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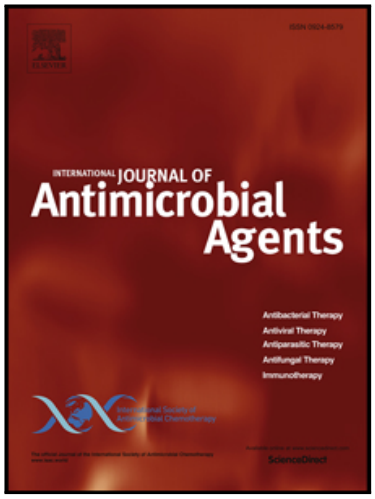

Real-world use of liposomal amphotericin B: a large retrospective, observational, multicenter study

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Highlights

- Large multicenter real-world study (389 patients across 14 Italian centers) describing current use of liposomal amphotericin B (L-AmB) in diverse clinical settings
- L-AmB was frequently used as empirical therapy, in heterogeneous patient populations with substantial comorbidity and clinical severity
- Observed mortality rates (35.7% at 30 days; 61.4% at 90 days) were consistent with the complexity of the treated population
- NYHA and SOFA scores were independent predictors of both short- and long-term mortality, highlighting the role of baseline comorbidity and acute illness severity.
- Treatment discontinuation due to adverse events occurred in 6.7% of patients in this cohort.

Original article

Real-world use of liposomal amphotericin B: a large retrospective, observational, multicenter study

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Running title: Real-world use of liposomal amphotericin B

Abstract

Background: Invasive fungal diseases (IFDs) cause significant morbidity and mortality, and liposomal amphotericin B (L-AmB) is a key treatment option for both IFDs and visceral leishmaniasis. Despite extensive clinical experience, real-world data on L-AmB use across diverse clinical settings remain limited.

Methods: The AMPHO-SITA study was a multicenter, retrospective observational study conducted across 14 Italian centers (September 2020-October 2024). Adult patients receiving at least one dose of L-AmB were included. Baseline demographics, comorbidities, infection and L-AmB treatment characteristics, and outcomes were collected. IFDs were classified according to EORTC/MSGERC and FUNDICU definitions, and visceral leishmaniasis according to international guidelines. Primary descriptive endpoints were patient characteristics, infection characteristics, 30-day mortality, and 90-day mortality. Secondary analyses assessed factors associated with mortality using multivariable Cox regression models.

Results: Among 389 patients (median age 61 years; 37.8% female), 41.3% had hematological malignancies and 52.4% required ICU admission. L-AmB was administered empirically in 53.0%, as targeted therapy in 47.0% of cases, and as combination therapy in 12.4%. Proven or probable IFDs were diagnosed in 52.2% of patients; visceral leishmaniasis was diagnosed and treated in 4.1%. Cumulative 30-day and 90-day mortality for IFD patients were 35.7% (95% confidence interval [CI] 28.5-42.9) and 61.4% (95% CI 51.8-69.6), respectively. NYHA score and SOFA score showed an independent association with both 30-day and 90-day mortality in multivariable models.

Conclusions: L-AmB remains an important antifungal option in real-life, across diverse clinical scenarios and in heterogeneous patient populations with substantial comorbidity and clinical severity, while overall retaining a manageable safety profile.

Keywords: liposomal amphotericin B, invasive fungal disease, candidemia, aspergillosis, leishmaniasis, mortality

Introduction

Invasive fungal diseases (IFDs) remain a major cause of morbidity and mortality worldwide, particularly among immunocompromised and critically ill patients [1]. Their management remains challenging, owing to diagnostic difficulties, the emergence of azole-resistant pathogens, and the growing clinical complexity of affected patient populations [2,3], underscoring the urgent need for broad-spectrum antifungal agents with favorable safety profiles.

Liposomal amphotericin B (L-AmB), a lipid-based formulation of amphotericin B, has emerged as a cornerstone therapy for severe IFDs, being recommended as first-line treatment by international guidelines for mucormycosis [4] and cryptococcosis [5], while representing an alternative or second-line option for invasive aspergillosis and invasive candidiasis, depending on the clinical setting, previous antifungal exposure, susceptibility patterns, and local epidemiology [6]. Beyond IFDs, it is also recommended for the treatment of leishmaniasis [7]. The progressive shift in clinical practice toward lipid formulations of amphotericin B over the past decades has been primarily driven by their improved safety profile, with up to a 50% reduction in nephrotoxicity compared with the deoxycholate formulation [8], while preserving potent fungicidal activity [9], good tissue penetration [10], and a broad spectrum activity [6].

Despite extensive clinical experience with L-AmB, a knowledge gap persists between evidence from controlled trials, where deoxycholate amphotericin B has often served as the standard comparator [11], and characteristics of L-AmB use in real-world practice, particularly in intensive care settings, where patients are more complex and frequently present with multiple comorbidities and polypharmacy [12]. Within this framework, it is also of note that L-AmB has shown significant fluctuations in clinical utilization trends over the last decade [13], underscoring the need for real-life studies to evaluate the current patterns of use and therapeutic positioning of its liposomal formulation.

In this multicenter retrospective observational study, we aimed to describe the real-world use of L-AmB in Italian hospitals, focusing on patient characteristics, clinical indications for treatment, and clinical outcomes.

Materials and methods

Setting and objectives

The AMPHO-SITA multicenter, retrospective study was conducted within the MULTI-SITA project from the Italian Society of Anti-Infective Therapy (SITA), i.e., a platform dedicated to conducting observational studies on invasive fungal and bacterial diseases. The AMPHO-SITA study was conducted across 14 Italian centers. The retrospective study period was from 1 September 2020 to 31 October 2024. Patients receiving at least one dose of L-AmB during the study period were included in the study. L-AmB administration leading to study inclusion was for empirical or targeted treatment; patients receiving L-AmB as antifungal prophylaxis were not included. Exclusion criteria were <18 years of age at time of L-AmB administration, already included due to another previous L-AmB treatment within the study period, enrollment in investigational studies.

The primary objective of the AMPHO-SITA study was to describe the use of L-AmB in Italian hospitals (the respective descriptive, primary endpoints were: characteristics of patients treated with L-AmB, characteristics of infections treated with L-AmB, 30-day mortality, and 90-day mortality). A secondary objective was to assess factors for mortality (time-to-event endpoints: 30-day mortality; 90-day mortality) in L-AmB-treated patients belonging to the following: (*i*) patients with proven IFD according to the European Organization for Research and Treatment of Cancer/ Mycoses Study Group Education and Research Consortium (EORTC/MSGERC) definitions [14] or (*ii*) patients with probable IFD according to the EORTC/MSGERC definitions [14]; or (*iii*) patients with probable rare yeast

infection according to international guidelines [15]; or (*iv*) intensive care unit (ICU) patients with probable IFD according to the invasive FUNgal Diseases in adult patients in intensive care unit (FUNDICU) definitions among ICU patients without EORTC/MSGERC host factors [16]; or (*v*) patients with leishmaniasis according to international guidelines [7].

