4-24-2020

Liposomal Amphotericin B and Flucytosine Versus Micafungin in Treatment of Candida Endocarditis

Anton Matthew Yanker
Yale Physician Associate Program, anton.yanker@yale.edu

LIPOSOMAL AMPHOTERICIN B AND FLUCYTOSINE VERSUS MICAFUNGIN IN TREATMENT OF CANDIDA ENDOCARDITIS

A Thesis Presented to
The Faculty of the School of Medicine
Yale University

In Candidacy for the degree of
Master of Medical Science

April 2020

Anton Matthew Yanker, PA-SII

Dr. Matthew Grant, MD

Table of Contents

List of Tables

Abstract

Candida endocarditis is a life-threatening, opportunistic fungal infection of the endocardium. The mortality rate of Candida endocarditis is approximately 50% and has been increasing in incidence over recent years. Historically, amphotericin B and flucytosine has been considered the standard of treatment for candida endocarditis, but is limited by safety concerns of amphotericin B, primarily nephrotoxicity and hepatotoxicity. Echinocandins, such as micafungin, have demonstrated similar efficacy in other forms of invasive candidiasis with better safety profiles, but there have been no large-scale or direct comparison trials. In this review, we summarize existing data between micafungin, an echinocandin, vs. amphotericin B and flucytosine in the setting of candida endocarditis. The information extrapolated will determine if micafungin is an appropriate comparator to amphotericin B and flucytosine for the primary treatment of candida endocarditis.

Chapter 1 – Introduction

1.1 Background

Fungal infective endocarditis is now increasing in incidence due to a growing number of at-risk patients, which include intravenous drug users and immunocompromised individuals.[1,2] Worldwide, the prevalence rate of infective endocarditis in 2016 was 6.7 per 100,000 persons, with an incidence rate of 15.8 per 100,000 persons.[3] Approximately 2-4% of these cases are fungal infective endocarditis, primarily through complications of fungaemia, which, in recent years, has increased by 128%.[2,4] The most common causative agent, *Candida albicans*, manifests within three subsets of endocarditis: native valve endocarditis, prosthetic valve endocarditis, and cardiac-device related endocarditis.[1,5] Current guidelines endorsed by the Infectious Diseases Society of America (IDSA) recommend that these subsets of endocarditis be treated with surgical replacement of the infected valves and/or cardiac devices;[1] however, two separate meta-analyses performed by the International Collaboration of Endocarditis-Prospective Cohort Study showed that among a cohort study of seventy cases, mortality rates between medical therapy alone and adjunctive surgical therapy after medical therapy were similar.[4,6] In conjunction with this data provided by the two largest prospective studies of Candida infective endocarditis, not all patients are eligible for surgical intervention due to a myriad of reasons, which elicits an outstanding need for standardized, effective pharmacologic management.[4,6]

In regard to current pharmacologic guidelines, the IDSA recommends that patients are initially started on either lipid formulations of amphotericin B, with or without flucytosine, or a high-dose echinocandin (i.e. caspofungin 150 mg daily,

micafungin 150 mg daily, or anidulafungin 200 mg daily).[4,7] Upon clearance of fungaemia, step-down therapy to an azole is recommended after susceptibility testing, followed by valve and/or cardiac device replacement; however, in the instance of surgical contraindications, fluconazole is also used as the main agent for long-term suppression therapy.[4] Yet, despite following these recommendations, mortality for patients have been estimated to be as high as 80% in published cases, prompting a need for a more comprehensive understanding of the disease and its treatment modalities.[4,8]

Amphotericin B had historically been the standard of care for several opportunistic fungal infections including invasive candidiasis, cryptococcal meningitis, and aspergillosis.[9] It was the first broad antifungal agent developed for treating such diseases through its ability to bind to ergosterol in the fungal cell membrane, leading to the formation of pores, ion leakage, and, ultimately, fungal cell death.[9] Although demonstrating an ability to target most Candida strains, except for *Candida lusitaniae*, amphotericin B not only has been known to produce common and severe toxicities, but also has been ineffective at penetrating into fibrin clots and vegetations associated with Candida endocarditis biofilms.[1,7] Furthermore, antifungal monotherapy, specifically with amphotericin B, without adjunctive surgery has been associated with the poorest patient outcomes when compared to medical antifungal combination therapy with or without adjunctive surgery.[1] However, with liposomal formulations of amphotericin B, which is comprised of hydrogenated soy phosphatidylcholine, distearoyl phosphatidylglycerol, and cholesterol, this agent has shown an ability to maintain its antifungal properties by penetrating the extracellular membrane to target fungal cells, as well as reducing dose-limiting toxicities

associated with amphotericin B, most markedly nephrotoxicity, infusion-related reactions, and hepatotoxicity.[9] Additionally, the liposomal formulation of amphotericin B, compared to amphotericin B deoxycholate, has demonstrated better abilities at addressing the issues associated with the development of biofilms.[9]

Additionally, flucytosine, another antifungal agent, is commonly given in combination with other antifungal agents, most commonly amphotericin B while treating refractory Candida infections, including Candida endocarditis and endophthalmitis.[7] Current literature suggests that positive clinical outcomes are more associated with combination antifungal therapy of amphotericin B and flucytosine compared to antifungal monotherapy of either amphotericin B or flucytosine.[1] In fact, flucytosine monotherapy has been shown to rapidly produce drug-resistant strains of *Candida*.[1] The success from this combination therapy stems from the synergistic antifungal effects provided through the addition of flucytosine, which, has demonstrated broad antifungal activity against most *Candida* species, except for *Candida krusei*.[1,7]

While this combination therapy has shown promising clinical results, the ability of these antifungal agents to penetrate biofilms of *Candida* species other than *Candida albicans* is poor compared to the abilities of the echinocandins.[10] Echinocandins are a newer class of antifungal agents that block the production of 1,3-β-D-glucan, which is an essential component of the fungal cell wall, and have demonstrated their activity against almost all strains of *Candida* species.[7] Each of the three existent echinocandins, micafungin, caspofungin, and anidulafungin, has demonstrated efficacy in the treatment of invasive candidiasis.[7] In addition to several case reports and case series that demonstrate the efficacy of echinocandins in treating

Candida endocarditis, there have been *in vitro* studies that have also shown successful elimination of model Candida biofilms that mimic vegetations residing in the endocardium.[1,6,10] The importance of penetrating these biofilms is clinically significant because the biofilms are associated with drug resistance, which ultimately lead to treatment failure.[10] Through eradication of the biofilms in the heart or affected intravascular devices, it may be possible for Candida endocarditis to be treated without the need for adjuvant surgery, which would possibly allow for preservation of the infected intravascular devices and/or remove the necessity for valve surgery in the setting of Candida endocarditis.[10]

Another benefit of this class of medication is its safety—compared to liposomal amphotericin B, the echinocandins have fewer adverse effects, most notably lacking nephrotoxicity.[7] Since echinocandins primary route of elimination from the body is through nonenzymatic degradation, they do not require dosage adjustments for patients with renal insufficiency or dialysis;[7] however, caspofungin and micafungin undergo minimal hepatic metabolism, with only caspofungin having dosage reduction recommendations for patients with moderate to severe hepatic insufficiency.[7]

Current treatment recommendations supported by the IDSA are based off of a meta-analysis of currently available literature; however, the evidence for their recommendations are derived only from case reports and case series.[1] There have been no prospective or randomized trials performed that compare amphotericin B combination therapy to echinocandin-based therapy in the setting of Candida endocarditis.[1,6] With such scarce clinical data within this realm of invasive fungal disease, a prospective, randomized, double-blind, international multi-centre, non-

inferiority trial is proposed to compare the efficacy and safety between liposomal amphotericin B with flucytosine and high-dose micafungin for the primary treatment of Candida endocarditis. Since amphotericin B with flucytosine is limited in its use through adverse events, micafungin would provide an appropriate alternative therapy in the primary treatment of Candida endocarditis.

1.2 Problem Statement

Current recommendations endorsed by the ISDA for the primary treatment of Candida endocarditis is either liposomal amphotericin B, with or without flucytosine, or a high-dose echinocandin;[7] however, there are currently no clinical data or trials comparing the efficacy of these recommendations.[6] Amphotericin B-based therapy has long been considered the gold standard of treatment for Candida endocarditis due to its historical use and multiple cases of documented success, but remains cumbersome in its use due to its nephrotoxicity, even after the development of its less nephrotoxic liposomal formulation.[6] However, with an attractive safety profile, *in vitro* studies demonstrating success against Candida biofilm models, and comparison studies revealing non-inferiority to the amphotericin B-based therapy in the setting of candidemia, echinocandins show potential of being an alternative primary treatment for Candida endocarditis.[6] The absence of prospective, randomized control trials warrants a study to determine the safety and efficacy between these two treatments in the setting of Candida endocarditis.

1.3 Goals and Objectives

The proposed study aims to determine if micafungin would be an appropriate primary alternative pharmacologic treatment to liposomal amphotericin B with flucytosine in the setting of Candida endocarditis. The goal of this study will be to

evaluate the outcomes between micafungin-based therapy and liposomal amphotericin B-based therapy for the primary treatment of Candida endocarditis by (i) determining non-inferiority of all-cause mortality from initiation of medication to day fifty-six, (ii) measuring time to clearance of candidemia, (iii) assessing safety through drug-related adverse effects, and (iv) quantifying the incidence of relapse following initiation of maintenance therapy.

