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ORIGINAL RESEARCH ARTICLE

Amphotericin B in Pediatrics: Analysis by Age Stratification Suggests a Greater Chance of Adverse Events from 13 Months of Age Onwards

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Abstract

Background and Objective Amphotericin B deoxycholate (AMB-D) remains an antifungal agent with great therapeutic value in pediatric patients. The currrent consensus is that its use in neonates is safer than in older children. However, childhood presents different periods of development that deserve to be evaluated more precisely. Our goal was to assess the usage profile of AMB-D in stratified pediatric age groups, adapted according to the National Institute of Child Health and Human Development classification.

Methods This retrospective cross-sectional observational study was conducted at a Brazilian tertiary children's hospital between January 2014 and December 2019. Data of patients who received at least two doses of intravenous AMB-D while hospitalized were extracted from electronic health files. Information on patient demographics, underlying diseases and comorbidities, laboratory examinations, fungal infection diagnosis, and AMB-D use were gathered following specific criteria. Nonparametric tests were applied, such as the chi-square test to compare proportions and Fisher's exact test to assess the association between categorical variables or contingency tables.

Results One hundred and twenty-seven (127) medical records were stratified as preterm neonatal (birth <37 weeks postmenstrual age), term neonatal (birth–27 days), infants (28 days–12 months), toddlers (13 months–2 years), early childhood (3–5 years), middle childhood (6–11 years), and early adolescence (12–18 years). The criteria for the indication of AMB-D followed empirical use as the main indication ($n = 74$; 58.26%), proven and probable fungal infection ($n = 39$; 30.71%), and medical suspicion ($n = 14$; 11.02%). *Candida* spp. was the main etiologic agent isolated in cultures, with the highest frequency of *C. albicans* ($n = 18$; 40%), followed by *Candida parapsilosis* ($n = 14$; 31.11%), and *Candida tropicalis* ($n = 6$; 13.33%). Very few acute infusion-related adverse effects were observed during the administration of AMB-D in pediatric patients. We found an unfavorable impact of AMB-D use in patients from 13 months of age onwards suggesting this group as a turning point for a greater chance of adverse events, and not soon after the neonatal period.

Conclusions Clinical or observational studies based on age stratification are essential to accurately elucidate whether potentially toxic drugs can be used safely in the pediatric population. Our search for a turning point was shown to contribute to the accuracy of the study, as it provided data on the impact of D-AMB in specific pediatric age groups.

1 Introduction

After 60 years of use, amphotericin B deoxycholate (AMB-D) remains the treatment of choice for several potentially fatal invasive fungal diseases (IFD), including opportunistic and endemic mycoses, and those affecting the pediatric population [1, 2]. Invasive candidiasis and candidemia remain major causes of morbidity and mortality, notably among immunocompromised and those hospitalized long term [3–7]. Epidemiologically, *Candida* spp. are the most common yeasts and along with other pathogens that cause IFD with potentially high mortality rates and poor response even to new antifungal therapies [7–10].

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Key Points

Most of the original research on amphotericin B deoxycholate in pediatrics goes back decades with some limitations in the number of patients.

To the best of our knowledge, this is the first observational study based on an age-stratification strategy and included a significant number of patients, totaling 127.

Until our findings, the discussion of acute side effects related to the infusion and toxicity of amphotericin B deoxycholate was primarily focused on neonates, with an overall worse scenario in older children.

We found an unfavorable impact of the conventional formulation from 13 months of age onwards, suggesting that this age group is a turning point for a greater chance of adverse events.

With this, we encourage other centers to investigate and support this approach in identifying a more accurate age group to better understand where prescribing amphotericin B deoxycholate is safer or less harmful in pediatrics.

Acute infusion-related side effects (IRSE) and toxicity associated with AMB-D have been widely reported, including renal, hepatic, and hematologic disorders [11, 12]. Nephrotoxic effects are mediated by changes in membrane permeability and vasoconstriction. Infusion-related side effects are believed to be triggered by the release of inflammatory cytokines by mononuclear phagocytic cells. The intensity appears to be related to the availability of the antifungal agent to react with the target cells, regardless of the extent of exposure [13, 14].

Despite their significantly higher costs, lipid-based formulations have been developed to reduce toxicity and used in clinical practice with relatively similar efficacy [15]. Nevertheless, because of undesirable adverse events (AEs) and the availability of new drugs, there is a consensus that AMB-D should be avoided in pediatric patients. However, previous data demonstrated that AMB-D AEs are less prominent in neonates than in older children and adults. Hence, AMB-D remains as the first-line antifungal agent for the youngest population [2, 16, 17]. Studies have indicated that this tolerability is due to the pharmacokinetics of the drug, including higher active clearance rates and a lower volume of distribution, resulting in lower plasma concentrations. However, other studies argue that AEs are directly proportional to the patient's age and AMB-D dosage when compared with other formulations [18–25].

In pharmacovigilance, drug safety has been relatively less explored in children than in adults. However, there is still a lack of data to support the suggested impact of pharmacokinetics on differences in the toxicity of AMB formulations [17]. The available studies are mostly single-center studies with a limited number of patients and focus mainly on neonates [19, 26–29]. Additionally, it is necessary to consider that childhood presents different periods of development that need to be evaluated more precisely. None of the previous publications evaluated the effects of AMB in a robust group of non-neonate patients, nor did they compare the results between different pediatric age groups. Therefore, gaps remain on the safety of drug administration in non-neonate patients.

We characterized and compared the occurrence of AMB-D AEs in different pediatric age ranges to provide more sustainable evidence on this topic and support doctors' decision making. In addition, we assessed the usage profile as well as the impact of AMB-D in stratified age groups, adapted according to the National Institute of Child Health and Human Development classification [30]: preterm neonatal (birth < 37 weeks of postmenstrual age), term neonatal (birth–27 days), infants (28 days–12 months), toddlers (13 months–2 years), early childhood (3–5 years), middle childhood (6–11 years), and early adolescence (12–18 years).