Definitions and data collected for the study

Baseline characteristics recorded at the time of L-AmB initiation included: age and sex; previous hospital admission within the preceding 6 months; prior ICU stay within 6 months; admission from a long-term care facility; diabetes mellitus; New York Heart Association (NYHA) functional class; chronic kidney disease, defined as an estimated glomerular filtration rate <60 mL/min/1.73 m²; chronic intermittent hemodialysis; autoimmune disorders; and the age-adjusted Charlson Comorbidity Index. Information on prior exposures included antifungal therapy, antibiotic treatment, corticosteroid therapy, chemotherapy and other immunosuppressive treatments within the previous 6 months, as well as major surgery performed within 3 months before L-AmB initiation. Host factors for probable invasive mould disease were identified according to EORTC/MSGERC definitions [14], including prolonged neutropenia (>10 days), hematological malignancy (HM), extended corticosteroid therapy (≥0.3 mg/kg for at least 3 weeks within the previous 60 days), treatment with T-cell immunosuppressive agents (e.g. calcineurin inhibitors, tumor necrosis factor-α inhibitors, lymphocyte-targeting monoclonal antibodies, or immunosuppressive nucleoside analogues within the previous 90 days), B-cell-targeting therapies such as Bruton tyrosine kinase inhibitors, primary severe immunodeficiency, or steroid-refractory grade III–IV graft-versus-host disease involving major organs. For critically ill patients not meeting EORTC/MSGERC host criteria, additional host factors for probable invasive pulmonary aspergillosis were evaluated according to FUNDICU definitions, including influenza, coronavirus disease 2019 (COVID-19), moderate-to-severe chronic obstructive pulmonary disease, uncontrolled HIV

infection, or active solid malignancy [16]. For the purpose of the study, probable infections due to rare yeasts were defined based on the criteria proposed by Chen and colleagues [15], i.e., in presence of all of the following: (i) compatible clinical and/or radiological features suggestive of deep-seated infection; and (ii) mycological evidence, defined as the isolation of a rare yeast species by culture or its detection by molecular methods from a non-sterile site, such as bronchoalveolar lavage fluid (BALF), tissue biopsy, or other clinically relevant specimens [15]. Other clinical data collected as they were at L-AmB initiation included the Sequential Organ Failure Assessment (SOFA) score, presence of septic shock, and evidence of acute kidney injury (AKI), defined according to Kidney Disease: Improving Global Outcomes (KDIGO) criteria. The need for organ support therapies, including invasive mechanical ventilation, continuous renal replacement therapy (CRRT), extracorporeal membrane oxygenation (ECMO) and information on ICU admission during follow-up was also documented. Treatment-related variables included the indication for L-AmB administration (empirical versus targeted therapy), use of combination antifungal therapy, concomitant administration of nephrotoxic agents, timing and duration of antifungal treatment. Data on concomitant infections and antibiotic therapy were also recorded. Mycological, histopathological, and clinical/radiological data required for the classification of invasive fungal infections and visceral leishmaniasis were systematically collected. For fungal infections, cases were classified as proven or probable according to EORTC/MSGERC criteria [14] and, when appropriate, according to FUNDICU definitions [16], while classification visceral leishmaniasis was based on Infectious Diseases Society of America (IDSA)/American Society of Tropical Medicine and Hygiene (ASTMH) guidelines [7]. Final classification was centrally reviewed by investigators at the coordinating center after completion of data collection and prior to statistical analysis. Safety data included the occurrence of adverse events (AEs) considered related to L-AmB, including also serious

AEs (SAEs), if any, as judged by the local investigators based on the collected retrospective data.

Statistical analysis

The characteristics of patients and infections treated with L-AmB were reported by means of proportions (%) and medians with interquartile range (IQR) for categorical and continuous variables, respectively. The 95% confidence interval (CI) was calculated for the estimates of both proportions and median values. In the first case we used the Wilson score method [17]; while for the medians we used percentile bootstrap with 5000 resamples obtained by random sampling with replacement from the original dataset [18]. Cumulative mortality (30-day and 90-day) was calculated through the Kaplan-Meier method in patients with proven/probable IFD, overall and stratified by probable vs. proven IFD. Cumulative mortality was additionally estimated stratifying by candidemia or invasive candidiasis vs. invasive aspergillosis vs. other IFD. Comparisons were performed by means of the log-rank test. Cumulative mortality (30-day and 90-day) was also calculated through the Kaplan-Meier method in patients with visceral leishmaniasis. For all cumulative mortality models, the time of origin was defined as the day of L-AmB initiation. Right-censoring was applied in case of loss to follow-up (e.g., discharge, transfer to another hospital). With regard to the secondary analysis of factors associated with mortality in patients with proven/probable IFD or visceral leishmaniasis, multiple imputation was first performed by chained equations (MICE) according to Rubin's approach [19]. Ten imputed datasets were generated using logistic regression (for categorical variables) and predictive mean matching (for continuous variables). Parallel models were built for assessing factors associated with 30-day mortality and 90-day mortality. For both these endpoints, the potential association of demographic and clinical variables with mortality was initially assessed through univariable Cox regression models. Then, all variables showing a p-value <0.10 in univariable comparisons

were included in an initial multivariable Cox regression model, and further selected for inclusion in the final multivariable Cox regression model (model A for 30-day mortality and model C for 90-day mortality) by means of a stepwise backward procedure. Variables included in models A and C were also included in additional multivariable Cox regression models including centre as shared frailty (model B for 30-day mortality and model D for 90-day mortality) [20]. The analyses were conducted using SAS software (version 9.4, SAS Institute Inc., Cary, NC, USA).

Ethics

The MULTI-SITA project was approved by the ethics committee of the coordinating centre (Liguria Region Ethics Committee, registry number 390/2020). The amendment authorizing the conduct of the AMPHO-SITA study within the MULTI-SITA project was approved by the Liguria Region Ethics Committee on 17 March 2025. The other participating centres followed the local ethical committees requirements. Collection of an informed consent specific for the present study was waived due to the retrospective nature of the study.