The primary study outcome, all-cause mortality from initiation of medication to day fifty-six of treatment, will be used to determine non-inferiority between the two proposed pharmacological regimens. The secondary outcomes will consist of measuring time to clearance of candidemia, assessing the safety profile through adverse side effects, and identifying the incidence of Candida endocarditis relapse after the initiation of step-down maintenance therapy. The information collected through the secondary outcomes will allow for a better understanding of optimal dosing for the two antifungal regimens.

1.4 Hypothesis

We hypothesize that micafungin will be non-inferior in all-cause mortality to day fifty-six in the treatment of Candida endocarditis when compared to liposomal amphotericin B with flucytosine.

1.5 Definitions

Infective endocarditis was defined according to the modified Duke criteria.[11] Probable and proven Candida endocarditis were defined according to both the modified Duke Criteria and EORTC/MSG.[5,12] Candida endocarditis-related death was defined as the patient having signs of endocarditis at the time of death, meaning

positive blood cultures for *Candida* species and/or one other major criterion, or three minor criteria per the modified Duke criteria.[11,13]

Table 1. *Definition of Candida Endocarditis per Modified Duke Criteria*

Proven Candida Endocarditis	Probable Candida Endocarditis
Pathologic criteria: 1. *Candida* species demonstrated by culture or histologic examination of a vegetation, embolized vegetation, or intracardiac abscess specimen; or 2. Pathologic lesions—vegetation or intracardiac abscess confirmed by histologic examination showing active endocarditis	1. 1 major and 1 minor criterion; or
Clinical criteria: 1. 2 major criteria; or 2. 1 major criterion and 3 minor criteria; or 3. 5 minor criteria	2. 3 minor criteria

[11,14,15]

Table 2. *Major and Minor Criteria in the Modified Duke Criteria*

Major Criteria	Minor Criteria
1. Persistently positive blood cultures: a. ≥2 positive blood cultures of blood samples drawn >12h apart; or b. ≥3 or >4 separate cultures of blood with first and last samples drawn at least 1h apart	1. Predisposing lesion or IV drug use
2. Evidence of endocardial involvement: a. Echocardiography positive for infective endocarditis b. New valvular regurgitation	2. Fever >38.0°C
	3. Vascular phenomena—major arterial emboli, septic pulmonary infarcts, etc.

	4. Immunologic phenomena—glomerulonephritis, Roth's spots, etc.
	5. Microbiological evidence—positive blood cultures not meeting major criterion or serologic evidence of an active infection with an organism known to cause infective endocarditis
	6. Echocardiographic findings consistent with infective endocarditis, but do not meet major criteria

11,14,15

Probable and proven candidemia were defined according to the criteria set by the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG).[12] Time to clearance of candidemia was defined as having demonstrated clinical stability and clearing *Candida* from the bloodstream with initiation of maintenance azole therapy.[7] Candida endocarditis relapse was defined as a new episode of endocarditis due to the same *Candida* species in patients that completed their assigned IV treatment and achieved time to clearance of candidemia.[13]

Table 3. *Definitions for Invasive Candida Infections*

Category	Definition
Proven Candidemia	Proof of invasive Candida disease by demonstration of Candida-specific elements in diseased tissue of most conditions
Probable Candidemia	Host factor, clinical features, and evidence of Candida are present

12,16

The safety profiles of each study regimen were defined according to the Patient-Reported Outcomes Version of the Common Terminology Criteria for Adverse Events (PRO-CTCAE).[17-19]

Table 4. *Grades from Common Terminology Criteria for Adverse Events*

Grades	Definition
Grade 1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
Grade 2	Moderate; minimal, local, or non-invasive intervention indicated; limiting age-appropriate instrumental activities of daily living
Grade 3	Severe or medically significant, but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living
Grade 4	Life-threatening consequences; urgent indication indicated
Grade 5	Death related to adverse events

17-19

References

1. Steinbach WJ, Perfect JR, Cabell CH, et al. A meta-analysis of medical versus surgical therapy for Candida endocarditis. *J Infect.* 2005;51(3):230-247.
2. Tacke D, Koehler P, Cornely OA. Fungal endocarditis. *Curr Opin Infect Dis.* 2013;26(6):501-507.
3. Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet.* 2017;390(10100):1211-1259.
4. Baddley JW, Benjamin DK, Jr., Patel M, et al. Candida infective endocarditis. *Eur J Clin Microbiol Infect Dis.* 2008;27(7):519-529.
5. Lefort A, Chartier L, Sendid B, et al. Diagnosis, management and outcome of Candida endocarditis. *Clin Microbiol Infect.* 2012;18(4):E99-E109.
6. Arnold CJ, Johnson M, Bayer AS, et al. Candida infective endocarditis: an observational cohort study with a focus on therapy. *Antimicrob Agents Chemother.* 2015;59(4):2365-2373.
7. Pappas PG, Kauffman CA, Andes DR, et al. Clinical Practice Guideline for the Management of Candidiasis: 2016 Update by the Infectious Diseases Society of America. *Clin Infect Dis.* 2016;62(4):e1-50.
8. Benjamin DK, Jr., Miro JM, Hoen B, et al. Candida endocarditis: contemporary cases from the International Collaboration of Infectious Endocarditis Merged Database (ICE-mD). *Scand J Infect Dis.* 2004;36(6-7):453-455.
9. Stone NR, Bicanic T, Salim R, Hope W. Liposomal Amphotericin B (AmBisome((R))): A Review of the Pharmacokinetics, Pharmacodynamics, Clinical Experience and Future Directions. *Drugs.* 2016;76(4):485-500.
10. Kuhn DM, George T, Chandra J, Mukherjee PK, Ghannoum MA. Antifungal susceptibility of Candida biofilms: unique efficacy of amphotericin B lipid formulations and echinocandins. *Antimicrob Agents Chemother.* 2002;46(6):1773-1780.
11. Li JS, Sexton DJ, Mick N, et al. Proposed modifications to the Duke criteria for the diagnosis of infective endocarditis. *Clin Infect Dis.* 2000;30(4):633-638.
12. De Pauw B, Walsh TJ, Donnelly JP, et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. *Clin Infect Dis.* 2008;46(12):1813-1821.
13. Rivoisy C, Vena A, Schaeffer L, et al. Prosthetic Valve Candida spp. Endocarditis: New Insights Into Long-term Prognosis-The ESCAPE Study. *Clin Infect Dis.* 2018;66(6):825-832.
14. Pasha AK, Lee JZ, Low SW, Desai H, Lee KS, Al Mohajer M. Fungal Endocarditis: Update on Diagnosis and Management. *Am J Med.* 2016;129(10):1037-1043.
15. Gould FK, Denning DW, Elliott TS, et al. Guidelines for the diagnosis and antibiotic treatment of endocarditis in adults: a report of the Working Party of

the British Society for Antimicrobial Chemotherapy. *J Antimicrob Chemother.* 2012;67(2):269-289.
16. Alothman AF, Al-Musawi T, Al-Abdely HM, et al. Clinical practice guidelines for the management of invasive Candida infections in adults in the Middle East region: Expert panel recommendations. *Journal of Infection and Public Health.* 2014;7(1):6-19.
17. Kluetz PG, Chingos DT, Basch EM, Mitchell SA. Patient-Reported Outcomes in Cancer Clinical Trials: Measuring Symptomatic Adverse Events With the National Cancer Institute's Patient-Reported Outcomes Version of the Common Terminology Criteria for Adverse Events (PRO-CTCAE). *Am Soc Clin Oncol Educ Book.* 2016;35:67-73.
18. Basch E, Reeve BB, Mitchell SA, et al. Development of the National Cancer Institute's patient-reported outcomes version of the common terminology criteria for adverse events (PRO-CTCAE). *J Natl Cancer Inst.* 2014;106(9).
19. U.S. Department of Health and Human Services NIoH, National Cancer Institute. Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0. 2017.

Chapter 2 – Review of the Literature

2.1 Introduction

A thorough review of the literature was conducted between December 2019 to April 2020 using Ovid (Medline, Embase) and Pubmed. Limitations were placed on publication year with the use of each database to only include studies from the last five years. Publications cited within these articles that did not fall within the time limitations were also considered for inclusion in the current analysis. Additionally, only articles written and/or translated into English were reviewed. Duplicated versions of articles were removed as well. Titles and abstracts were then reviewed to determine relevance to our proposed study.

Articles and cases were included in the current analysis if they met the following inclusion criteria: (i) met criteria for proven or probable Candida endocarditis, (ii) used an echinocandin-based therapy or a liposomal amphotericin B-based therapy, and (iii) contained specific information about the outcome of the patient(s). Articles and cases were excluded if they (i) received concurrent amphotericin B-based and echinocandin-based therapy, (ii) received less than seven days of systemic antifungal therapy, or (iii) received surgical intervention only.