Furthermore, we strived to find a "watershed," a period of age in childhood that could indicate a turning point where the benefit of using AMB-D outweighs the risk. Consequently, lipid-based formulations could be directed for particular ages to reduce toxicity.

2 Material and Methods

2.1 Study Design

We conducted a retrospective cross-sectional observational study between January 2014 and December 2019 involving the medical records of patients who received at least two doses of intravenous AMB-D while hospitalized at a Brazilian tertiary public-private children's hospital. The institution has approximately 400 beds and offers more than 30 services in different specialties, including transplants. This study was approved by the research ethics committee of the hospital, waiving the requirement for informed consent.

We reviewed patients' medical records from registration to discharge or death during their hospitalization for AMB therapy. Data were extracted from electronic health files and collected using REDCap electronic data capture tools hosted at our institution [31, 32]. Information on patient demographics, underlying diseases and comorbidities, laboratory examinations, fungal infection diagnosis, and AMB-D use were gathered following specific criteria.

Proven and probable IFD were classified according to the European Organization for Research and Treatment of Cancer/National Institute of Allergy and Infectious Diseases Mycoses Study Group consensus group, based on microbiology, imaging, and clinical findings [33, 34]. Additional data were considered in the open field when any information reported by the physician about the patient's daily evolution was relevant.

The present children's hospital was accredited in 2019 at the maximum level (III) by the National Accreditation Organization [35, 36]. Consequently, different methods are used for drug pharmacovigilance, including spontaneous reporting and monitoring prescription events. The spontaneous reporting of AEs plays an important role in drug surveillance for the pediatric population, as a caregiver (usually a parent) monitors all steps at the bedside, from identification to possible reactions that may occur during or after drug administration. In addition, the nursing professional responsible for the infusion of AMB-D remains attentive to acute events and is aware of the potential toxicity of the drug. Any signs or symptoms that may be related to the administration of the antifungal agent should be recorded, in addition to informing the attending physician. Thus, to be true to the identification of AEs and aware of the limitations of the study's retrospective nature, medical records were carefully consulted so as not to omit information mainly on neonatal patients. Acute IRSE occur most frequently with initial doses during administration; thus, nursing records were also checked to avoid missing any notes.

In addition to infusion, renal, hepatic, and other organ functions require monitoring. Each laboratory parameter was analyzed separately for each stratified age group, as the study center had specific reference values for neonates and distinct pediatric age groups. In short, we identified laborarory parameters that were adequate and inadequate immediately before and after AMB-D exposure. Kidney function during AMB-D exposure was evaluated using serum urea, creatinine, and potassium profiles. Similar analyses were performed to identify hepatic and hematologic toxicity using alanine transaminase, aspartate transaminase, gamma-glutamyl transferase, bilirubin, hemoglobin, neutrophils, and platelets as markers.

Amphotericin B dosages at the present study site are recommended on an "mg kg^{-1}" basis. The AMB-D regimen can range from 0.5 to 1.5 mg kg^{-1}/daily for neonates and pediatric individuals. The AMB-D infusion duration may be reduced to a minimum of 2 hours if the 4- to 6-h infusion is well tolerated.

2.2 Statistical Analyses

Data were analyzed using R version 4.1.0, IBM SPSS Statistics for Windows version 25.0, and MedCalc version 20.027 [37–39]. Some values were expressed as the median and range for a continuous variable and as an absolute frequency and percentage of the group from which they were derived. A nonparametric chi-square test was applied to compare proportions and a Fisher's exact test to assess the association between categorical variables or contingency tables (i.e., groups of newborns and non-neonates, occurrence of AEs). We generated descriptive charts using Microsoft Excel® [40] for some significant (considering 5% significance) relevant results.

We also analyzed laboratory parameters to identify whether the drug had a direct impact from the time it was administered. The chi-square test was used to evaluate the toxicity of AMB-D on target organ functions. The measure was the relationship between the number of patients who presented normal values of a given exam adjusted to the reference values of each age group and the total number of patients who underwent the exam in the following period: baseline (the day before the first AMB-D dose), day (D) 3, D7, and D14 of AMB-D therapy, and end of treatment (last day of AMB-D use). The results represent the percentage of adequation for each parameter. Therefore, it was possible to minimize the bias of patients who already had any compromised function before receiving AMB-D.

In some cases, owing to the small sample size of a specific stratified age group, *p*-values were provided for descriptive purposes only. In other cases, for better statistical equality and credibility, we re-divided patients into two groups: neonates and non-neonates. The purpose was to understand if there was any divergence between the groups. Finally, we presented the 95% confidence intervals for *p*-values, and differences in proportions in the tables and graphs, to explain the observed values.

3 Results

3.1 General Data

This study included children aged ≤ 18 years who underwent AMB-D therapy. Initially, 206 medical records were identified. Fifteen records were excluded because of a single exposure to the drug, seven for previous exposure, three for duplicate data, and one for the lack of essential data. Another 53 patients were excluded because they were receiving AMB lipid-based formulation therapy. Patients who started treatment with AMB-D but switched to another AMB formulation after a few days upon medical request were considered, as the first exposure to AMB-D was the most important for the study. In total, 127 medical records were included in the study. Data were stratified by age group to present a more homogenous comparison of variables (Table 1).

The main age group receiving AMB-D therapy was preterm neonates, or birth at < 37 weeks' postmenstrual age, with variation between 24 and 37 weeks of gestational age, corresponding to 35.43% of the total. Eleven were classified as very low birth weight (< 1.5 kg) [1.06–1.36] and ten as extremely low birth weight (< 1.0 kg) [0.620–0.980]. In neonates, the main underlying disease was gastrointestinal disorder (52.8%). Older children present with a frequency of more than 50% for a variety of other conditions, such as cardiological, genetic, ophthalmological, and neurological diseases. Approximately 89% of all patients received AMB-D in the intensive care unit. No significant difference between the sexes was found despite female individuals representing 53.5% of the population treated with AMB-D.