Results

Overall, 389 patients who received L-AmB for any indication were included in the study (supplementary figure S1). Their median age was 61 years (IQR 51–69) and 37.8% were female (147/389). As shown in Table 1, 41.3% of patients had hematological malignancies, 16.3% were hematopoietic cell transplantation recipients, 15.8% had solid tumours, and 8.8% were solid organ transplant recipients. Prior exposure to antifungal therapy was common (46.1%), particularly to azoles (26.8%) and to echinocandins (25.3%). Overall, 78/389 patients (20.1%) initiated L-AmB treatment in the ICU, with an additional 126/389 patients required ICU admission at some point during the follow-up (33.0%).

L-AmB was administered empirically in 53.0% of cases (206/389), and as targeted treatment in 47.0% (183/389). L-AmB was administered in combination with other antifungals in 12.4% of cases (48/385), mostly with an echinocandin (25/48, 52.1%). As shown in Table 2, 144/389 patients (37.0%) had proven IFD while 59/389 (15.2%) had probable IFD. L-AmB was administered for the treatment of visceral leishmaniasis in 16/389 patients (4.1%). Among patients who received empirical L-AmB, 36/206, 17.5% were subsequently diagnosed with proven IFD and 1 case (0.5%) with visceral leishmaniasis, while 39/206 (18.9%) were later found to have probable IFD. Overall, considering both patients receiving targeted L-AmB and patients receiving empirical L-AmB with subsequent IFD diagnosis, the most frequent proven IFD and probable IFD were candidemia [83/389, 21.3%, predominantly caused by *Candida parapsilosis* (43/83, 51.8%)] and invasive aspergillosis [52/389, 13.4%, with *Aspergillus fumigatus* being the most frequently identified species (18/52, 34.6%)], respectively. Detailed characteristics of proven/probable IFD, including available microbiological data, and visceral leishmaniasis treated with L-AmB in the present cohort are reported in Table 2 and supplementary Table S1-S2.

Among proven IFDs, candidemia was the most frequent diagnosis (83/144, 57.6%), predominantly caused by *Candida parapsilosis* (43/83, 51.8%) and *Candida albicans* (15/83, 18.1%). Invasive aspergillosis accounted for 34/144 (23.6%) cases, with *Aspergillus fumigatus* being the most frequently identified species. Further species-level details, including other mould infections, are provided in Supplementary Table S1."

Cumulative mortality (30-day and 90-day) in patients with proven/probable IFD treated with L-AmB, overall and stratified according to proven vs. probable IFD and type of IFD is displayed graphically in figure 1A (overall), in figure 1B (stratified by proven vs. probable IFD), and in supplementary figure S1 (stratified by IFD type). Cumulative 30-day mortality was 35.7% (95% CI 28.5-42.9) overall, 34.7 (95% CI 26.1-43.5) vs. 37.6% (95% CI 24.8-50.3) in patients with proven IFD vs. probable IFD, respectively (log-rank test, p =

0.528), and 40.9% (95% CI 30.2-51.3) vs. 32.2% (95% CI 19.9-45.1) vs. 26.9% (95% CI 13.1-42.8) in patients with invasive candidiasis vs. invasive aspergillosis vs. other IFD, respectively (log-rank test, p = 0.501). Regarding cumulative 90-day mortality, it was 61.4% (95% CI 51.8-69.6) overall, 59.1% (95% CI 47.1-69.3) vs. 66.2% (95% CI 48.7-79.0) in patients with proven IFD vs. probable IFD, respectively (log-rank test, p = 0.225), and 64.7% (95% CI 49.8-76.2) vs. 62.3% (95% CI 44.4-75.9) vs. 50.8% (95% CI 28.0-69.8) in patients with invasive candidiasis vs. invasive aspergillosis vs. other IFD, respectively (log-rank test, p = 0.525). The cumulative 30-day mortality and 90-day mortality were 6.3% (95% CI 0.4-25.5) and 37.5% (95% CI 0.5-83.3) in patients with visceral leishmaniasis.

The results of univariable and multivariable analyses of factors associated with 30-day mortality in patients with probable/proven IFD or visceral leishmaniasis are displayed in Table 3. In the final multivariable model (model A), female sex (hazard ratio [HR] 1.89, 95% CI 1.14-3.12, p = 0.013), New York Heart Association (NYHA) score (HR 1.52, 95% CI 1.11-2.08, p = 0.009), and SOFA score (HR 1.21, 95% CI 1.15-1.28, p <0.001) retained an independent association with 30-day mortality. Regarding the direction of effects in model B, including also centre as shared frailty, they were consistent with those of model A (Table 3). The results of univariable and multivariable analyses of factors associated with 90-day mortality in patients with probable/proven IFD or leishmaniasis are displayed in supplementary table S3. In the final multivariable model (model C), NYHA score (HR 1.51, 95% CI 1.18-1.95, p = 0.001), SOFA score (HR 1.16, 95% CI 1.10-1.24, p <0.001), presence of AKI (HR 1.84, 95% CI 1.14-2.99, p = 0.013), CRRT (HR 0.49, 95% CI 0.24-0.99, p = 0.045) and ECMO (HR 2.88, 95% CI 1.14-7.27, p = 0.026) retained and independent association with 90-day mortality. Direction of effects in model D, including also centre as shared frailty, were consistent with those of model C (supplementary table S3).

In the entire study cohort, the median duration of L-AmB treatment was 13 days (IQR, 6–23). L-AmB treatment was discontinued due to possible drug-related AEs reported by

investigators in 24/389 patients (6.7%), for the following reasons: nephrotoxicity (n = 18), electrolyte abnormalities (n = 4), lumbar pain (n =1) and hepatotoxicity (n = 1).

Discussion

In this large multicenter, real-life, observational study conducted across Italian hospitals, L-AmB was mostly used for the treatment of proven or probable IFD (52.2%), while a much smaller but non-negligible proportion of patients received L-AmB for visceral leishmaniasis (4.1%), confirming its role in diverse clinical settings [6]. Notably, as many as 53.0% of patients started L-Amb as empirical therapy. To our knowledge, this is the largest real-world study describing the use of L-AmB across a broad range of clinical indications. Besides the various clinical indications, also the population treated with L-AmB in our cohort was highly heterogeneous and complex, with a substantial burden of comorbidities, a predominance of immunocompromised patients, and more than half of patients requiring ICU admission during follow-up. Possible drug-related AEs were reported in a minority of cases (6.7%).

