline was normal; > 5.0–20.0 × baseline if baseline was abnormal GGT: > 5.0–20.0 × ULN if baseline was normal; > 5.0–20.0 × baseline if baseline was abnormal	Creatinine > 3.0 × baseline; >3.0–6.0 × ULN	Sodium: > 150 mmol/l Potassium: > 5.8 mmol/l Total calcium: > 3 mmol/l Magnesium: > 1.3 mmol/l

ALAT – alanine aminotransferase, AspAT – aspartate aminotransferase, GGT – gamma-glutamyltransferase, ULN – upper limit of normal

tion. If an event occurred after starting the treatment, it was presumptively recognized as LAmB-related adverse effects.

STATISTICAL ANALYSES

Statistical analysis was conducted using Statistica version 13.2 software. Normality of continuous variables was assessed with Shapiro-Wilk test. Mean and standard deviation were used for normally distributed variables, and median and interquartile range for variables that did not follow normal distribution. Categorical variables were compared using chi-squared and Fisher exact tests. *P*-value < 0.05 was considered statistically significant.

TABLE 3. Microbiological data summary

Factor	*n*
Confirmation status of fungal infection	
Confirmed	19
Probable	40
Samples from which micro-organisms were isolated	
Stool	10
Urine	2
Lung sputum	2
Histopathology	1
Gastrointestinal tract	1
Unspecified	3
Confirmed fungal species	
Candida albicans	3
Candida krusei	2
Saccharomyces spp.	2
Aspergillus spp.	2
Leishmania spp.	1
Candida glabrata	3
Candida spp.	3
Candida dubliniensis	2
Candida parapsilosis	1

TABLE 4. Use of antifungal prophylaxis and quantitative compilation of prophylactic medication used

Factor	*n*
Use of antifungal prophylaxis	
No prophylaxis	8
Prophylaxis	51
Medication used in antifungal prophylaxis	
Nystatin	30
Fluconazole	14
Posaconazole	4
Amphotericin	1
Micafungin	2

RESULTS

The study analyzed 59 patients' data, and 52 complications were recorded. The characteristics of the study group are presented in Table 1.

Acute lymphoblastic leukemia was the most common underlying disease in the study group (36%). The microbiological summary is shown in Table 3. The median duration of neutropenia was 19 days. In 8 patients, a probable IFD was established based on serological techniques' clinical criteria.

The antifungal prophylaxis is summarized in Table 4, and the therapy is presented in Table 5. Among patients who underwent polytherapy, only 7 (23%) received more than 2 additional drugs. The daily dose of LAmB and cumulative dose are presented in Figures 1 and 2. A positive response to therapy was achieved in 51 patients.

Adverse events during LAmB therapy did not seem to be related to the cumulative dose of LAmB (Table 6). Due to the lack of significant differences in LAmB cumulative dose among patients with and without specific adverse events, there was no basis for calculating odds ratio. The attempt to connect the presence of adverse events as a predictor of a fatal outcome is presented in Table 7.

Only the presence of kidney adverse events was related to the fatal outcome; however, the relationship was weak.

The lower dose of LAmB (3 mg per kg or less) was administrated in 50 patients, and 4 mg per kg or more in 9 patients (Figure 1). The higher dose per kg of LAmB was unrelated to better outcomes, specific, or total adverse events or death (Table 8).

TABLE 5. Additional medication used in antifungal therapy

Factor	*n*
Comparison of monotherapy vs. polytherapy for antifungal treatment	
Monotherapy with liposomal amphotericin B	28
Antifungal polytherapy	31
Additional medication used in antifungal therapy	
Fluconazole	1
Posaconazole	7
Caspofungin	12
Micafungin	8
Voriconazole	4

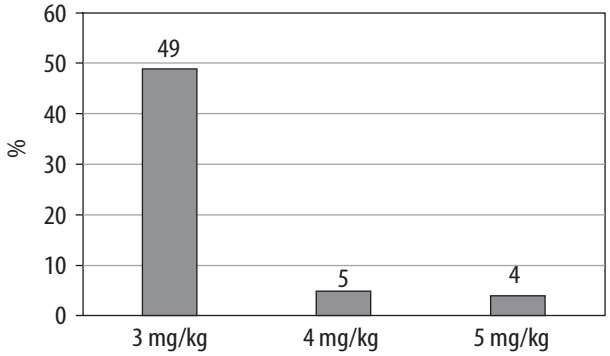

FIGURE 1. Classification of patients by liposomal amphotericin B dose per kg body weight

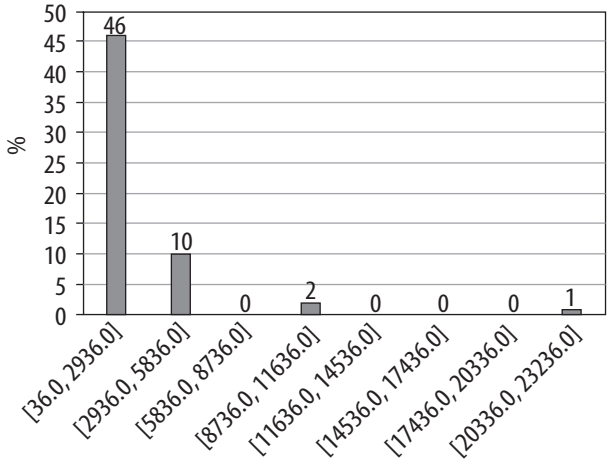

FIGURE 2. Cumulative dose of liposomal amphotericin B (quantitative summary of results is shown in each range). The median cumulative dose was 1.050 mg