The key terms that were utilized include *Candida*, *endocarditis*, *amphotericin B*, *micafungin*, and *echinocandin*. Of the 141 articles, 38 were fully reviewed to be included within the literature review. The purpose of this literature review is to justify the protocol-specific determinations of our study.

2.2 Overview of Candida Endocarditis

Fungal endocarditis has an alarmingly high mortality ranging from 30-80%, with 53-68% of all cases being *Candida* species, affecting primarily neutropenic and

critically ill, non-neutropenic patients, especially in the nosocomial setting.[1-8] *Candida albicans* has been identified as being the most common species causing candidemia and Candida endocarditis.[2,6-8] Prior to 1980, mortality rates for Candida endocarditis were reported to be as high as 80%, but through advancements of antifungal agents and cardiac surgeries, mortality rates have fallen from 46% to 30% within the last decade.[3]

Risk factors for the development of Candida endocarditis include not only all variables that predispose patients to candidemia—central venous catheters, parenteral nutrition, immunosuppression, and prior surgical procedures, but also ones that predispose patients to endocarditis, such as intravenous drug use/abuse, prosthetic heart valves, valvular abnormalities, transcatheter aortic valve replacement, congenital heart abnormalities, previous endocarditis infections, and pacemaker/cardiac defibrillator placement.[3,4,6,7,9] Out of all of these risk factors, the one that poses the highest threat to the development of Candida endocarditis is the presence of prosthetic heart valves, which is increasing due to the utilization of transcatheter aortic valve replacement and an aging population.[3,9] The valves that are most affected are typically the aortic valve, followed by the mitral valve.[3,5] Based on these risk factors, it is predicted that the incidence of Candida endocarditis will increase due to the increasing number of elderly patients worldwide, increasing number of immunocompromised patients worldwide, and increasing frequency of intravascular device placement.[5,7]

The clinical presentation of Candida endocarditis is a combination of the signs and symptoms associated with candidemia, infectious endocarditis, and coagulopathies associated with endocarditis.[3,4,6,10] The presence of a new or changing

murmur in conjunction with the development of large vegetations with proceeding venous thromboembolic events are cardinal findings of Candida endocarditis.[3,6,10]

Candida endocarditis should be highly suspected in patients with multiple blood cultures positive for *Candida* species and a major venous thromboembolic event; however, it should be noted that not every blood culture will be positive for *Candida* species in the setting of candidemia due to their nature of being slow-growing, obligate aerobes and the sensitivity for detection being between 50-75%.[3,4,6,7] Additionally, the use of transthoracic echocardiography and/or transoesophageal echocardiography is an extremely useful tool in making the diagnosis of Candida endocarditis, especially in occult infections, through their ability to identify vegetations in the heart.[3,6,10] Although both types of echocardiography are sufficient evidence for the diagnosis of Candida endocarditis, only the use of transoesophageal echocardiography can reasonably exclude the diagnosis of Candida endocarditis due to its increased sensitivity and specificity compared to transthoracic echocardiography.[6,10]

In addition to causing significant fungal infections, *C. albicans* is known for being extremely difficult to treat clinically due to its ability to create biofilms.[9,11,12] These biofilms promote resistance to conventional antifungal therapies, at times, leading to clinical failure.[9,11-13] Echinocandins and liposomal amphotericin B have been identified as the most effective therapy against biofilms, but there has been evidence of *in vivo* development of resistance to these novel antifungals.[9,11,12] Azoles, which include fluconazole, voriconazole, itraconazole, and posaconazole, were initially thought to be a safe and effective therapy in the treatment of Candida endocarditis, but data showed that there was a high frequency for failure and relapse, resulting in a discontinuation of this treatment standard.[3,14] Instead, it was discovered

that the most effective use of these medications was after clearance of candidemia and subsequent susceptibility testing of the *Candida* species. [4,6,13,15-17] These medications are better served in the step-down therapy of maintaining remission for Candida endocarditis because of their fungistatic properties.[4,6,13,15-17]

2.3 Current Treatment Guidelines

Guidelines in the treatment of Candida endocarditis written by both the IDSA and European Society of Microbiology and Clinical Infectious Diseases (ESCMID) now recommend either an amphotericin B-based or an echinocandin-based therapy paired with adjunctive surgery.[6,15,18] These recommendations specifically indicate either liposomal amphotericin B (3-5 mg/kg daily IV), with or without flucytosine (25 mg/kg QID PO), or a high-dose echinocandin (caspofungin 150 mg daily IV, micafungin 150 mg daily IV, or anidulafungin 200 mg daily IV).[15] Antifungal therapy should be administered for six to eight weeks, but not less than four weeks.[6]

Due to the high mortality rates and poor prognosis of patients that are treated with medical treatment alone, fungal endocarditis is considered an indication for cardiac surgery and/or valve replacement.[5,6,18] However, these guidelines are based on evidence provided only by case reports, case series, and clinical experience since there are no randomised control trials exploring the most effective treatment strategy for this infectious disease.[18] Additionally, in cases where cardiac surgery is not an option, it is unknown what the optimal primary medical management would be for Candida endocarditis.[2] This gap in the literature has even been noted by the IDSA, acknowledging that their current guidelines are based on low-quality evidence.[15]

To date, there have only been observational studies that compare the efficacy of an amphotericin B-based therapy to an echinocandin-based therapy for Candida

endocarditis.[18] Furthermore, there have been no prospective, randomised trials comparing the efficacy of an amphotericin B-based therapy to an echinocandin-based therapy in the primary treatment of Candida endocarditis.[4,18]

A retrospective study conducted by Steinbach et al. found that survival likelihood increased with the use of surgery and an antifungal therapy compared to antifungal therapy alone.[2] However, a prospective study conducted by Baddley et al. had data showing that mortality rates were similar between patients receiving either surgery and an antifungal therapy (33.3%) or antifungal therapy alone (27.8%) (p=0.26).[19] Additionally, there have been a number of studies that demonstrate treatment success with novel antifungal agents, such as echinocandins, in medical therapy alone.[4] Surgical management of Candida endocarditis has been linked to a high incidence of venous thromboembolic events, such as embolic haemorrhagic or ischaemic stroke.[4] These complications have been involved in 60% of cases.[4]

2.4 Liposomal Amphotericin B

Prior to the development of further classes of antifungal agents, the standard-of-care treatment for life-threatening systemic fungal infections caused by species of *Candida*, *Aspergillus*, and *Fusarium* has been an amphotericin B-based regimen, which is part of a class of antifungals known as polyenes.[4,17,18,20] The polyene structure of amphotericin B forms complexes with ergosterol, which is an essential component of fungal membranes, interrupting the integrity of the cell membrane and causing leakage of essential cellular components.[20,21]

In instances where adjunctive surgery is not an option, patients would receive extended courses of amphotericin B deoxycholate, which has been associated with a high mortality rate and amphotericin B-induced nephrotoxicity.[2,4,14,17,20] Because of

these dose-dependent side effects, a need for formulations that reduce toxicity and transport the agent efficiently to specific locations, while still maintaining its antifungal effects arose.[20] This led to the creation of three additional formulations of amphotericin B—liposomes, emulsions, and nanoparticles.[20]

Most medical centres are currently using lipid-based amphotericin B formulations, more specifically, liposomal amphotericin B, which has a narrower toxicity profile when compared to amphotericin B deoxycholate.[3,14,20] It has been postulated that the reason liposomal amphotericin B is less nephrotoxic is due to the drug's large molecular size and neutral charge, resulting in a more rapid and specific distribution to tissues and organs with large reticuloendothelial composition (i.e. liver, spleen, lungs, lymphatics), which spares the kidneys, allowing increased deliverance of amphotericin B to certain sites of infection.[15,21]

Flucytosine is commonly paired with liposomal amphotericin B because it provides synergistic fungicidal effects, but if used in high doses or for an extended time period, there is a risk for bone marrow toxicity.[3,4] Additionally, similar to liposomal amphotericin B, flucytosine requires dosage adjustments for patients with renal insufficiency.[15]

2.5 Clinical Implications of Echinocandins

With developments in antifungal therapy, a new class of medications, echinocandins, created an increase in options for the treatment of Candida endocarditis.[17,18,22] Echinocandins have shown a decrease in mortality rate compared to other antifungal agents when used in the setting of candidemia and other invasive Candida infections.[23] A panel of experts from the United States of America, Middle East region, and Italy have stated in their recommendations that echinocandins are the

preferred treatment of proven or probable candidemia, especially in the setting of critically ill patients or patients with previous exposure to azoles.[1,10,23] Furthermore, first-line treatment guidelines of Candida endocarditis in United Kingdom have shifted towards high-dose echinocandins instead of liposomal amphotericin B.[10,24]

Echinocandins not only have the benefit of being active against a broad spectrum of *Candida* species, but they also have a low tendency to cause drug-drug interactions and contain a less severe adverse effect profile.[4,9,10,13,14,16,17,22,25] Echinocandins implement their fungicidal capabilities through their ability to inhibit beta-glucan synthesis, thus disrupting the integrity of the fungal cell wall.[13,17,18] With the disruption of cell wall integrity, intracellular osmotic pressure becomes unstable, causing fungal cell lysis.[26] The absence of cell walls in mammalian cells is thought to be a contributing factor to their attractive safety profile.[14,17,26] The improved tolerability of these antifungals allows for prolonged, high-dose treatments when necessary.[4,24]