3.2 Invasive Fungal Infection and AMB-D Treatment

Of the 127 medical records analyzed, 35.43% had proven fungal infection, with a survival rate of 66.66%, and no evidence of residual disease after the end of therapy, except for those who had their treatment suspended because of a medical decision to change antifungal therapy ($n = 4$; 3.15%). The median length of hospital stay, daily dosage, and other information are shown in Table 1. The pediatric patients received a maximum AMB-D dosage of 1.5 mg/kg/day. A gradual increase or decrease in dosing for posology adaptation was observed in 67 records (52.7%). The actual number of days of AMB-D use (excluding possible breaks and occasional suspensions with returns during treatment) and its cumulative dose ranged from 3 to 42 days and from 3.30 to 890.50 mg, respectively. When testing the correlation between groups of proven and non-proven fungal infections, we found that in the preterm neonatal age group, it was possible to associate the length of hospital stay (181 days vs 97 days; $p = 0.024$) with the death rate (46% vs 22%; $p = 0.017$).

Considering the criteria for AMB indication, empirical use was the most common ($n = 74$; 58.26%), followed by proven and probable [31, 32] ($n = 39$; 30.71%), and medical suspicion ($n = 14$; 11.02%). Doctors reported the most common diagnoses of proven disease as "sepsis" with 18 cases, "candidemia" with ten cases, and "urinary tract infection" with nine cases (Table 2). *Candida* spp. was the main etiologic agent isolated in cultures, with the highest frequency of *C. albicans* ($n = 18$; 40%), followed by *C. parapsilosis* ($n = 14$; 31.11%), and *C. tropicalis* ($n = 6$; 13.33%). Fungal specimens were isolated from normally sterile sites such as peripheral blood ($n = 32$; 71%), catheter tip ($n = 12$; 26.6%), and central venous catheter ($n = 5$; 11%). Fifteen patients (33.3%) had positive urine culture results. Of the 46 patients with proven IFD, 15 lost their lives. Finally, the 127 patients received antifungal therapy with at least two doses of AMB-D alone (94; 74.01%), or in combination with an azole (24; 18.90%), or an echinocandin (9; 7.09%).

3.3 Searching for a "Watershed" for Greater Occurrence of AEs

Acute IRSEs during AMB-D administration were observed mainly in older children, totaling four occurrences (3.1%). Fever, itching or rash, trembling or chills, and nausea or vomiting were cited. Fifty percent of all patients received prophylactic premedication, including antipyretics, antihistamines, and corticosteroids, to prevent the onset of acute IRSE. The age-stratified Chart 1A shows the respective occurrences of acute IRSE, setting a turning point from toddlers (aged 13 months–2 years). Neonates (preterm and term) and infants (with zero occurrences) were pooled to compare with older children, showing significant associations between the two. Laboratory parameters of toxicity were individually classified as adequate or inadequate according to the age reference value. After a general comparison, another turning point was traced in the toddlers, suggesting a greater chance of target-organ toxicity from this age group forward (Chart 1B).

In our study, toxicity was assessed using the proportion of adequation of urea, creatinine, potassium, and other laboratory parameters. For nephrotoxicity, the urea proportion of adequation for neonates at baseline was 59%, meaning that less than 60% of patients were within normal levels (according to reference values) before receiving AMB-D. The proportion followed D3 (54%), D7 (65%), and D14 (48%) until the end of treatment (52%), with no significant variation during therapy length. However, the same parameter for older children revealed another pattern after AMB-D use, starting with 73.5% of adequation at baseline, followed by a sequence of significant decreases of adequation at D3 (53%), D7 (52%), D14 (31%), and end of treatment (46%) [$p = 0.0055$] (Table 3).

There was no considerable variation in creatinine levels, although a small percentage of patients (12, 9.4%) showed signs of acute kidney injury or developed oliguria/edema. Six belonged to the neonate group: 4/45 (8.9%) preterm neonates and 2/27 (7.4%) term neonates. The other six represented older children: 5/25 (20%) in infants and 1/7 (14.3%) in the early childhood group. For creatinine proportion of adequation, no significant differences were found between the neonates and non-neonates (Table 3). Serum potassium levels showed no significant changes in the neonate group during treatment, apart from an expressive decrease of up to 27% at the end of treatment for older children ($p = 0.0072$). Only a third or less of the laboratory results for the liver function, such as alanine transaminase, aspartate transaminase, gamma-glutamyl transferase, and bilirubin, were available (not shown). Hematological parameters, such

Table 1 Demographic data and clinical characteristics of pediatric patients receiving D-AMB therapy from 2014 to 2019 at a Brazilian tertiary public-private children's hospital divided by age stratification

Variables	Age groups[a] Total $n = 127$; 100%													
	Neonates		Older children											
	Preterm neonatal < 37 wk PMA $n = 45$; 35.4%	Term neonatal Birth–27 d $n = 27$; 21.2%	Infants 28 d–12 mo $n = 25$; 19.7%		Toddler 13 mo–2 y $n = 9$; 7.1%		Early childhood 3 y–5 y $n = 7$; 5.5%		Middle childhood 6 y–11 y $n = 8$; 6.3%		Early adolescence 12 y–18 y $n = 6$; 4.7%	
Sex, F/M	28 F 17M	10 F 17 M	15 F	10 M	4 F	5 M	5 F	2 M	3 F	5 M	3 F	3 M
Weight, median (kg) [range]	1.6 [0.6–6]	3.1 [2–7.8]	3.5 [1.2–13]		12.1 [9.5–16]		21 [12–38]		24 [18–74.8]		39 [28–56]	
Underlying diseases, *n* (%)												
Diabetes mellitus	–	–	–		–		–		–		–	
Hypertension	–	–	2 (8)		–		–		–		1 (17)	
Neoplasm/ hematologic disease	–	3 (11)	2 (8)		2 (22)		2 (29)		3 (38)		–	
Gastrointestinal disease	29 (64)	9 (33)	11 (44)		1 (11)		1 (14)		–		–	
Hepatopathy/ liver disease	1 (2)	2 (7)	–		1 (11)		–		–		1 (17)	
Nephropathy/ kidney disease	9 (20)	4 (15)	2 (8)		1 (11)		–		–		1 (17)	
Rheumatological disease	–	–	–		2 (22)		–		–		1 (17)	
Neurological disease	7 (16)	7 (26)	6 (24)		2 (22)		4 (57)		1 (13)		3 (50)	
Immune/autoimmune disease	1 (2)	2 (7)	1 (4)		2 (22)		–		–		–	
Solid organ transplant	–	–	–		–		–		1 (13)		1 (17)	
Others	11 (24)	15 (56)	16 (64)		5 (56)		2 (29)		3 (38)		2 (33)	
HSCT, *n* (%)	–	1 (3.7)	2 (8)		2 (22.2)		–		–		1 (16.6)	
Febrile neutropenia, *n* (%)	2 (4.4)	–	2 (8)		1 (11.1)		–		1 (12.5)		–	
Fungal disease, *n* (%)												
Possible	32 (71)	12 (44)	15 (60)		5 (56)		2 (29)		4 (50)		6 (100)	
Probable	–	1 (4)	–		–		2 (29)		2 (25)		–	