are not dose-dependent, meaning that increasing the cumulative dose does not necessarily increase the risk of adverse events. This is crucial for clinical practice as it implies that dosing adjustments may not mitigate adverse events. Our findings are in line with a work of Stone *et al.* [17], who found that the adverse events associated with LAmB did not show a clear dose-response relationship. Patients receiving varying cumulative doses did not exhibit significantly different rates of nephrotoxicity or other adverse events, suggesting that the toxicity profile of LAmB may be more related to individual patient factors rather than cumulative dose. Another study supporting the idea that adverse events are not necessarily dose-dependent was conducted by Sunakawa *et al.* [18]. In a multicenter study involving patients with IFIs, they indicated that while higher doses of LAmB were effective in managing infections, the incidence of adverse events, particularly nephrotoxicity, was not significantly higher in patients receiving higher cumulative doses compared with those on lower doses [18]. Contrary, a study on non-LAmB by Fisher *et al.* [19] showed that higher daily doses and cumulative doses of non-LAmB were commonly associated with increased nephrotoxicity. They observed that nephrotoxicity risk doubled with each 0.10 mg/kg/day increase in daily dosage.

In this study, 19 out of 59 fungal infections were classified as proven; however, in the majority of cases, fungal isolates were obtained from stool and sputum samples, which are physiologically non-sterile. The use of such materials inherently limits the diagnostic certainty, as these sites can be colonized by fungi without causing invasive

TABLE 6. Analysis of relationship between liposomal amphotericin B cumulative dose (mg) and complications

Adverse event (AE)	No adverse events, median (IQR)	With adverse event, median (IQR)	*p*-value
Any AE	1,080 (550–2,112)	1,045 (427–3,916)	0.067
Liver AE	1,302 (442–2,700)	585 (393.5–1,161.5)	0.140
Kidney AE	1,080 (525–2,700)	975 (310–1,458)	0.305
Electrolytes AE	829.5 (442–2,100)	1,273 (437–3,987)	0.249

TABLE 7. Chi-square test of adverse events and deaths

Adverse event (AE)	*p*-value	Fi (power of dependence)
Any AE	0.08775	N.A.
Liver AE	0.46066	N.A.
Kidney AE	0.03073	0.2837
Electrolytes AE	0.05581	N.A.

TABLE 8. Differences in adverse events, deaths, and response depending on liposomal amphotericin B dose

Type of adverse event	Lower dose of AmBisome (≤ 3 mg per kg), *n* = 50	Higher dose of AmBisome (≥ 4 mg per kg), *n* = 9	*p*-value
Any	36 (72.0%)	6 (66.7%)	0.3733
Liver	13 (26.0%)	3 (33.3%)	0.3251
Kidney	17 (34.0%)	3 (33.3%)	0.4837
Electrolites	25 (50.0%)	4 (44.4%)	0.3785
Death	17 (34.0%)	4 (44.4%)	0.2743
Response to therapy	44 (88.0%)	7 (77.8%)	0.2053

DISCUSSION

The study found no significant differences in the cumulative dose of LAmB among patients with and without specific or any adverse events. This suggests that adverse events

disease. This limitation should be taken into account when interpreting the results, as it may lead to potential overestimation of proven fungal infections.

Although there was a weak relationship between the presence of kidney adverse events and fatal outcomes, it is important to note that this relationship was not strong enough to be conclusive. Kidney function monitoring remains essential during amphotericin B therapy, but our findings suggest that other factors may also play a significant role as a cause of mortality. One of the most important issues is a formulation of amphotericin B. Liposomal formulations (e.g., AmBisome) have been shown to have a lower risk of nephrotoxicity compared with conventional formulations. This is due to better renal tolerance and reduced direct nephrotoxic effects [20]. Amphotericin B causes nephrotoxicity through direct tubular toxicity, arteriolar vasoconstriction, and reduced renal blood flow. These mechanisms lead to a decreased glomerular filtration rate and potential renal failure [21]. Pre-existing renal conditions, concurrent nephrotoxic drugs, and overall health status significantly impact nephrotoxicity. Patients with compromised renal function are at higher risk, but adequate hydration and sodium supplementation can mitigate nephrotoxic effects. Hydration status influences renal function and can protect against amphotericin B-induced damage [22]. Co-administration of other nephrotoxic drugs, such as aminoglycosides or flucytosine, can influence the extent of nephrotoxicity. Folk *et al.* [23] reported that certain drug combinations may exacerbate renal impairment.

Nephrotoxicity associated with amphotericin B is a multifactorial issue influenced by drug formulation, dosing, patient-specific factors, hydration status, and co-administered medications. Understanding of these factors is crucial for minimizing nephrotoxic risks and effective treatment of fungal infections.

As this was a single-center observational study, further research is needed to explore the underlying factors contributing to adverse events and mortality in patients receiving LAmB. Investigating patient-specific characteristics, such as pre-existing conditions, co-medications, and genetic factors, could provide more comprehensive insights. Additionally, larger, multi-center studies could help validate the current findings.

The ultimate goal of any therapeutic regimen is to maximize efficacy while minimizing adverse events. The findings suggest that with LAmB, careful attention should be given to balancing these two aspects, particularly in high-risk populations. Personalized treatment plans, which consider the patient's overall health status and risk factors for adverse events, may enhance the overall safety and efficiency of therapy.

CONCLUSIONS

The study provides evidence that the cumulative dose of LAmB does not significantly impact the incidence of adverse events or therapeutic outcomes. The weak association between kidney adverse events and fatal outcome highlights the complexity of patient responses to therapy. Clinicians are advised to consider lower initial doses and closely monitor patients to tailor treatments effectively. Further research is warranted to better understand the factors influencing patient outcomes and to optimize LAmB treatment protocols.

DISCLOSURES

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