The frequent use of L-AmB as empirical therapy in our cohort likely reflects the severity of illness in this population, characterized by high baseline SOFA scores, septic shock in 20.9% of patients, and a high rate of prior antifungal exposure (46.1%), mostly classes other than polyenes. Considering that the most common documented infections were invasive candidiasis and invasive aspergillosis, the use of L-AmB in our cohort is consistent with its role as a first-line alternative or a second-line option in selected scenarios, as in case suspected antifungal resistance, prior azole exposure, or refractory disease [21–24]. Of the 206 who initiated L-AmB empirically, only 37 (18%) were subsequently diagnosed with a proven IFD or visceral leishmaniasis and notably, 39 (18.9%) were classified as having a probable IFD, supporting the presence of a clinical suspicion of IFD despite the absence of definitive microbiological documentation fulfilling criteria for proven infection. This diagnostic uncertainty may have contributed to the choice of a broad-spectrum

antifungal agent with good tissue penetration such as L-AmB [12,25,26]. Moreover, the role of L-AmB in acute settings, such as septic shock, was previously evaluated in a large observational study of 141 patients, where early administration was associated with improved outcomes compared with delayed treatment, highlighting its potential benefit for timely empirical use [27]. Beyond the empirical setting, a substantial proportion of patients (47.0%) received L-AmB as targeted therapy. Although L-AmB does not generally represent a first-line option for some of the documented IFDs, the clinical heterogeneity of our population, together with the different microbiological scenarios encountered, may have influenced its use as targeted treatment. However, the specific rationale for antifungal selection at the individual patient level was not captured due to the retrospective nature of the study.

Cumulative mortality in our study population was high and comparable to that reported in previous IFD cohorts [28,29]. In multivariable analyses, SOFA score, reflecting severity of acute conditions, and NYHA score, reflecting chronic heart function impairment, were independent predictors of both 30-day and 90-day mortality, in line with the expected unfavorable prognostic effect of both severe acute presentation and chronic diseases in patients with IFD [30–32]. Conversely, factors identified as independent predictors of either short- or long-term mortality require further exploration. For example, female sex, which was associated with 30-day but not 90-day mortality. This finding should be interpreted cautiously and warrants further investigation in dedicated studies.

In the overall study population, the median duration of L-AmB treatment was only 13 days despite a high frequency of IFD like invasive aspergillosis requiring longer treatment. This finding likely reflects the frequent use of the drug as empirical therapy in our cohort, but possibly also switch to oral formulations of other antifungal classes. Although detailed information on subsequent antifungal de-escalation or step-down therapy was available in our retrospective cohort, this interpretation is consistent with recent reports. In one study

evaluating L-AmB as initial therapy for invasive aspergillosis in 105 patients compared to azoles in 296 patients, treatment was changed more frequently than with azoles (63.8% vs 17.2%, P < 0.001), most often due to a planned switch to oral therapy (71.6%), and less commonly because of toxicity (10.4%) [33]. In our cohort, discontinuations due to investigator-reported drug-related AEs were uncommon, especially if considering the high burden of baseline comorbidities and the frequent concomitant use of nephrotoxic agents, although the relatively short median duration of L-AmB exposure may have contributed to this finding. Overall, these results are in line with the previously reported good tolerability profile of L-AmB in real-life settings, also in comparison with other lipid formulations [34].

This study has some limitations that should be acknowledged. First, its retrospective design may have introduced some information bias, also involving detailed safety data. Second, we were unable to systematically capture retrospectively the specific clinical rationale for selecting L-AmB over alternative antifungal agents, both as empirical and targeted therapy, as well as detailed information on antifungal dosing and subsequent de-escalation, was not systematically captured. Third, while the fact that the cohort was heterogeneous (i.e., patients from different clinical settings) and included different indications for L-AmB and treatment strategies is a strength for the primary descriptive endpoints of clearly depicting how L-AmB is used in real-life, but it is also limitation for the secondary analysis of identifying predictors of mortality, that could not be reliably tailored to specific IFDs. Fourth, microbiological data were not uniformly available across centers, and also susceptibility tests were performed according to local protocols, precluding a more in-depth assessment of epidemiological data regarding antifungal resistance patterns. Finally, our findings should be interpreted within the context of a healthcare system where access to L-AmB is relatively high compared with many other regions of the world.

In conclusion, the present large multicenter real-world study provides contemporary data on how L-AmB is used in Italian hospitals, where it remains an important antifungal

option across diverse clinical scenarios and heterogeneous patient populations with substantial comorbidity and clinical severity. Despite being used in such complex scenarios, our results support the manageable safety profile of L-AmB in real-world practice.

Transparency declarations

Outside the submitted work, Matteo Bassetti has received funding for scientific advisory boards, travel and speaker honoraria from Cidara, Gilead, Menarini, MSD, Mundipharma, Pfizer and Shionogi. Outside the submitted work, Daniele Roberto Giacobbe reports investigator-initiated grants from Pfizer, Shionogi, Menarini, Advanz Pharma, bioMérieux, Tillotts Pharma, and Gilead Italia, travel support from Pfizer, bioMérieux, and Shionogi, and speaker/advisor fees from Pfizer, bioMérieux, Advanz Pharma, Shionogi, MSD, and Menarini. Outside the submitted work, Claudia Bartalucci reports speaker fees from bioMérieux. Outside the submitted work, Emanuele Pontalil has received funding for scientific advisory boards and travel from Advanz Pharma, Gilead, Innoviva, Mundipharma and Viiv. Outside the submitted work, Erica Franceschini reports travel support from Pfizer and Takeda and speaker fees from MSD. Outside the submitted work, Andrea Cortegiani has received fee for scientific advisory board and/or speaker honoraria from bioMérieux, Gilead, Menarini, Mundipharma, and Pfizer. The remaining authors report no conflicts of interests.

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Table 1. Demographic and clinical characteristics of patients treated with liposomal amphotericin B