Table 1 (continued)

Variables	Age groups[a] Total *n* = 127; 100%						
	Neonates		Older children				
	Preterm neonatal < 37 wk PMA *n* = 45; 35.4%	Term neonatal Birth–27 d *n* = 27; 21.2%	Infants 28 d–12 mo *n* = 25; 19.7%	Toddler 13 mo–2 y *n* = 9; 7.1%	Early childhood 3 y–5 y *n* = 7; 5.5%	Middle childhood 6 y–11 y *n* = 8; 6.3%	Early adolescence 12 y–18 y *n* = 6; 4.7%
Proven	13 (29)	14 (52)	10 (40)	4 (44)	3 (43)	2 (25)	–
LOS median (d) [range]	92 [19–469]	76 [35–429]	95 [13–272]	55 [33–132]	56 [38–153]	76.5 [14–209]	34.5 [10–47]
Deaths, *n* (%)	13 (29)	11 (41)	7 (28)	2 (22)	1 (14)	2 (25)	4 (66)
Department of D-AMB treatment, *n* (%)							
Pediatric ward	–	2 (8.4)	1 (4)	1 (11.1)	2 (28.6)	2 (25)	–
Onco-hematological ward	–	–	–	1 (11.1)	–	3 (37.5)	–
ICU	45 (100)	25 (92.6)	24 (96)	6 (66.6)	5 (71.4)	2 (25)	6 (100)
Other	–	–	–	1 (11.1)	–	1 (12.5)	–
D-AMB dosage median (mg/day) [range]	2 [0.9–6]	3.5 [2.3–7.8]	3.3 [1.2–9]	11.5 [3.3–17]	20.3 [6–38]	18 [15.5–21]	37.5 [15–84]
D-AMB dose accumulated median (mg) [range]	26.4 [3.3–177.8]	40 [6.8–210]	37.6 [10.5–135]	112 [27–247.5]	286.5 [87–420]	304 [162.8–484.5]	259 [100.5–890.5]
D-AMB real number of days use median (days) [range]	12 [3–28]	13 [4–42]	12 [3–29]	11 [3–27]	14.5 [9–21]	16 [7–21]	6.5 [3–13]

d days, *D-AMB* amphotericin B deoxycholate, *F* female, *HSCT* hematopoietic stem cell transplant, *ICU* intensive care unit, *LOS* length of stay, *M* male, *mo* months, *PMA* postmenstrual age, *wk* weeks, *y* years

[a]Adapted from the National Institute of Child Health and Human Development [30]

Table 2 Relation of pediatric patients diagnosticated with proven fungal infections, therapeutical regimens, and outcome

Pediatric age groups[a]		Patient	Medical diagnosis of proven fungal infection[b]	Etiologic agent	Isolated sample[c]	Combined therapy	D-AMB real use (d)	Outcome
Neonates	< 37 wk PMA	1	Disseminated candidiasis	*C. glabrata*	Urine; blood		14	Death
		2	Disseminated candidiasis	*C. albicans*	Blood; cath. tip		12	Death
		3	Fungal sepsis	*C. albicans*	Blood; cath. tip	D-AMB + ECN	58	Death
		4	Fungal sepsis	*Candida* spp.	Blood		25	I.H.D
		5	Fungal sepsis	*C. glabrata*	Blood	D-AMB + ECN	24	I.H.D
		6	Candidemia	*C. albicans*	Blood; cath. tip		23	Death
		7	Fungal sepsis	*C. parapsilosis*	Blood		24	I.H.D
		8	Fungal sepsis	*C. albicans*	Blood	D-AMB + Azole	21	I.H.D
		9	Fungal sepsis	*C. albicans*	Urine		3	Death
		10	Fungal sepsis	*C. parapsilosis*	Blood; cath. tip		6	Death
		11	Fungal UTI	*C. parapsilosis*	Urine	D-AMB + ECN	12	I.H.D
		12	Fungal UTI	*Candida spp.*	Urine	D-AMB + Azole	25	I.H.D
		13	Fungal sepsis	*C. parapsilosis*	Blood; c.v.c; cath. tip		20	I.H.D
	Birth–27 d	14	Fungal sepsis	*C. albicans*	Blood		10	Death
		15	Septic shock	*C. albicans*	Blood; dialysis cath.		14	I.H.D
		16	Candidemia	*C. albicans*	Blood; cath. tip		5	Death
		17	Fungal endocarditis	*C. parapsilosis*	Urine; blood		28	I.H.D
		18	Fungal UTI	*C. parapsilosis*	Urine		8	I.H.D
		19	Fungal sepsis	*Trichosporon asahii*	Blood; c.v.c		25	Death
		20	Fungal UTI	*C. albicans*	Urine	D-AMB + Azole	7	I.H.D
		21	Fungal sepsis	*C. parapsilosis*	Blood	D-AMB + Azole	18	I.H.D
		22	Candidemia	*C. albicans*	Urine; blood		4	I.H.D
		23	Fungal UTI	*C. parapsilosis*	Urine		9	Death
		24	Fungal UTI	*C. tropicalis*	Urine		11	Death
		25	Fungal UTI	*C. albicans*	Urine	D-AMB + Azole	6	I.H.D
		26	Fungal sepsis	*C. albicans*	Blood		14	I.H.D
		27	Fungal sepsis	*C. guilliermondii*	Blood		29	I.H.D
Non-neonates	28 d–12 mo	28	Disseminated candidiasis	*C. tropicalis*	Urine; blood		21	I.H.D
		29	Candidemia	*C. parapsilosis*	Urine; blood; cath. tip		25	Death
		30	Resistant candidiasis	*C. albicans*	Cath. tip; blood	D-AMB + ECN	31	I.H.D
		31	Candidemia	*C. parapsilosis*	Blood	D-AMB + Azole	12	I.H.D
		32	Candidemia	*C. parapsilosis*	Blood; c.v.c; cath. tip	D-AMB + Azole	20	I.H.D
		33	Fungal sepsis	*C. albicans*	Blood	D-AMB + ECN	8	I.H.D
		34	Fungal sepsis	*C. tropicalis*	c.v.c; blood; cath. tip	D-AMB + ECN	13	Death
		35	Candidemia	*C. albicans*	Blood; implemented cath.		27	I.H.D