The first echinocandin approved for the treatment of invasive candidiasis was caspofungin in 2003, followed by micafungin and anidulafungin in 2005 and 2006, respectively.[18,26] Unfortunately, echinocandins cannot be used to treat all types of Candida infections because they do not have the ability to reach therapeutic concentrations for infections of the eyes, central nervous system, and urine.[15]

2.6 Echinocandin Therapy Success in Candidemia

A prospective, double-blind, randomised control trial was conducted in patients with candidemia (n=244) to evaluate non-inferiority between treatment with caspofungin (n=109) or amphotericin B (n=115).[17] Duration of treatment between the two groups was similar (p=0.60) with the caspofungin group having a mean treatment

length of 12.1 days (median 11.0 days) and amphotericin B having a mean treatment length of 11.7 days (median 10.0 days).[17]

In the modified intention-to-treat analysis, it was noted that the 12.7% difference in proportion for treatment efficacy amongst non-neutropenic patients was without statistical significance between the caspofungin group (73.4%) and the amphotericin B group (61.7%) (95.6% CI, -0.7-26.0; p=0.09).[17] Additionally, relapse rates of candidemia were similar between the caspofungin group (6.4%) and the amphotericin B group (7.0%).[17] However, when evaluating the successful outcomes among patients that met prespecified criteria for evaluation, which were patients within the modified intention-to-treat population in conjunction with no concomitant antifungal therapies, no protocol violations, an appropriate evaluation at the end of treatment, and receipt of study treatment for at least five days, the caspofungin group was favoured (80.7%) over the amphotericin B group (64.9%), with a statistically significant difference of 15.4% (95.6% CI, 1.1-29.7; p=0.03).[17]

Through evaluation of safety and tolerability, it was noted that all drug-related adverse events of statistical significance demonstrated favourability of caspofungin.[17] Overall, the caspofungin group had a statistically significant lower proportion of clinical events (28.9%, vs 58.4% in the amphotericin B group; p=0.002).[17] Patients receiving amphotericin B had a higher rate of experiencing chills (26.4%, vs. 5.3% in the caspofungin group; p=0.003) and fever (23.2%, vs. 7.0% in the caspofungin group; p=0.01).[17] Furthermore, amphotericin B had a statistically larger proportion of laboratory abnormalities (54.0%, vs. 24.3% in the caspofungin group; p=0.002), including elevated blood urea nitrogen (15.8%, vs. 1.9% in the caspofungin group; p=0.02), elevated serum creatinine (22.6%, vs. 3.7% in the caspofungin group;

p=0.05), and decreased serum potassium (23.4%, vs. 9.9% in the caspofungin group; p=0.04).[17]

Due to the number of adverse effects experienced by the patients in this study, it should be noted that the amphotericin B group had a larger proportion of patients that experienced toxic effects (16.5%, vs. 2.8% in the caspofungin group; p=0.03), resulting in a change of antifungal therapy.[17]

All-cause mortality rate was similar between the caspofungin group (34.2%) and the amphotericin B group (30.4%) (p=0.53).[17] Similarly, after a post hoc analysis was performed to determine mortality secondary to candidemia, the rates were similar between the caspofungin group (4.4%) and the amphotericin B group (7.2%) (p=0.57).[17]

Based on the results from this study, superiority cannot be established between caspofungin and amphotericin B in the treatment of invasive candidiasis; instead, it can be extrapolated that caspofungin is non-inferior to amphotericin B. However, what can be established is that caspofungin had a significantly lower number of adverse events compared to amphotericin B.

Although these results support the caspofungin as an alternative to amphotericin B in the treatment of invasive Candida infections, there are two factors that limit its generalizability to our study. First, amphotericin B deoxycholate was used, which is known to have a more severe adverse effect profile compared to the liposomal amphotericin B formulation. Secondly, the use of these antifungals was to treat candidemia and not Candida endocarditis, but what must also be considered is that data from a prospective cohort (n=187) showed that at least 4.2% of patients with candidemia have Candida endocarditis.[10]

2.7 Drawbacks to Echinocandins

Although echinocandins may seem like a novel class of antifungals that may operate as a panacea for invasive fungal infections, it should be noted that like other therapies designed to treat infections, resistance may develop. This has been shown through a case report involving a patient in France that demonstrated failure in treatment with caspofungin for Candida endocarditis.[27]

C. glabrata was cultured originally and after susceptibility testing, it was determined that the isolates were susceptible to echinocandins, leading to an initial treatment with caspofungin.[27] After four weeks of treatment with caspofungin, the patient developed a second infection, which was isolated and identified as *C. tropicalis*.[27] Treatment was continued with caspofungin, but on hospital day ninety-three, the patient developed a concurrent candidemia with *C. albicans*.[27] Susceptibility testing of the *C. tropicalis* and *C. albicans* demonstrated resistance to all echinocandins caused by a missense mutation that changed the coding of the beta-glucan synthase, rendering the echinocandins ineffective.[27] Due to this development, the patient had a treatment change from caspofungin to liposomal amphotericin B with flucytosine, followed by surgical resection of the vegetation that ultimately led to a curative outcome to Candida endocarditis and candidemia.[27] This report raises concerns for the use of echinocandins because it was previously thought that only *C. parapsilosis* demonstrated slight resistance to echinocandins.[4,8,9]

2.8 Animal Studies Supporting Echinocandins

A prospective, randomised control trial involving rats (n=18) infected with Candida endocarditis comprised of *C. albicans* compared the efficacy between caspofungin and liposomal amphotericin B.[28] The rats were randomised into three treatment groups based on their intervention—caspofungin, liposomal amphotericin

B, and placebo.[28] Fungal density of the extracted vegetations, the primary outcome, was measured based on average absorbance of blood cultures from the rats and showed a significant statistical difference ($p<0.05$) between the rats that were treated with an antifungal, either caspofungin or liposomal amphotericin B, and the rats that received a placebo.[28]

While the rats that were treated with an antifungal showed no statistically significant differences, the rats treated with placebo only showed a statistically lower absorbance, which was similar to the positive control: 0.878 (placebo) versus 0.865 (positive control).[28] Conversely, the rats treated with caspofungin had an average absorbance (0.230), which was similar to the rats treated with liposomal amphotericin B (0.251).[28]

The lack of statistically significant difference in the two antifungal treatment groups lend to the hypothesis that an echinocandin would be non-inferior to liposomal amphotericin B in the treatment of Candida endocarditis. Furthermore, histological comparison of the fibrinous vegetations were characterized between the rats treated with caspofungin and the rats treated with liposomal amphotericin B.[28] While both antifungal treatment groups had reduction in colony size, the caspofungin treatment group also showed a disruption in the structural integrity of the hyphae, indicating damage to the membranes of the remaining *C. albicans*.[28] Unlike the caspofungin treatment group, the liposomal amphotericin B treatment group did not cause any distortions to the hyphae, which shows that liposomal amphotericin B only affects the membrane permeability of the *Candida* species.[28]

2.9 Caspofungin, Micafungin, and Anidulafungin

Caspofungin has the highest number of indications of the three echinocandins, closely followed by micafungin.[23] While both caspofungin and micafungin are approved for treatment of candidemia or invasive candidiasis in both non-neutropenic and neutropenic adult and paediatric patients, caspofungin has additional indications through its use in salvage therapy for invasive aspergillosis and empirical treatment of febrile neutropenia.[23]

Although caspofungin has been the echinocandin most used in the treatment of Candida endocarditis publications, it is unknown which of the three echinocandins is preferred because there is no evidence directly comparing superiority over one another.[3,10,23] The Clinical and Laboratory Standards Institute (CLSI) M27-A3 and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) were developed to establish standardized methodologies in the testing of susceptibility of fungi against available antifungal agents.[15] Based on minimum inhibitory concentrations, pharmacokinetic data, pharmacodynamic data, and animal data, interpretative breakpoints for susceptibility were established for several antifungal agents against, in decreasing order of incidence, five of the most common *Candida* species—*C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei*.[15] Based on this current data, it is assumed that the three different echinocandins are equivalent in their fungicidal effects.[3,10]

Table 5. *Clinical Breakpoints for Echinocandins Against Common Candida Species*

Candida Species	Echinocandin	Susceptibility	Intermediate	Resistance
C. albicans	Anidulafungin	≤0.25	0.5	≥1
	Caspofungin	≤0.25	0.5	≥1
	Micafungin	≤0.25	0.5	≥1
C. glabrata	Anidulafungin	≤0.12	0.25	≥0.5
	Caspofungin	≤0.12	0.25	≥0.5
	Micafungin	≤0.06	0.12	≥0.25

C. parapsilosis	Anidulafungin Caspofungin Micafungin	≤2 ≤2 ≤2	4 4 4	≥8 ≥8 ≥8
C. tropicalis	Anidulafungin Caspofungin Micafungin	≤0.25 ≤0.25 ≤0.25	0.5 0.5 0.5	≥1 ≥1 ≥1
C. krusei	Anidulafungin Caspofungin Micafungin	≤0.25 ≤0.25 ≤0.25	0.5 0.5 0.5	≥1 ≥1 ≥1