Variables[a]	No. of patients (389)	%	95% CI
Demographics			
Age in years, median (IQR)	61 (51-69)		59-63
Female sex	147	37.8	33.1-42.7
Comorbidities and medical history			
Prior hospitalization	208	54.6	49.6-59.5
Prior ICU admission	65	17.2	13.8-21.4
Admission from LTCF	7	1.8	0.9-3.7
Diabetes mellitus	72	18.8	15.2-23.0
NYHA score, median (IQR)	1 (1-2)		1-1
Chronic kidney disease	41	10.7	8.0-14.1
Chronic intermittent hemodialysis	15	3.9	2.4-6.3
Chronic liver disease	32	8.4	6.0-11.6
Autoimmune disease	26	6.8	4.7-9.7
SOT	34	8.8	6.4-12.0
HM[b]	160	41.3	36.6-46.3
HCT	63	16.3	12.9-20.3
Solid tumour	61	15.8	12.5-19.8
HIV	7	1.9	0.9-3.9
COPD	48	12.8	9.8-16.5
Neutropenia (Neutrophil count <500/mm3)	112	28.8	24.5-33.5
Age-adjusted Charlson comorbidity index, median (IQR)	4 (2-5)		3-4
Prior exposition to antifungal therapy	172	46.1	41.1-51.2
Prior azoles	99	26.8	22.5-31.5
Prior fluconazole or itraconazole[c]	30	8.1	5.7-11.3
Prior voriconazole[c]	14	3.8	2.3-6.3
Prior isavuconazole[c]	27	7.3	5.1-10.4
Prior posaconazole[c]	37	10.0	7.3-13.5
Prior echinocandins	94	25.3	21.2-30.0
Prior liposomal amphotericin B	6	1.6	0.8-3.5
Prior antibiotic therapy	275	73.7	69.0-77.9
Prior chemotherapy	123	32.2	27.7-37.0
Prior corticosteroids	121	33.2	28.5-38.1
Prior immunotherapy	52	13.8	10.7-17.6
Prior major surgery	109	28.1	23.9-32.8
Prior fungal colonization	22	6.1	4.1-9.0
Setting of L-AmB prescription			
Onco-hematological ward	132	33.9	29.4-38.8
Internal medicine ward	57	14.7	11.5-18.5
ICU	78	20.1	16.4-24.3
Surgical ward	74	19.0	15.4-23.2
Others	48	12.3	9.4-16.0
Days from hospital admission to L-AmB initiation, median (IQR)	22 (8-48)		19-26
Clinical presentation			
SOFA score, median (IQR)	5 (2-8)		4-5
Presence of septic shock	81	20.9	17.1-25.2
Presence of CVC	347	89.4	86.0-92.1
Presence of AKI	122	31.4	27.0-36.2

CRRT	39	10.1	7.5-13.5
ECMO	11	2.8	1.6-5.0
Characteristics of L-AmB administration			
Empirical therapy	206	53.0	48.0-57.9
Targeted treatment	183	47.0	42.1-52.0
Combination therapy with other antifungals[d]	48	12.4	9.5-16.1
Concomitant infections[e]	220	56.9	51.9-61.7
Concomitant antibiotic therapy	301	77.6	73.2-81.5
Concomitant pharmacological therapy with nephrotoxic agents	43	11.7	8.8-15.3

Abbreviations: AKI, acute kidney injury; COPD, chronic obstructive pulmonary disease; CI, confidence interval; CRRT, continuous renal replacement therapy; CVC: central venous catheter; ECMO, extra- corporeal membrane oxygenation; HCT, haematopoietic stem cell transplantation; HIV, human immunodeficiency virus; HM, hematological malignancies; IQR, interquartile range; NYHA, New York Heart Association; LTCF, long-term care facility; SOT, solid organ transplantation.

[a]Results are presented as no. of patients/total of patients unless otherwise indicated.
[b]Hematological malignancies: acute myeloid leukaemia (n=71), non-Hodgkin lymphoma (n=25), acute lymphoblastic leukaemia (n=19), Others (n=45)
[c]Non-mutually exclusive.
[d]Agents used in combination with liposomal amphotericin B: echinocandins (n=25/48), fluconazole or itraconazole (n=4/48), posaconazole (n=1/48), isavuconazole (n=2/48), voriconazole (n=9/48), others (n=5, of them 4 flucytosine and 1 nystatin), missing data on antifungal type (n=5).
[e]Concomitant infections: bloodstream infections (n=89/389), Pneumonia (n=53/389), Others (n=78/389).

Number of missing values per variable were as follows: Prior hospitalization (n=8/389); Prior ICU admission (n=12/389); Admission from LTCF (n=1/389); Diabetes mellitus (n=5/389); NYHA score (n=1/389); Chronic kidney disease (n=4/389); Chronic intermittent hemodialysis (n=2/389); Chronic liver disease (n=6/389); Autoimmune disease (n=5/389); Solid organ transplantation (n=2/389); Hematological malignancies (n=2/389); Hematopoietic stem cell transplantation (n=2/389); Solid tumour (n=3/389); HIV infection (n=20/389); COPD (n=13/389); Age-adjusted Charlson comorbidity index (n=1/389); Prior exposition to antifungal therapy (n=16/389); Prior azoles (n=19/389); Prior fluconazole or itraconazole (n=19/389); Prior voriconazole (n=19/389); Prior isavuconazole (n=19/389); Prior posaconazole (n=19/389); Prior Echinocandins (n=18/389); Prior liposomal amphotericin B (n=19/389); Prior antibiotic therapy (n=16/389); Prior chemotherapy (n=7/389); Prior corticosteroid therapy (n=24/389); Prior immunotherapy (n=11/389); Prior major surgery (n=1/389); Prior fungal colonization (n=27/389); Days from hospital admission to L-AmB initiation (n=8/389); SOFA score (n=1/389); Presence of septic shock (n=1/389); Presence of CVC (n=1/389); Presence of AKI (n=1/389); CRRT (n=2/389); ECMO (n=2/389); Combination antifungal therapy (n=3/389); Concomitant infections (n=2/389); Concomitant antibiotic therapy (n=1/389); Concomitant nephrotoxic drugs (n=20/389).

Table 2. IFD diagnosed according to EORTC/MSGERC 2020 or FUNDICU criteria and Leishmaniasis according to international guidelines, leading to liposomal amphotericin B prescription

Disease	No. of patients N (%)[a]	Microbiological criteria for diagnosis[b]	Host factors/radiological criteria (reported for probable IFI)
Proven infection	**144[c]**		
Candidemia	83 (57.6)	Causative agents identified on blood culture (n=83); molecular detection on blood (n=1)	-
Deep seated candidiasis	11 (7.6)	Causative agents identified on CSF culture (n=3); histology on renal biopsy confirmed by culture (n=2); liver biopsy confirmed by PCR (n=1); endo-vascular (n=1), bone (n=2) biopsy culture; intraocular (n=1), intrabdominal aspirate (n=1) culture	-
Rare yeasts fungemia	8 (5.6)	Causative agents identified on blood culture (n=8)	-
Cryptococcosis	6 (4.2)	Causative agents identified by blood culture (n=3); culture on CSF (n=3); histology on skin biopsy confirmed by culture (n=1); CrAg positive on blood and CSF (n=4)	
Other invasive yeast infection	4 (2.8)	Histology on liver biopsy without culture or PCR confirmation (n=1); bone (n=1), hepatic abscess (n=2) biopsy culture	-
Invasive aspergillosis	9 (6.3)	Causative agents identified on CSF/brain abscess culture (n=2); culture and PCR on pleural fluid (n=1); histology on lung biopsy confirmed by PCR (n=3); lung biopsy culture (n=3)	-
Mucormycosis	4 (2.8)	Causative agents identified on histology on rhino-sinusal (n=2), lung (n=1) and cutaneous (n=1) biopsy confirmed by culture	-
Other mould invasive infections	4 (2.8)	Causative agents identified on histology on skin biopsy (n=1) and rhino-sinusal biopsy (n=1) confirmed by culture; histology on lung biopsy without culture or molecular confirmation (n=2)	-
Visceral leishmaniasis	16 (11.1)	Causative agents identified on peripheral blood (n=10) and/or bone marrow aspirate (n=7) by PCR; CSF (n=1), bone marrow (n=1) and abdominal (n=2) biopsy without molecular confirmation	-
Probable infection	**59[d]**		