Table 2 (continued)

Pediatric age groups[a]	Patient	Medical diagnosis of proven fungal infection[b]	Etiologic agent	Isolated sample[c]	Combined therapy	D-AMB real use (d)	Outcome
	36	Candidemia	*C. pelliculosa*	Cath. tip; blood		14	I.H.D
	37	Fungal sepsis	*C. albicans*	Blood		20	I.H.D
13 mo–2 y	38	Candidemia	*C. parapsilosis*	Blood		15	I.H.D
	39	Fungal sepsis	*C. parapsilosis*	C.v.c	D-AMB + Azole	14	I.H.D
	40	Fungal UTI	*C. tropicalis*	Urine		3	Death
	41	Fungal UTI	*C. tropicalis*	Urine		6	I.H.D
3 y–5 y	42	Candidemia	*C. albicans*	Biopsies (not discriminated)	D-AMB + Azole	15	I.H.D
	43	Fungal sepsis	*C. parapsilosis*	Blood		14	Death
	44	Disseminated candidiasis	*C. tropicalis*	Blood	D-AMB + Azole	35	I.H.D
6 y–11 y	45	Fungal septic arthritis	*Acremonium* spp.	Synovial liquid	D-AMB + Azole	21	I.H.D
12 y–18 y	-	-	-	-	-	-	-

C. Candida, *cath* catheter, *c.v.c,* central venous catheter, *d* days, *D-AMB* amphotericin B deoxycholate, *ECN* echinocandin, *I.H.D* improved hospital discharge, *mo* months, *PMA* postmenstrual age, *UTI* urinary tract infection, *wk* weeks, *y* years

[a]Adapted from the National Institute of Child Health and Human Development[30]

[b]Medical diagnosis as described in the reviewed medical records

[c]Sequence of positive samples in laboratory tests over time

as hemoglobin and platelets, also showed significant differences between the neonate and non-neonate groups.

Potentially toxic drugs concomitant with AMB-D were also evaluated. The highest frequency was observed with diuretics (65.3%), followed by vancomycin (61.4 %), mostly in newborns, especially in the preterm group (41.9 %), and toddlers (19.3%). Sixty-one patients were given two or more potentially toxic drugs during AMB-D therapy, following the same characteristics as the groups mentioned above. However, no other significant statistical data on toxicity have been established.

4 Discussion

To date, the discussion of acute AEs related to the infusion and toxicity of AMB-D has primarily focused on neonates, with an overall worse scenario in older children. We found an unfavorable impact of the conventional formulation from 13 months of age onwards, suggesting that this age group is a turning point for a greater chance of AEs. To the best of our knowledge, this is the first observational study based on an age-stratification strategy and included a significant number of patients, totaling 127.

Previously published data suggested that differences in AMB-D toxicity in children arise from the amount administered and should not be based on available toxicity data in adults [41]. Indeed, there is still no clear understanding of at what age the benefit of the drug could overcome the risk of its use, or vice versa, or how to identify the age to foresee possible harm caused by the drug in the pediatric population [17]. Age is considered a potential confounder of many associations, as it is often associated with exposure and conditions in different situations. However, it can be controlled through pairing or stratification [42].

It is challenging to compare our proposal with studies that did not follow the same methodology. Yet, many of the studies shared their findings on toxicity in general pediatric patients (Table 4). Significant events related to drug exposure may not be assessed when studies encompass broad age groups, given the variations in a child's developmental physiological and pharmacological stages. A consensus has accepted that among neonates, the drug is not capable of severe target organ toxicity and has fewer AEs than in older children, implying a limit for safe use from the day of birth to approximately 28–30 days of age [33, 41, 44].

In the neonatal population, IFD is often a cause of death, especially in those with very low birth weight who require more invasive support, and multiple and prolonged courses

A)

Age Groups (n)	Occurrence of infusion-related side effects			Turning point	Occurence, by Group		Fisher's exact test
	Yes	No	% (Yes)		Yes	No	
< 37 weeks PMA (45)	0	45	0.0%	Group A (up to 12 months)	0	97	p-value: **0.0027***
birth - 27 days (27)	0	27	0.0%				
28 days - 12 months (25)	0	25	0.0%				
13 months - 2 years (9)	1	8	11.1%	Group B (13 months or more)	4	26	CI 95%, to p-value: [0 ; 0.4395]
3 - 5 years (7)	1	6	14.3%				
6 - 11 years (8)	1	7	12.5%				
12 - 18 years (6)	1	5	16.7%				

B)