15

Additionally, there have been no studies that directly observe and analyse the differences in several pharmacokinetic and pharmacodynamic factors.[23] However, there has been one retrospective study (n=66) showing no statistically significant differences in mortality rate when treating candidemia or invasive candidiasis with either micafungin (29.1%) or caspofungin (45.9%).[8]

While caspofungin has been the echinocandin most often used in case reports, there is some evidence that micafungin would be a more appropriate alternative. Micafungin has similar indications in its use of candidemia and Candida endocarditis in relation to caspofungin, but it has the added benefit of being indicated in newborns.[23] Additionally, unlike caspofungin, micafungin does not require a loading dose or dosage adjustments for patients with moderate to severe hepatic insufficiency, making it an attractive alternative for patients with multiple comorbidities.[14,15] Additionally, compared to micafungin, caspofungin has been shown to have the most drug-drug interactions of all echinocandins, specifically affecting the pharmacokinetic profiles of cyclosporine, tacrolimus, and rifampin.[26] Unfortunately, over the last two decades, caspofungin and micafungin have been used as both first-line treatment and prophylaxis of candidemia and subsequent infections, leading to the breeding of echinocandin-resistant *Candida* species.[8,27]

2.10 Micafungin in Candidemia

A prospective, double-blind, randomised control trial (n=531) was conducted to determine if micafungin (n=264) would be non-inferior to liposomal amphotericin B (n=267) in the setting of candidemia.[14] The primary outcome that was being investigated between all populations was the response rate of overall treatment success, which was quantified by both a clinical and mycological response at the conclusion of antifungal therapy.[14]

The populations of these groups were further separated into three analysis populations: per-protocol population (n=202 in the micafungin group, vs. n=190 in the liposomal amphotericin B group), intention-to-treat population (n=264 in the micafungin group, vs. n=267 in the liposomal amphotericin B group), and the modified intention-to-treat population (n=247 in the micafungin group, vs. n=247 in the liposomal amphotericin B group).[14] This specific study had chosen to include results from both the intention-to-treat and the modified intention-to-treat populations due to them both being identified as critical information to draw a conclusion of non-inferiority.[14]

When comparing the results from the intention-to-treat population, defined as those that received at least one dose of the study drug, there was no statistically significant difference in treatment success when comparing micafungin (71.6%) to liposomal amphotericin B (68.2%).[14] Additionally, after stratification of neutropenic status, the difference between non-neutropenic patients was 3.9% (CI 95%, -3.9-11.6).[14]

The results of the modified intention-to-treat population, which were those that received at least one dose of the study drug and had confirmed Candida infection,

demonstrated no statistically significant difference in treatment success (4.5%) between micafungin (74.1%) and liposomal amphotericin B (69.6%) (CI 95%, -3.5-12.4).[14] Furthermore, stratification by neutropenic status showed a difference of 4.9% between non-neutropenic patients, which was still not statistically significant (CI 95%, -3.0-12.8).[14]

Finally, the results from the per-protocol population, which was the primary efficacy population, showed similar overall treatment success between the micafungin group (89.6%) and the liposomal amphotericin B group (89.5%), with a difference in proportions of 0.1% (95% CI, -5.9-6.2).[14] After stratification by neutropenic status, the difference in proportion between non-neutropenic patients was 0.7% (95% CI, -5.3-6.7).[14]

Regarding candidemia, similar rates of success were observed between the micafungin group (90.6%) and the liposomal amphotericin B group (90.8%).[14] Mortality rates were similar over the entirety of the study of the intention-to-treat population of micafungin (40%) and liposomal amphotericin B (40%).[14] When examining if the cause of death was directly related to the fungal infection, it was noted that, again, the micafungin group (13%) was similar in comparison to the liposomal amphotericin B group (9%) (p=0.22).[14] When specifically looking at Candida endocarditis, there was a difference in treatment success, but without statistical significance, between the micafungin group (1/1, 100%) and the liposomal amphotericin B group (3/4, 75.0%).[14] More patients would need to be evaluated in this setting to allow for an attainment of statistical significance, as well as generalizability.

When comparing the treatment-related adverse effects, it was noted that, overall, there was not a significant difference in adverse effects between micafungin (43.2%) and liposomal amphotericin B (50.9%) (p=0.082).[14] Additionally, micafungin did not demonstrate any statistically significant differences in serious adverse effects (4.2%, vs. 7.5% in liposomal amphotericin B; p=0.138) or treatment discontinuation (4.9%, vs. 9.0% in liposomal amphotericin B; p=0.087).[14] However, with micafungin, there was a statistically significant lower rate of rigors (0.8%, vs. 6.4% with liposomal amphotericin B group; p=0.0006), increased blood creatinine (1.9%, vs. 6.4% with liposomal amphotericin B group; p=0.015), back pain (0.4%, vs. 4.5% with liposomal amphotericin B group; p=0.003), and infusion-related reactions (17.0%, vs. 28.8% with liposomal amphotericin B group; p=0.001).[14]

Based on the results of treatment success between the three analysis populations, it has been shown that micafungin is non-inferior to liposomal amphotericin B in the primary treatment of candidemia and invasive candidiasis. Inferences based on these two antifungal therapies cannot be made in reference to Candida endocarditis due to the limitation of having only five patients within the study.[14] However, although non-inferior to liposomal amphotericin B, micafungin showed superiority in safety profile compared to liposomal amphotericin B, specifically in renal function and infusion-related events. This data related to safety profile bridges the gap in literature from the study comparing caspofungin and amphotericin B deoxycholate because there is now evidence comparing echinocandins and liposomal amphotericin B.

2.11 Case Reports Using Liposomal Amphotericin B in Candida Endocarditis

While conducting our literature review, we summarized recent case reports that used a liposomal amphotericin B-based treatment regimen in the setting of

Candida endocarditis. All included cases had a diagnosis of Candida endocarditis through multiple, positive blood cultures and vegetations visualized either by transthoracic and/or transoesophageal echocardiography. All cases reported successful treatment of Candida endocarditis with or without use of adjunctive surgery, which should be noted as a potential bias that the researchers have identified. Table 5 contains a brief summary of information regarding each case report.

Table 6. *Case Reports Using Liposomal Amphotericin B*

Reference	Age/ Sex	*Candida* Species	Therapy	Surgery	Outcome
Bauer[7]	64/M	*C. tropicalis*	L-AMB 5 mg/kg/d + 5-FU 25 mg/kg QID for 26 days Dose reduction of L-AMB to 3 mg/kg for rising Cr for 30 days	-	14-month follow up without relapse
Gardiner[29]	56/M	*C. parapsilosis*	Ani 200 mg for 4 days L-AMB 5 mg/kg/d + 5-FU 500 mg QID for 17 days Discontinuation of L-AMB due to renal toxicity Ani 200 mg + fluc 800 mg for 6 weeks	-	Day 149 found dead from heroin overdose Post-mortem examination: no evidence of fungal endocarditis
Checchia[30]	56/M	*C. krusei*	AmB 50 mg/d for 4 weeks Ani 100 mg for 24h	Heart transplantation after amp-deox	6-week follow up without relapse
Pipa[31]	49/M	*C. tropicalis*	L-AMB for 8 weeks	MV and AV mechanical prosthesis during L-ampB therapy	8-week follow up without relapse

5-FU – Flucytosine; AmB – Amphotericin Deoxycholate; Ani – Anidulafungin; AV – Aortic Valve; Cr – Creatinine; Fluc – Fluconazole; L-AMB – Liposomal Amphotericin B; MV – Mitral Valve; QID – quater in die

2.12 Liposomal Amphotericin B with Flucytosine in Candida Endocarditis

In a recent retrospective study (n=46) of Candida endocarditis, thirty-one patients (67%) received antifungal therapy alone.[32] Of the thirty-one patients, seventeen (55%) received liposomal amphotericin B with flucytosine and fourteen (45%) received an echinocandin, either caspofungin or anidulafungin.[32] From the subset of patients that were receiving liposomal amphotericin B with flucytosine, ten developed renal insufficiency, leading to its discontinuation in three patients.[32] Although more patients developed renal injury in this treatment group, it was noted through univariate analysis that compared to all other induction antifungal therapies, liposomal amphotericin B with or without flucytosine (26% survival without flucytosine; 33% survival with flucytosine) was associated with a lower six-month mortality rate.[32] During review of six-month survival rate by multivariate analysis, it was noted that patients receiving liposomal amphotericin B-based monotherapy had a higher survival rate than those receiving an echinocandin-based monotherapy (95% CI, 1.03-838.10; aOR 13.52).[32] Through the evidence provided by this study, the data supports the recommendation of having the primary pharmacological therapy for Candida endocarditis consisting of a liposomal amphotericin B-based therapy, more specifically, one that includes the use of flucytosine.