Pneumocystosis	3 (5.1)	Causative agents identified on BALF by PCR (n=3)	**Host factors:** HM (n=1), HIV infection (n=2), corticosteroids (n=1) **Radiological criteria:** finding consistent with PjP (n=2)
Other invasive yeast infection	2 (3.4)	Causative agents identified on BALF culture (n=2)	**Host factors:** HM (n=1), autoimmune disease (n=1), T-cell immunosuppressants (n=1), corticosteroids (n=1), **Radiological criteria:** finding consistent with pulmonary infection (n=2)
Invasive aspergillosis according to EORTC/MSGERC 2020	43 (72.9)	Causative agent identified by culture on sputum (n=6), tracheal aspirate (n=4), BALF (n=7), rhino-sinusal aspirate (n=1) culture. Serum GM ≥1.0 (n=15); GM on BALF ≥1.0 (n=24)	**Host factors**: HM (n=18); allogenic HCT (n=8); SOT (n=11), T-cell immunosuppressants (n=15), B-cell immunosuppressants (n=4), corticosteroids (n=19), **Radiological criteria**: finding consistent with IPA (n=42), rhino-sinusal infection (n=1)
Additional IPA cases in critically ill patients according to FUNDICU criteria	6 (10.2)	Causative agent identified by culture on BALF (n=2) culture; serum GM >0.5 (n=2); GM on BALF ≥1.0 (n=3)	**Host factors**: COVID-19 (n=3), Moderate/severe COPD (n=1), uncontrolled solid tumours (n=2) **Radiological criteria**: finding consistent with IPA (n=6)
Mucormycosis	3 (5.1)	Causative agent identified BALF (n=1), tracheal aspirate (n=2) culture	**Host factors:** HM (n=1), HCT (n=2), SOT (n=1), T-cell immunosuppressants (n=2), corticosteroids (n=1), **Radiological criteria**: finding consistent with pulmonary mucormycosis (n=3)
Other mould invasive infections	4 (6.8)	Causative agent identified on BALF culture (n=4)	**Host factors**: HM (n=2), SOT (n=2), T-cell immunosuppressants (n=1), corticosteroids (n=4)

			Radiological criteria: finding consistent with mould pulmonary disease (n=4)

Abbreviations: BALF, bronchoalveolar lavage fluid; COPD, chronic obstructive pulmonary disease; COVID-19, coronavirus disease 2019; CrAg, cryptococcal antigen; CSF, cerebrospinal fluid; GM, galactomannan; HCT, hematopoietic cell transplantation; HIV, human immunodeficiency virus; HM, hematological malignancy; IPA, invasive pulmonary aspergillosis; PCR, polymerase chain reaction; PjP, *Pneumocystis jirovecii* pneumonia; SOT, solid organ transplantation.

[a]Individual patients could present concomitant microbiological and radiological criteria and host factors; consequently, counts reported for single variables are not mutually exclusive and may exceed the overall number of patients.

[b]PCR-based microbiological criteria according to EORTC/MSGERC 2020 could not be systematically assessed due to the retrospective design, which precluded evaluation of consecutive positive results. However, a subset of patients (n=28) had a single positive PCR for *Aspergillus* spp. on respiratory samples, including sputum (n=2), tracheal aspirate (n=4), bronchoalveolar lavage fluid (n=19), and pleural fluid (n=1). Five of these cases were excluded from the final classification of proven or probable invasive aspergillosis. Among them, two patients met EORTC/MSGERC host factor criteria (hematological malignancies), and three fulfilled FUNDICU criteria (influenza), although PCR positivity alone is not considered a mycological criterion in the latter. Positive PCR for *Candida* spp. on blood was the sole microbiological finding in 2 patients; however, these cases were not classified as proven candidemia, as molecular assays are not currently included among diagnostic criteria, despite their increasing clinical relevance. Additionally, *Candida* spp. was isolated from peritoneal fluid in 19 cases, of which 14 were not classified as proven or probable IFD. Due to the retrospective design, key clinical details were unavailable, including the sampling method and timing of peritoneal drain placement, precluding classification; these cases were therefore excluded from the analysis.

Further details on the fungal species identified and available antifungal susceptibility data are provided in Supplementary Table S1.

[c]Coinfections in proven diseases: One patient included experienced concomitant candidemia (positive blood culture) ad disseminated cryptococcosis (culture isolation of *Cryptococcus* spp. on skin biopsy with concomitant serum CrAg positive)

[d]Coinfections in probable diseases: One patient experienced concomitant IPA and *Scedosporium* pulmonary infection (culture positive of both pathogens on BALF); one patient experienced concomitant IPA and *Mucor spp.* pulmonary infection (culture positive of both pathogens on BALF); one patient experienced a mould pulmonary infection due to mixed species (*Penicillum* spp and *Fusarium* spp)

Table 3. Univariable and multivariable analysis of factors associated with 30-day mortality in patients with proven/probable IFD or visceral leishmaniasis (203 patients, 63 events)

Variables	Univariable analysis		Model A*		Model B**	
	HR (95% CI)	p-value	aHR (95% CI)	p-value	aHR (95% CI)	p-value
Age in years	1.01 (0.99-1.03)	0.1671				
Female sex	1.64 (1.00-2.71)	0.0516	1.89 (1.14-3.12)	**0.0132**	1.77 (1.06-2.95)	**0.0281**
Prior hospitalization	1.07 (0.65-1.76)	0.7988				
Prior ICU admission	0.90 (0.48-1.69)	0.7511				
Admission from RSA	1.03 (0.14-7.46)	0.9733				
Diabetes mellitus	0.79 (0.41-1.52)	0.4819				
NYHA score	1.31 (0.98-1.75)	0.0724	1.52 (1.11-2.08)	**0.0093**	1.43 (1.02-2.02)	**0.0399**
Chronic kidney disease	0.95 (0.45-1.98)	0.8814				
Chronic liver disease	0.71 (0.27-1.87)	0.4832				
Autoimmune disease	1.85 (0.84-4.05)	0.1264				
SOT	0.40 (0.15-1.10)	0.0755				
Hematological malignancy	1.29 (0.77-2.15)	0.3370				
HSCT	1.02 (0.49-2.15)	0.9516				
Solid tumour	0.91 (0.49-1.71)	0.7695				
HIV	0.85 (0.21-3.47)	0.8193				
COPD	1.05 (0.50-2.20)	0.9073				