Age Groups (n)	Laboratory parameters adequacy			Turning point	Inadequacy, by Group	Chi-squared test
	Inadequate	Adequate	% (Inadequate)		% Inadequacy	
< 37 weeks PMA (409)	160	249	39.1%	Group A (up to 12 months)	37.8%	p-value: **0.0467***
birth - 27 days (251)	93	158	37.1%			
28 days - 12 months (228)	83	145	36.4%			
13 months - 2 years (91)	39	52	42.9%	Group B (13 months or more)	44.3%	Diference: 6.5% CI 95%, to difference: [0.1% ; 13.0%]
3 - 5 years (70)	27	43	38.6%			
6 - 11 years (82)	36	46	43.9%			
12 - 18 years (57)	31	26	54.4%			

Chart 1 Searching for a turning point after amphotericin B deoxycholate exposure, based on age stratification according to the National Institute of Child Health and Human Development [30]. **A** Percentage of occurrence of infusion-related side effects. **B** Comparison of proportion of laboratory parameters from the reference values of each pediatric age group. *Significant results considering 5% significance. *IC 95%* confidence interval, *PMA* postmenstrual age

of antimicrobial agents. In addition, it is associated with significant morbidity, damage to target organs, and compromised neurological development. In this context, AMB-D has been extensively used to treat neonatal IFD. Retrospective studies published after 2001 concluded that AMB-D delivers a survival rate greater than 75%, with a time to eradication between 6 and 10 days of therapy [23]. Unfortunately, there is a lack of data on older children to confirm the effectiveness and tolerability of the drug.

In our study, 60% of all confirmed diagnoses were neonates with *Candida* spp. accounting for 96.3% of the infections. Gastrointestinal diseases represented the main underlying disease, with extended hospital stays, including some reaching many months or even years of permanence. The survival rate after AMB-D treatment was 66.7%. In the preterm neonatal group, we found a correlation in the length of hospital stay between the subgroups of proven or unproven fungal infection and the mortality rate, indicating longer hospital stays and deaths in the group with proven fungal infection when compared with patients without this diagnosis.

Adverse events of AMB-D in neonates are considerably less common than in older children and adults, resulting from the immaturity of their immune system and low cytokine production [29]. Ages following the neonatal period are poorly evaluated, making it difficult to know how safe the use of AMB-D is in older children. Wilson et al. described a high frequency of chills, fever, and nausea in an age range of 1–17 years [29]. Other authors reported similar symptoms without specifying stages of child development [18, 24]. Therefore, it is difficult to predict the stage at which these symptoms become more frequent in children aged 12 months to 17 years.

Recently, an Australian study appeared to be the first to compare two groups of patients based on age adjusted for "90 days or more" and "less than 90 days" of life. It concluded that the use of AMB-D in younger groups did not determine glomerular toxicity, and acute IRSE only occurred in the group aged > 90 days [18]. However, despite the progress, the heterogeneity in dividing pediatrics into two sample groups prevents the study from drawing more specific conclusions about other parties.

Table 3 Percentage of adequation of each laboratory parameter in the assessment of D-AMB toxicity on target-organ functions

Lab tests	Urea			Creatinine			Potassium		
D-AMB therapy follow-up	Neonates	Non-neonates	*p*-Value	Neonates	Non-neonates	*p*-Value	Neonates	Non-neonates	*p*-Value
% Baseline	58.8	73.5	0.102	77.9	89.6	0.102	52.1	63.8	0.211
% D3	53.6	53.2	0.966	81.8	91.3	0.16	43.5	44.7	0.899
% D7	65	52.4	0.204	90.9	86.1	0.477	50	47.1	0.792
% D14	48.5	31.0	0.164	93.5	89.3	0.567	44.1	26.9	0.174
% EOT	52.2	46.0	0.509	80.6	84.1	0.645	46.8	35.6	0.249
p-value	0.4421	**0.0055*(A)**		0.706	0.4363		0.5435	**0.0072*(B)**	
Lab tests	Hemoglobin			Neutrophils			Platelets		
D-AMB therapy follow-up	Neonates	Non-neonates	*p*-Value	Neonates	Non-neonates	*p*-Value	Neonates	Non-neonates	*p*-Value
% Baseline	68.7	56.6	0.199	46	51.2	0.601	32.8	46.9	0.125
% D3	46.7	56	0.334	46.4	50	0.737	25	48.8	**0.013*(D)**
% D7	60.4	47.2	0.222	53.2	44.4	0.469	36	44.1	0.458
% D14	62.5	40.7	0.098	58.6	65	0.655	53.3	40.9	0.427
% EOT	68.4	46.7	**0.028*(C)**	56	54.8	0.916	38.6	51.3	0.22
p-value	0.9715	0.3308		0.2931	0.7612		0.5028	0.6834	

CI confidence interval, *D-AMB* amphotericin B deoxycholate, *EOT* end of treatment, *Lab* laboratory

The proportion comparison test was defined as the relation between the number of patients who were within the normality standards of a given exam adjusted to the reference values of each age group and the total number of patients who had collected the exam in determined period of D-AMB treatment (baseline, day [D] 3, D7, D14, and EOT). The results are shown in a simplified manner for viewing. The comparison of adequation of the neonates and non-neonates' groups are presented with *p*-values horizontally, at each stage of therapy follow-up; and vertically, analyzing each group itself with *p*-values calculated between baseline and the EOT, reflecting the exposure to D-AMB

(A): *p*-value between baseline and EOT. Difference 27.5% and 95% CI 8.2–44.1

(B): *p*-value between baseline and EOT. Difference 28.2% and 95% CI 7.8–45.6

(C): *p*-value between neonates and non-neonates at EOT. Difference 21.7% and 95% CI 2.5–39.0

(D): *p*-value between neonates and non-neonates at D3. Difference 23.8% and 95% CI 5.0–40.9

Based on our experience with age stratification, it was possible to identify groups that developed acute IRSE, identified as toddlers (aged 13 months–2 years), followed by middle childhood aged (6–11 years), early childhood (aged 3–5 years), and early adolescence (aged 12–18 years). Bringing this added information into clinical practice might be relevant and useful, avoiding a gap in detailed information about the stages of children's development. In addition, prescribing prophylactic premedication to 50% of our patients may also be the reason for better control of IRSE.