A meta-analysis totalling 879 cases of reported Candida endocarditis between 1966-2002 reviewed 418 reports.[2] Through the authors' inclusion criteria of definitive Candida endocarditis, 105 reports containing a total of 163 patients were reviewed.[2] In order to reflect current medical practices, cases after 1980 (n=92), which was the decade where echocardiography technology emerged, were summarized with greater

detail by including location of cardiac valve involvement, type of infected valve, and specific treatment details.[2]

These cases were divided based on pharmacological and/or surgical treatment—medical antifungal monotherapy (n=15), medical antifungal combination therapy (n=19), and medical antifungal therapy with adjunctive surgery (n=58).[2] Within the medical antifungal monotherapy group, 53.3% (8/15) had reported successful outcomes.[2] The most commonly used antifungal was amphotericin B (53.3%), which had the second highest reported successful outcome (75.0%).[2] The second most commonly used antifungal was fluconazole (40.0%), which resulted in a successful outcome of 16.7%.[2] Flucytosine monotherapy had the highest reported treatment success (100%); however, it should be noted that only one patient was treated with flucytosine monotherapy.[2]

Within the medical antifungal combination therapy group, 63.2% (12/19) had successful reported outcomes.[2] The most commonly used medical antifungal combination therapy was amphotericin B with flucytosine (73.7%), which had the third highest reported successful outcome (63.2%), following amphotericin B with fluconazole (66.7%) and amphotericin B with rifampin and flucytosine (100%).[2] However, similar to the monotherapy group, it should be noted that the number of patients treated with amphotericin B with fluconazole and amphotericin B with rifampin and flucytosine were comprised of three and one patients, respectively.[2]

With the use of meta-regression analysis techniques and using mortality as the outcome, mortality was highly associated in patients that were treated with antifungal monotherapy (95% CI, 0.39-5.81; Prevalence Odds Ratio (POR) 1.49).[2] The findings from this meta-analysis suggest that when treating Candida endocarditis with medical

therapy alone, combination therapy is preferred to monotherapy alone.[2,4] Additionally, although the medical antifungal combination therapy of amphotericin B with flucytosine had the third highest rate of successful reported outcomes, the two medical antifungal combination therapies with higher reported successful outcomes were not sufficiently powered, making them lack statistical significance.

While this meta-analysis shows a higher rate of treatment success with amphotericin B-based combination therapy, it must be noted that this study did not include the use of any echinocandin-based therapy.

2.13 Case Reports Using Echinocandins in Candida Endocarditis

During our review of recent literature, we summarize case reports that use echinocandins in the setting of Candida endocarditis. The case of the 69-year-old male was given a clinical diagnosis of Candida endocarditis via two major criteria (persistently positive blood cultures and evidence of endocardial involvement) because the transoesophageal echocardiogram only revealed fibrin stranding.[13] This patient's clinical diagnosis was further supported by the presence of three minor criteria (fever above 38.0°C, predisposing lesion, and echocardiographic findings consistent with infective endocarditis, but do not meet major criteria).[13]

All other cases had a diagnosis of Candida endocarditis through multiple, positive blood cultures and transthoracic and/or transoesophageal echocardiography. The majority of case reports resulted in treatment success of Candida endocarditis with and without the use of adjunctive surgery, which should be noted as a potential bias that the researchers have identified. More details are provided on these cases in Table 7.

Table 7. *Case Reports Using Echinocandins*

Reference	Age/ Sex	*Candida* Species	Therapy	Surgery	Outcome
Morioka[9]	80/M	*C. parapsilosis*	L-AMB 3 mg/kg/d for 8 days Discontinuation of L-AMB due to rising Cr Mica 150 mg/d + fluc 200 mg/d for 6 weeks	AV bioprosthesis during mica and fluz	6-month follow up without relapse
Ahuja[13]	69/M	*C. parapsilosis*	L-AMB 5 mg/kg/d Discontinuation of L-AMB due to rising creatinine Mica 150 mg + fluc 400 mg for 12 weeks	-	1-year follow up without relapse
Ahuja[13]	45/M	*C. parapsilosis*	Mica 150 mg + fluc 6 mg/kg for 2 weeks L-AMB 5 mg/kg/d + 5-FU 2500 mg Q6H + fluc 400 mg/d for 1 week Discontinuation of L-AMB due to rising Cr Mica 150 mg/d + fluc 400 mg/d + 5-FU 2500 mg/d followed by an increase in 5-FU to 2500 mg Q8H for 12 weeks	-	5-month follow up without relapse
Kubota[33]	31/F	*C. albicans*	L-AMB 200 mg/d for 4 days Discontinuation of L-AMB due to rising Cr L-AMB 200 mg/d for 14 days Discontinuation of L-AMB due to rising Cr Mica 150 mg/d + fluc 400 mg/d for 54 days	Removal of prosthetic valves during mica therapy	6-week follow up without relapse
Bandyopadhyay[34]	86/M	*C. tropicalis*	Caspo 70mg for 1 day Caspo 50mg for 10 days Fluc for 15 days	-	2-month follow up without relapse
Durante-Mangoni[35]	19/M	*C. albicans*	Caspo + fluc for 8 weeks	PM replacement	3-month follow up without relapse
Glavis-Bloom[36]	70/F	*C. glabrata*	Caspo 70mg for 2 days	-	Deceased day 31

			Caspo 100mg + 5-FU 37.5mg BID Discontinuation of caspo due to hepatotoxicity Mica + 5-FU for 3 weeks		
Glockner[37]	69/F	*C. albicans*	Ani 200mg for 1 day Ani 100mg for 30 days	-	Follow up (no timeframe) without relapse
Jagernauth[38]	54/M	*C. albicans*	Caspo for 3 months	PV/TV replacement	No follow-up
5-FU – Flucytosine; Ani – Anidulafungin; Caspo – Caspofungin; Cr – Creatinine; Fluc – Fluconazole; L-AMB – Liposomal Amphotericin B; Mica – Micafungin; PM – Pacemaker; PV – Pulmonary Valve; TV – Tricuspid Valve					

2.14 Review of Studies to Identify Possible Confounding Variables

Throughout the extensive literature review, it has been noted that there are several confounding variables that have influenced the generalizability of conducting this study. Most of these confounding variables will be attempted to be curbed through the use of a highly specific exclusion and inclusion criteria, as well as stratification of subgroups during statistical analysis.

Although *C. albicans* has historically been identified as the species of *Candida* to most often cause Candida endocarditis and candidemia, the prominent global use of azoles for prophylaxis and treatment has been associated with the epidemiological shift to other *Candida* species.[8,16] Local epidemiology describing the species of *Candida* need to be considered for empiric treatment of invasive Candida infections.[8,16] This will be done by stratification based on recruitment site.

Mortality is directly correlated with delays in both the identification of Candida endocarditis and choice of the most efficacious antifungal agent.[6,16]

Identifying the infectious source is extremely important in the management of Candida endocarditis and candidemia—whenever possible, infected medical devices need to be promptly removed and abscesses need to be drained.[10,14,16] Failure to quickly identify and address these infectious sources is correlated with increased mortality while swift intervention to achieve source control to reduce fungal inoculum is correlated with improved clinical outcomes.[6,10,16]

Only patients diagnosed with proven and probable Candida endocarditis will be included in the study to avoid bias related to the pathogenic characteristics specific to other fungal pathogens.[5] After identification of patients with proven Candida endocarditis, comorbid conditions that have been acknowledged as independent predictors of all-cause mortality will need to be taken into account. Acute heart failure has been recognised as an independent predictor of all-cause mortality, as well as glycaemic control in diabetic patients needing to be optimized.[5,16] Finally, if clinically possible, the use of immunosuppressive and/or antibacterial therapies should be decreased or stopped.[16]

2.15 Review of Relevant Methodology

To our knowledge, all published clinical trials investigating the efficacy and safety of an amphotericin B-based regimen or an echinocandin-based therapy have been through the use of randomised control trials or retrospective analyses.[2,5,8,14,17,18,22,28,32] Based on our goal of determining non-inferiority, our study was designed as a randomised control trial to allow for control of patients and treatment options.

Patients 16 years of age or older were eligible for recruitment into the study.[14] Non-neutropenic patients receiving an azole-based systemic antifungal prophylaxis

for three or more days within the last seven days were ineligible for recruitment into the study.[14] Neutropenic patients, defined as having an absolute neutrophil count less than 500 cells per microlitre, receiving antifungal prophylaxis were eligible for recruitment into the study.[14]

The modified Duke Criteria was developed in aiding the diagnosis of infective endocarditis; however, it was created through data from bacterial endocarditis.[4] There are currently no diagnostic criteria that are specific to the identification of fungal endocarditis.[4] Utilization testing for the Mannan antigen and the anti-mannan antibodies is another useful diagnostic tool due to the combined sensitivity of 83% and specificity of 86% for Candida endocarditis.[4,6] The downfall to this diagnostic test is that empiric treatment with antifungals can lower the levels of the Mannan antigen and anti-mannan antibodies.[4] Based on this information, the modified Duke criteria, positive candidemia cultures, and utilization of the transthoracic and/or transoesophageal echocardiogram will be used to determine Candida endocarditis diagnosis.