Neutropenia	1.28 (0.72-2.28)	0.4075				
Age-adjusted Charlson comorbidity index	1.03 (0.93-1.14)	0.5494				
Intermittent hemodialysis	1.28 (0.40-4.09)	0.6727				
Prior exposition to antifungal therapy	0.77 (0.47-1.27)	0.3030				
Prior fluconazole or itraconazole	0.85 (0.34-2.12)	0.7280				
Prior voriconazole	1.82 (0.57-5.84)	0.3138				
Prior isavuconazole	1.30 (0.59-2.85)	0.5165				
Prior posaconazole	0.32 (0.04-2.28)	0.2534				
Prior echinocandins	0.85 (0.50-1.44)	0.5361				
Prior liposomal amphotericin B	1.60 (0.50-5.12)	0.4248				
Prior antibiotic therapy	0.67 (0.40-1.14)	0.1393				
Prior chemotherapy	1.57 (0.91-2.71)	0.1069				
Prior corticosteroids	1.52 (0.92-2.53)	0.1036				
Prior immunotherapy	1.25 (0.63-2.45)	0.5233				
Prior major surgery	0.74 (0.42-1.29)	0.2846				
Prior fungal colonization	0.81 (0.26-2.52)	0.7198				
Type of ward		0.1799				
Onco-hematological ward	(Ref.)					
Internal medicine ward	0.61 (0.28-1.36)	0.2292				

ICU	0.98 (0.52-1.88)	0.9598				
Surgical ward	0.50 (0.23-1.08)	0.0764				
Others	0.48 (0.20-1.16)	0.1030				
Days from hospital admission to L-AmB initiation	1.00 (1.00-1.01)	0.4624				
SOFA score	1.20 (1.13-1.27)	**<0.0001**	1.21 (1.15-1.28)	**<0.0001**	1.23 (1.15-1.30)	**<0.0001**
Presence of septic shock	3.46 (2.10-5.73)	**<0.0001**				
Presence of CVC	1.94 (0.70-5.33)	0.2010				
Presence of AKI	2.71 (1.65-4.45)	**<0.0001**				
CRRT	1.94 (1.03-3.63)	**0.0398**				
ECMO	3.82 (1.64-8.87)	**0.0019**				
Empirical therapy (vs. Targeted)	1.20 (0.73-1.98)	0.4747				
Combination therapy with other antifungals	1.25 (0.69-2.26)	0.4658				
Concomitant infections	1.76 (1.04-3.00)	**0.0363**				
Concomitant antibiotic therapy	3.62 (1.65-7.95)	**0.0013**				
Concomitant pharmacological therapy with nephrotoxic agents	1.45 (0.69-3.03)	0.3218				
Proven infection (vs. Probable)	0.79 (0.47-1.33)	0.3653				
Infection type		0.4584				
Candidemia/invasive candidiasis	(Ref.)					

Invasive aspergillosis	0.81 (0.46-1.45)	0.4812				
Leishmaniasis	0.20 (0.03-1.45)	0.1115				
Mucormycosis	0.90 (0.22-3.75)	0.8877				
Other plus coinfections	0.65 (0.30-1.40)	0.2688				

Analyses conducted after multiple imputation, the reported p-values are from Cox regressions (see study methods). A p-value <0.05 was considered statistically significant and reported in bold.

Abbreviations: aHR, adjusted hazard ratio; AKI, acute kidney injury; COPD, chronic obstructive pulmonary disease; CI, confidence interval; CRRT, continuous renal replacement therapy; CVC: central venous catheter; ECMO, extra- corporeal membrane oxygenation; HCT, haematopoietic stem cell transplantation; HIV, human immunodeficiency virus; HM, hematological malignancies; HR, hazard ratio; NYHA, New York Heart Association; LTCF, long-term care facility; SOT, solid organ transplantation.

* Multivariable model with backward stepwise selection. Other variables included in the initial multivariable model were: SOT, Presence of septic shock, Presence of AKI, CRRT, ECMO, Concomitant infections, Concomitant antibiotic therapy.** Including variables retained in model A plus centre as shared frailty.

Figure 1A. Cumulative incidence of all-cause mortality at 30 (panel A) and 90 (panel B) days, with censoring marks, and numbers at risk in patients with proven/probable IFD

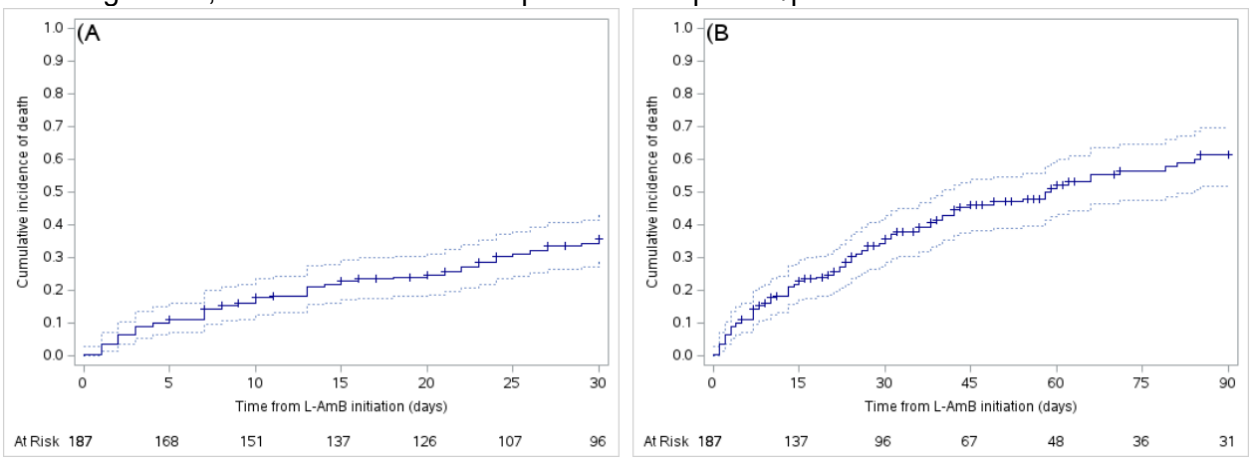


Figure 1A legend. *Panel A.* Cumulative mortality up to day 30 in patients with proven/probable IFD treated with liposomal amphotericin B (L-AmB). *Panel B.* Cumulative mortality up to day 90 in patients with proven/probable IFD treated with liposomal amphotericin B (L-AmB). For both panels, the time of origin was set at the day of L-AmB initiation. Death was the event of interest and right-censoring was applied at the end of follow-up (day 30 in panel A and day 90 in panel B) or previous loss of follow-up. For further details, see methods. IFD, invasive fungal diseases.