The most clinically significant and dose-limiting adverse effect that has long been reported in the literature is nephrotoxicity [18–20, 24, 26–29, 45–52]. Previously, it was associated with increased morbidity, mortality, renal replacement therapy, prolonged hospital stay, higher costs, and important long-term adverse effects in children [43, 53]. Over time, changes in AMB-D administration allowed for better tolerability. For example, sodium intake of 4 mEq/kg/day significantly reduced nephrotoxicity [25].

In our study, nephrotoxicity was tracked through creatinine, potassium, and urea levels, as well as diuresis alterations and edema. We adjusted all reference values for each age group to minimize any bias in patients who already had some compromised functions before receiving AMB-D. For statistical purposes, when analyzing a large group of older children, we found a significant adequation decrease in urea levels at the end of treatment. Specifically, they seem to be the most inadequate from early childhood onwards compared with younger children. Potassium levels also showed a significant decrease in the adequation during AMB-D therapy in older children.

Hepatotoxicity was assessed in only one-third of the patients. Less than 50% of the adequation at baseline was observed for the neonate and non-neonate groups, with no relevant variation during treatment. According to Andrew et al., liver toxicity occurred more frequently with the liposomal formulation than with AMB-D (83% vs 56%). Most of the AEs were of low grade; 35/44 (79.5%) patients had grade I or II alterations for any parameter (bilirubin, alanine transaminase, alkaline phosphatase, and gamma-glutamyl transferase) [18].

Blood disorders, including anemia and thrombocytopenia, have also been reported. However, they were most prominent in patients with previous hematological disarray and tended to decline within the days following therapy [20, 24, 45, 48, 50]. We found a significant difference between groups, with older children achieving a worse scenario of adequation for hemoglobin levels and toddlers indicating a more worrisome onset of a negative impact. Platelets also

Table 4 Overview of previous original studies on D-AMB in the pediatric population

Author	Year/country	Title	Method	Total *n*	Age groups (*n* D-AMB)	Survival/death	Adverse event findings
Wilson et al. [29]	1979/USA	Toxicity of amphotericin B in children with cancer	Retrospective transversal	20	1 y–18 y (20)	NA	Azotemia occurred during 23 of 24 treatment courses Frequent IRSE, anemia, hypokalemia, thrombocytopenia, and neutropenia during D-AMB therapy
Baley et al. [26]	1984/USA	Disseminated fungal infections in very low birth weight infants: therapeutic toxicity	Prospective transversal	10	14 d–4 mo (10) ($\bar{x}$ 28 wk Ga)	4/6	Six out of seven drug toxicity-related infants died Interruption of D-AMB therapy, with reinstitution at a lower dose, showed effectiveness in alleviating anuria
Faix et al. [27]	1984/USA	Systemic *Candida* infections in infants in intensive care nurseries: high incidence of central nervous system involvement	Retrospective transversal	27	11 d–3 mo (12)	12/0	Toxicity is common but usually reversible
Turner et al. [28]	1985/USA	Consequences of candidemia for pediatric patients	Retrospective transversal	45	Premature (8) 3 mo–11 y (3)	3/8	No evidence of nephrotoxicity Two patients had hypokalemia during treatment
Starke et al. [24]	1987/USA	Pharmacokinetics of amphotericin B in infants and children	Prospective transversal	10	17 d (1) 1 mo–8 mo (6) 6 y–15 y (3)	7/3	Most surviving patients had a transient rise, early in therapy, in BUN or Cr levels Other readily identifiable causes of pre-renal azotemia were present and contributed for nephrotoxicity
Baley et al. [19]	1990/USA	Pharmacokinetics, outcome of treatment, and toxic effects of amphotericin B and 5-fluorocytosine in neonates	Prospective transversal	13	< 36 wk Ga (12) 40 wk Ga (1)	8/5	Serum Cr value rise significantly during therapy and fell significantly at the end of therapy ($p < 0.05$) Hypokalemia was always transient and responded readily to additional potassium chloride
Butler et al. [45]	1990/USA	Amphotericin B as a single agent in the treatment of systemic candidiasis in neonates	Retrospective transversal	38	14 d–4 mo (36) ($\bar{x}$ 29 wk Ga)	30/8	The addition of flucytosine is unnecessary and could contribute to potential toxicity without a clear benefit D-AMB was tolerated among neonates but monitoring renal function is mandatory

Table 4 (continued)

Author	Year/country	Title	Method	Total *n*	Age groups (*n* D-AMB)	Survival/death	Adverse event findings
Glick et al. [47]	1993/USA	Neonatal fungemia and amphotericin	Retrospective transversal	36	30 d–6 mo	30/6	No evidence of toxicity from this drug regimen and no apparent treatment failures No changes in BUN and Cr before or during therapy and in total urinary output
Driessen et al. [54]	1996/South Africa	Fluconazole vs. amphotericin B for the treatment of neonatal fungal septicemia: a prospective randomized trial	Clinical	24	30 d–6 mo (11)	6/5	Fluconazole showed fewer side effects than D-AMB D-AMB developed high levels of direct bilirubin, ALP, and GGT Five in the D-AMB group had severe thrombophlebitis that progressed to skin abscesses as local side effects
Kingo et al. [49]	1997/USA	Lack of evidence of amphotericin B toxicity in very low birth weight infants treated for systemic candidiasis	Retrospective transversal	22	< 36 wk Ga–30 d (18)	14/4	Two patients had hypokalemia and two had alterations on renal function including decreased urine output Hypokalemia was transient and resolved without treatment Two patients with increased BUN before therapy had no evidence of renal toxicity during therapy
Fernandez et al. [46]	2000/USA	*Candida* meningitis in neonates: a 10-year review	Retrospective transversal	23	< 35 wk Ga (23)	17/6	Nephrotoxicity and electrolyte imbalance can be diminished by careful fluid management Increased BUN or Cr levels for whom D-AMB dosing was altered, rapid normalization of renal function was observed
Linder et al. [55]	2003/Israel	Treatment of candidemia in premature infants: comparison of three amphotericin B preparations	Retrospective transversal	56	≤ 33.5 wk Ga 30 d–6 mo (34)	29/5	No signs of nephrotoxicity or hepatotoxicity with any formulation Renal function normalized in 94.2% of the surviving infants given the lipidic formulation
Holler et. al [48]	2004/USA	Effects of fluid and electrolyte management on amphotericin B-induced nephrotoxicity among extremely low birth weight infants	Retrospective transversal	25	< 26 wk Ga 14–18 d (25)	23/2	D-AMB combined with adequate hydration and higher sodium intakes of >4 mEq/kg per day may provide effective protection against nephrotoxicity among extreme low birth weight infants