While on either the echinocandin-based or liposomal amphotericin B-based therapy, clinical success has been documented in patients that received treatments for six to eight weeks.[6] In the setting of Candida endocarditis, it would be considered inappropriate to treat with either medical therapy for less than four weeks.[6]

In order to determine cessation of candidemia, daily blood cultures must be drawn until sterilisation of the blood is noted.[6,10] Upon clearance of candidemia, the general consensus is to continue antifungal treatment for an additional two weeks, but there is no experimental data supporting this clinical decision.[10]

2.16 Conclusion

The existing evidence provided by a series of case studies, meta-analyses, and randomised control trials have demonstrated promise in the use of micafungin as a non-inferior alternative to liposomal amphotericin B with flucytosine for the treatment of Candida endocarditis. With micafungin providing several instances of successful treatment of Candida endocarditis, as well as a more tolerated side effect profile, this choice of echinocandin is the next logical choice for further investigation in the treatment of this deadly disease. Comparison of all-cause mortality from initiation of the assigned study medication to the end of treatment as the primary outcome, together with measuring time to clearance of candidemia, assessing safety profile through adverse side effects, and identifying relapse of Candida endocarditis after initiation of step-down therapy as secondary outcomes will provide the setting for this study to produce meaningful results.

References

1. Alothman AF, Al-Musawi T, Al-Abdely HM, et al. Clinical practice guidelines for the management of invasive Candida infections in adults in the Middle East region: Expert panel recommendations. *Journal of Infection and Public Health.* 2014;7(1):6-19.
2. Steinbach WJ, Perfect JR, Cabell CH, et al. A meta-analysis of medical versus surgical therapy for Candida endocarditis. *J Infect.* 2005;51(3):230-247.
3. Kauffman CA. Complications of Candidemia in ICU Patients: Endophthalmitis, Osteomyelitis, Endocarditis. *Semin Respir Crit Care Med.* 2015;36(5):641-649.
4. Pasha AK, Lee JZ, Low SW, Desai H, Lee KS, Al Mohajer M. Fungal Endocarditis: Update on Diagnosis and Management. *Am J Med.* 2016;129(10):1037-1043.
5. Siciliano RF, Gualandro DM, Sejas ONE, et al. Outcomes in patients with fungal endocarditis: A multicenter observational cohort study. *Int J Infect Dis.* 2018;77:48-52.
6. Ammannaya GKK, Sripad N. Fungal endocarditis: what do we know in 2019? *Kardiol Pol.* 2019.
7. Bauer BK, Schulze AB, Loher A, Reinke F, Eckardt L. Candida tropicalis defibrillator endocarditis: A case report and review of current literature. *Med Mycol Case Rep.* 2019;25:1-9.
8. Shorr AF, Wu C, Kothari S. Outcomes with micafungin in patients with candidaemia or invasive candidiasis due to Candida glabrata and Candida krusei. *J Antimicrob Chemother.* 2011;66(2):375-380.
9. Morioka H, Tokuda Y, Oshima H, et al. Fungal endocarditis after transcatheter aortic valve replacement (TAVR): Case report and review of literature. *J Infect Chemother.* 2019;25(3):215-217.
10. Mellinghoff SC, Cornely OA, Jung N. Essentials in Candida bloodstream infection. *Infection.* 2018;46(6):897-899.
11. Ramage G, Robertson SN, Williams C. Strength in numbers: Antifungal strategies against fungal biofilms. *International Journal of Antimicrobial Agents.* 2014;43(2):114-120.
12. Agnelli C, Guinea J, Valerio M, Escribano P, Bouza E, Munoz P. Infectious endocarditis caused by Candida glabrata: Evidence of in vivo development of echinocandin resistance. *Revista Espanola de Quimioterapia.* 2019;32(4):395-397.
13. Ahuja T, Fong K, Louie E. Combination antifungal therapy for treatment of Candida parapsilosis prosthetic valve endocarditis and utility of T2Candida Panel(R): A case series. *IDCases.* 2019;15:e00525.
14. Kuse ER, Chetchotisakd P, da Cunha CA, et al. Micafungin versus liposomal amphotericin B for candidaemia and invasive candidosis: a phase III randomised double-blind trial. *Lancet.* 2007;369(9572):1519-1527.
15. Pappas PG, Kauffman CA, Andes DR, et al. Clinical Practice Guideline for the Management of Candidiasis: 2016 Update by the Infectious Diseases Society of America. *Clin Infect Dis.* 2016;62(4):e1-50.
16. Whitney LC, Bicanic T. Treatment principles for Candida and Cryptococcus. *Cold Spring Harb Perspect Med.* 2014;5(6).

17. Mora-Duarte J, Betts R, Rotstein C, et al. Comparison of caspofungin and amphotericin B for invasive candidiasis. *N Engl J Med.* 2002;347(25):2020-2029.
18. Arnold CJ, Johnson M, Bayer AS, et al. Candida infective endocarditis: an observational cohort study with a focus on therapy. *Antimicrob Agents Chemother.* 2015;59(4):2365-2373.
19. Baddley JW, Benjamin DK, Jr., Patel M, et al. Candida infective endocarditis. *Eur J Clin Microbiol Infect Dis.* 2008;27(7):519-529.
20. Lemke A, Kiderlen AF, Kayser O. Amphotericin B. *Appl Microbiol Biotechnol.* 2005;68(2):151-162.
21. Linden PK. Amphotericin B lipid complex for the treatment of invasive fungal infections. *Expert Opin Pharmacother.* 2003;4(11):2099-2110.
22. Reboli AC, Rotstein C, Pappas PG, et al. Anidulafungin versus fluconazole for invasive candidiasis. *N Engl J Med.* 2007;356(24):2472-2482.
23. Scudeller L, Viscoli C, Menichetti F, et al. An Italian consensus for invasive candidiasis management (ITALIC). *Infection.* 2014;42(2):263-279.
24. Tattevin P, Revest M, Lefort A, Michelet C, Lortholary O. Fungal endocarditis: Current challenges. *International Journal of Antimicrobial Agents.* 2014;44(4):290-294.
25. Koehler P, Tacke D, Cornely OA. Our 2014 approach to candidaemia. *Mycoses.* 2014;57(10):581-583.
26. Patil A, Majumdar S. Echinocandins in antifungal pharmacotherapy. *J Pharm Pharmacol.* 2017;69(12):1635-1660.
27. Grosset M, Desnos-Ollivier M, Godet C, Kauffmann-Lacroix C, Cazenave-Roblot F. Recurrent episodes of Candidemia due to Candida glabrata, Candida tropicalis and Candida albicans with acquired echinocandin resistance. *Medical Mycology Case Reports.* 2016;14:20-23.
28. Victorio GB, Bourdon LMB, Benavides LG, et al. Antifungal activity of caspofungin in experimental infective endocarditis caused by Candida albicans. *Mem Inst Oswaldo Cruz.* 2017;112(5):370-375.
29. Gardiner BJ, Slavin MA, Korman TM, Stuart RL. Hampered by historical paradigms - echinocandins and the treatment of Candida endocarditis. *Mycoses.* 2014;57(5):316-319.
30. Checchia TE, Moura LZ, Colatusso C, Veiga S, Fortes JA, Tuon FF. Heart transplantation and Candida endocarditis. *Transpl Infect Dis.* 2016;18(3):483-484.
31. Pipa S, Dias C, Ribeiro J, Gregorio T. Fungal endocarditis of native valves. *BMJ Case Rep.* 2018;11(1).
32. Rivoisy C, Vena A, Schaeffer L, et al. Prosthetic Valve Candida spp. Endocarditis: New Insights Into Long-term Prognosis-The ESCAPE Study. *Clin Infect Dis.* 2018;66(6):825-832.
33. Kubota K, Soma K, Uehara M, et al. Combined Surgical and Medical Therapy for Candida Prosthetic Endocarditis in a Patient with Repaired Tetralogy of Fallot. *Int Heart J.* 2018;59(4):877-880.
34. Bandyopadhyay S, Tiwary PK, Mondal S, Puthran S. Pacemaker lead Candida endocarditis: Is medical treatment possible? *Indian Heart J.* 2015;67 Suppl 3:S100-102.
35. Durante-Mangoni E, Nappi G. Giant endocarditis vegetation on a pace-maker lead. *Intern Emerg Med.* 2010;5(3):251-252.

36. Glavis-Bloom J, Vasher S, Marmor M, et al. Candida and cardiovascular implantable electronic devices: a case of lead and native aortic valve endocarditis and literature review. *Mycoses.* 2015;58(11):637-641.
37. Glockner A. Recurrent candidaemia and pacemaker wire infection with Candida albicans. *Mycoses.* 2011;54 Suppl 4:20-23.
38. Jagernauth S, Patel A, Baig K, De Souza A. Fungal endocarditis of the eustachian valve in carcinoid heart disease: a case report. *J Heart Valve Dis.* 2007;16(6):631-633.

Chapter 3 – Study Methods

3.1 Study Design

We will be performing a prospective, randomized, double-blind, international, multicentre, non-inferiority clinical trial comparing liposomal amphotericin B and flucytosine versus micafungin for the primary treatment of Candida endocarditis. Enrollment will be conducted between January 2021 to June 2022 based on convenience sampling. Study participants will be randomized to the control group (liposomal amphotericin B with flucytosine) or intervention group (micafungin) using a third-party, computerized random number generator. Data collection will be conducted between July 2022 to December 2022.