Figure 1B. Cumulative incidence of all-cause mortality at 30 (panel A) and 90 (panel B) days, with censoring marks, numbers at risk, and log-rank test in patients with proven vs. probable IFD

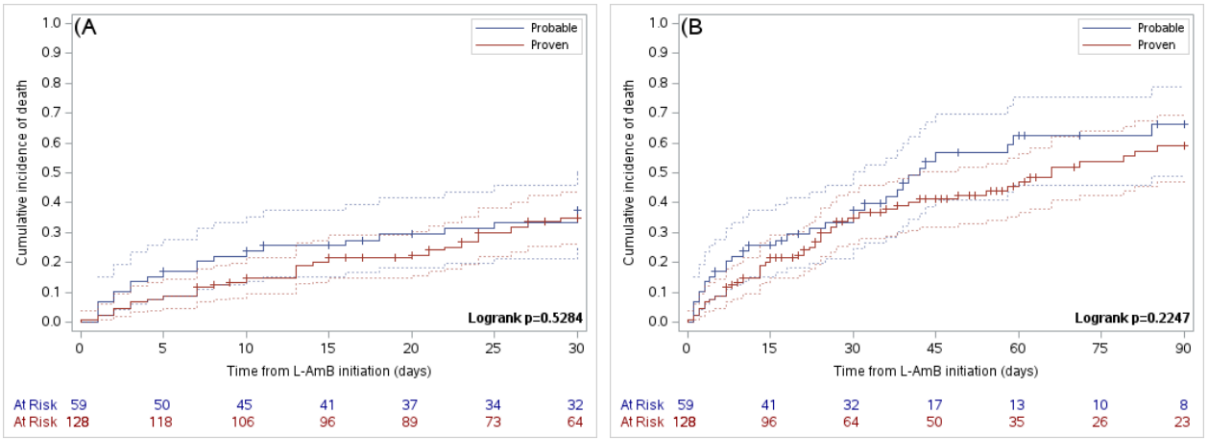
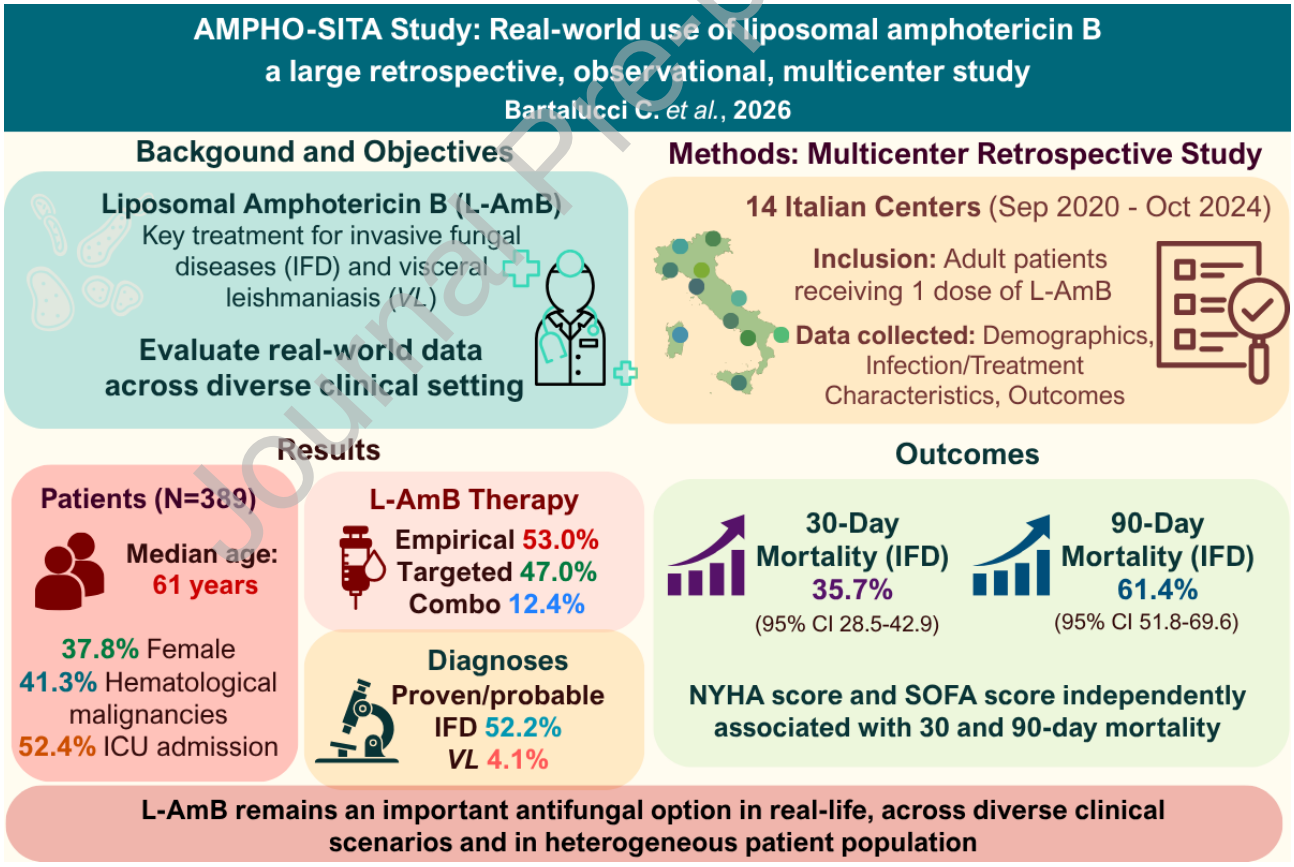


Figure 1B legend. *Panel A.* Cumulative mortality up to day 30 in patients with proven vs. probable IFD treated with liposomal amphotericin B (L-AmB). *Panel B.* Cumulative mortality up to day 90 in patients with proven vs. probable IFD treated with liposomal amphotericin B (L-AmB). For both panels, the time of origin was set at the day of L-AmB initiation. Death was the event of interest and right-censoring was applied at the end of follow-up (day 30 in panel A and day 90 in panel B) or previous loss of follow-up. For further details, see methods. IFD, invasive fungal diseases. Coinfections (n = 3) were classified as proven when both were proven (n= 1) and as probable when both were probable (n = 2).



Graphic abstract