Table 4 (continued)

Author	Year/country	Title	Method	Total *n*	Age groups (*n* D-AMB)	Survival/death	Adverse event findings
Jeon et al. [50]	2007/South Korea	A comparison of Ambisome® to amphotericin B for treatment of systemic candidiasis in very low birth weight infants	Prospective historical control multi-center	46	≤ 27 wk Ga 30 d–6 mo (20)	15/5	Ambisome® had lower renal and hepatic side effects 55% of the D-AMB group had a 50% increase in serum Cr level against 21% from the Ambisome® group. 100% increase in serum ALT level was 25% and 65% for the Ambisome® and D-AMB groups
Le et al. [51]	2009/USA	Nephrotoxicity associated with amphotericin B deoxycholate in neonates	Retrospective transversal	92	≤ 41 wk Ga 30 d–6 mo (92)	88/4	Nephrotoxicity was mostly mild and resolved by the end D-AMB does not appear to be associated with lasting measurable nephrotoxicity in neonates
Turcu et al. [52]	2009/USA	Influence of sodium intake on amphotericin B-induced nephrotoxicity among extremely premature infants	Retrospective historical control cohort	37	< 30 wk Ga–30 d (37)	34/3	Premature' with high sodium intake (4 mEq/kg/day) had less D-AMB-induced nephrotoxicity than the historical control group No association was found between hydration and D-AMB nephrotoxicity
Benjamin et al. [20]	2018/USA	A phase 3 study of micafungin vs. amphotericin B deoxycholate in Infants with invasive candidiasis	Clinical	30	< 27 wk Ga (2) ≥ 27 wk Ga (8)	9/1	The most common treatment-emergent adverse events were anemia and thrombocytopenia Both agents were safe and well tolerated
Andrew et al. [18]	2018/Australia	Adverse effects of amphotericin B in children; a retrospective comparison of conventional and liposomal formulations	Retrospective transversal	115	< 90 d (9) > 90 d (67)	NA	No IRSE occurred in children aged <90 d Differences in adverse effects is not as marked in children as reported in adults

ALP alkaline phosphatase, *ALT* alanine aminotransferase, *BUN* blood urea nitrogen, *Cr* creatinine, *d* days, *D-AMB* amphotericin B deoxycholate, *Ga* gestational age, *GGP* gamma-glutamyl transpeptidase, *IRSE* infusion-related side effects, *mo* months, *n* total number of patients per study, *NA* not available, *n D-AMB* number of patients who received D-AMB per study, $\bar{x}$ mean, *wk* weeks, *y* years

showed a statistically significant difference between the groups at baseline and D3 of AMB-D treatment, although the neonatal preterm was the most affected during treatment.

Diuretics and vancomycin were potentially toxic drugs concomitant with AMB-D, particularly in neonates in the preterm group. However, it was not possible to establish statistically significant data on these associations. All the patients studied by Andrew et al. who reported nephrotoxicity also received one or two concomitant nephrotoxic drugs [18]. However, Baley et al. reported seven cases of acute oliguria in most patients who were not under or previously exposed to other agents, implying that AMB-D alone was responsible for severe renal failure [26]. Studies by Wilson et al., Holler et al., Le et al., and Turcu et al. found no association between renal toxicity and the concomitant use of nephrotoxic drugs (aminoglycosides, gentamicin, tobramycin, vancomycin, indomethacin, and methotrexate) during AMB-D therapy [29, 48, 51, 52].

We achieved our purpose when all parameters were computed together for comparison between the stratified age groups, possibly because they were previously classified individually as adequate and not adequate. A turning point was observed in the 13 months–2 years old group, suggesting a greater chance of target-organ toxicity and acute IRSE occurrence from this age range onwards. We are unaware of any study that has used this methodology to compare observational data.

Limitations of our study include its retrospective design, missing laboratory data, and an unsatisfactory number of patients when stratified by older age. We also tried to minimize the lack of information about AEs, especially in neonates, by checking all records of doctors and nurses during drug prescription/administration; however, we are aware of the possibility of under-reporting and its impact on statistics.

5 Conclusions

Amphotericin B has been widely used to treat IFD. The literature points out that acute IRSE and toxicity in neonates are not as intense and frequent as in older children and adults, allowing the conventional formulation to be considered as first-line therapy for this population. Original research on this topic dates back decades and mainly consists of a single pediatric group or a limited number of patients separating neonates from older children. Our observational study based on age stratification proved essential to accurately elucidate whether potentially toxic drugs could be used safely in the pediatric population. Based on the National Institute of Child Health and Human Development classification, it was possible to find an unfavorable impact of the polyene drug from 13 months of age, suggesting that this range is a turning point for a greater chance of AEs. We encourage other centers to investigate and support this approach in identifying the childhood age that may indicate or better understand when prescribing conventional AMB-D is safer or less harmful in pediatric patients.

Declarations

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Conflict of Interest/Competing Interests FBC, JLB-C, FdAM, APMV, LS, and FdQ-T have no conflicts of interest that are directly relevant to the content of this article.

Ethics Approval Ethical approval was granted by the Ethics Committee of Hospital Pequeno Príncipe with the waiver of the consent form due to the retrospective nature of the study and the fact that the data collection form is part of the routine of care. Ethical approval number: 3,803,746.

Consent to participate Not applicable.

Consent for publication Not applicable.

Availability of data and materials The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Code availability Not applicable.

Authors' Contributions All authors contributed to the study conception and design. Material preparation, data collection, and analysis were also performed by all authors. The first draft of the manuscript was written by FBC and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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