3.2 Study Population and Sampling

Patients 16 years or older will be eligible if they are considered to meet the criteria of infective endocarditis set by the modified Duke criteria,[1] as well as criteria for probable or proven candidemia.[2] Key inclusion and exclusion criteria will be further discussed in the following sections, with full criteria being found in the appendix. Only patients that meet all inclusion criteria and none of the exclusion criteria will be eligible to participate in the study, which will include a preliminary screening visit that will include, but not be limited to, physical examinations and blood samples. Due to the rarity of Candida endocarditis, convenience sampling will be utilized with a 1:1 allocation to assign subjects to receive either amphotericin B with flucytosine or micafungin.

3.3 Inclusion Criteria

The following inclusion criteria will be disseminated to participating sites to screen potential study participants: patients 16 years or older that meet that pathologic

criteria and clinical criteria of definite infective endocarditis.[1] After initial screening, participants will be categorized into criteria for proven, probable, and possible Candida endocarditis.[2] Once complete, only the participants that have met the criteria for proven or probable Candida endocarditis will be included into the study. In addition to meeting these key inclusion criteria, patients will also be screened via blood sampling to determine renal function based on creatinine levels. A full list of inclusion criteria can be found in the appendix.

3.4 Exclusion Criteria

Key exclusion criteria will be distributed to participating sites. If either of the key exclusion criteria are met, the patient will be deemed ineligible for recruitment due to the contraindications of liposomal amphotericin B with flucytosine administration. These key exclusion criteria included patients with moderate-to-severe renal insufficiency, defined as a calculated creatinine clearance <50 mL/min.[3] Additional exclusion criteria includes known hypersensitivity or allergy history to the proposed study medications and/or their adjuvant components,[3] acute heart failure,[4] patients with poor glycaemic control,[5] and body weight less than forty kilograms.[6] Antifungal prophylaxis will be considered a relative exclusion criterion—non-neutropenic patients on systemic antifungal prophylaxis for three or more days within the last seven days will be excluded, but neutropenic patients on antifungal prophylaxis will be allowed to be included.[6] A full list of exclusion criteria can be found in the appendix.

3.5 Subject Protection and Confidentiality

This study will be reviewed by the Institutional Review Board (IRB) or independent ethics committees at each of the participating sites. All eligible patients will be given information verbally by study personnel. If interested, these patients will

then be given a study brochure that will detail all pertinent information to be enrolled in the study. Once the patient decides to partake in the study, he or she will be required to give verbal consent, as well as written consent by signing an IRB-approved consent form. This consent form will outline the purpose of the study, the two treatment groups, randomization procedures, requirements of the patient, timeline of the study, and possible treatment-related adverse effects. If the patient wishes to withdraw from the study at any point in time, the IRB-approved consent form will detail how to formally withdraw from the study. The study brochure and IRB-approved consent form will be included in the appendix.

In order to maintain confidentiality of patients, the research conducted in this study will adhere to policies and regulations set forth by the Health Insurance Probability and Accountability Act (HIPAA). All identifying patient information will be kept strictly confidential with IRB-approved patient database. Additionally, all study personnel will be required to undergo HIPAA training and certification. Documentation of this training and certification will be kept by the principal investigators.

3.6 Recruitment

In order to participate in the study, interested healthcare facilities will be required to have their governing bodies approve of the study protocols and any amendments that may be made. Once recruited, the participating study sites will facilitate the enrolment of patients to obtain the required sample size by providing the eligible, interested patients with further study information, including the study brochure. In the event that the patient is unable to make medical decisions for himself or herself, consent for enrolment into the study must be obtained by the legally authorized representative of the patient.

3.7 Study Variables and Measures

The intervention of this study will be the administration of micafungin at 150mg IV daily with a placebo PO QID to maintain blinding. The control for this study will be the standard of care, which is liposomal amphotericin B 4mg/kg IV daily with flucytosine 25 mg/kg PO QID.[7] Both interventions will be given for a minimum of six weeks, but no longer than eight weeks.[7]

The primary dependent variable will be an all-cause mortality from the day of assigned study drug regimen to day fifty-six. Day fifty-six was chosen due to the maximum therapy duration for Candida endocarditis being eight weeks.[7]

Secondary dependent variables will include time to clearance of candidemia, assessment of adverse effects, and determination for the incidence of relapse of Candida endocarditis after the initiation of step-down maintenance therapy. After identification of the *Candida* species, susceptibility testing will be conducted and the appropriate step-down therapy (PO voriconazole 200-300 mg (3-4 mg/kg) BID, long-acting posaconazole 300 mg daily, or fluconazole 400-800 mg (6-12 mg/kg) daily) will be administered.[7]

3.8 Blinding of Intervention

All personnel involved in the study were blinded to treatment allocation, except for two research pharmacists at each participating study site. One research pharmacist would be responsible for the preparation and dosing of medications, which would be blinded during administration via opaque coverings on medication administration sets. If placed in the intervention group (micafungin), the patient would also be given a placebo at the frequency of flucytosine administration in the

control group. The second research pharmacist would be responsible for reviewing drug accountability records.

3.9 Blinding of Outcome

The study participants will be blinded to the primary and secondary outcomes while being treated in the inpatient setting. The researchers will be blinded to the primary or secondary outcomes while treating the patients in the inpatient setting.

3.10 Assignment of Intervention

Patients will be randomly assigned to either receive 150mg of micafungin IV daily with a placebo PO QID or 4mg/kg of liposomal amphotericin B IV daily with 25mg/kg of flucytosine PO QID for 8 weeks. Randomisation will be conducted in a 1:1 ratio and stratification will be conducted based on treatment site, as well as baseline neutropenic status. In addition, a third-party computer program will be used to generate randomisation to ensure true random probability for treatment allocation at each site. This use of the third-party computer program will maintain the integrity of our results.

3.11 Adherence

Adherence to the allocated treatment would be maintained through research personnel. Study participants would not be expected to administer the medication themselves; therefore, interventions will only be administered within the inpatient setting of the study participants' hospitalisation. Supervision of adherence will be maintained through the use of study nurses at the study sites through their documentation of study medication administration. Additionally, prior to the first administration of the assigned treatment regimen, all indwelling catheters will be removed.

3.12 Monitoring of Adverse Events

Study participants will be informed to notify investigators of any adverse effects they are experiencing. Investigators will record the reported adverse effects on a standardised form and will categorise these adverse effects based on PRO-CTCAE. In addition to recording these reported adverse effects, study participants will also have daily morning assessment of their serum chemistry laboratories, such as complete blood count, complete metabolic panel, electrolyte panels, etc. and categorised based on the PRO-CTCAE.

3.13 Data Collection

All necessary data of the study participants will be recorded via the patient's online medical records, as well as a separate form in order to maintain accumulation of mandatory information for statistical analysis of the primary and secondary outcomes.

Assessment of probable and proven Candida endocarditis will be done at baseline, weekly during the treatment phase, and at the end of therapy using the modified Duke Criteria. Assessment of probable and proven candidemia will be done at baseline, weekly during the treatment phase, and at the end of therapy using the EORTC/MSG.

The primary endpoint is all-cause mortality at day fifty-six and based on whether the study participant had expired from any cause in the hospital. The secondary endpoint of measuring time to clearance of candidemia will be determined through two sets of negative blood cultures. Additionally, drug-related adverse effects will be documented based on categorisation of PRO-CTCAE throughout the entirety of the treatment phase. Finally, identification of relapse will be assessed for patients

that experience treatment success through the treatment phase but showed re-emergence of the same *Candida* isolates after completion of IV treatment.

3.14 Sample Size Calculation

We based our sample size calculation on all-cause mortality published by an observational cohort study that examined Candida endocarditis with a specific focus on therapy modalities.[8] However, due to the scarcity of data in regard to micafungin success specifically in Candida endocarditis, the rate of success in Candida endocarditis is from echinocandins as a class instead of individually.[8] In order to calculate sample size, a program called Sealed Envelope Limited 2012 – Power Calculator for Binary Outcome Non-Inferiority Trials was used.

Assuming liposomal amphotericin B with flucytosine has a successful response rate of 55% (45% mortality rate) and micafungin has a successful response rate of 64% (36% mortality rate), (α=0.05, β=0.2, δ=0.10) it was determined that a total of 82 patients per treatment group were needed to determine non-inferiority.[6,9-11] This would result in a need for roughly 164 patients in the study; however, we are planning to enrol a total of 223 patients through the assumption of the need to exclude 36% of patients from the per-protocol set.[6]

3.15 Statistical Analysis

Although this study will be conducting statistical analyses of the primary and secondary endpoints of the per-protocol population, the intention-to-treat (ITT) population and the modified intention-to-treat population (mITT) will be of most interest and significance. The ITT population, the primary efficacy population, will include all patients that were enrolled, randomised, and received at least one dose of either experimental treatment therapy.[3] The primary efficacy endpoint will be all-

cause mortality from the first dose of the assigned therapy to day fifty-six in the ITT population because this population would best represent the patients receiving antifungal treatment in a real-wold setting.[3,6] Additionally, the mITT population will be essential in drawing the conclusion of non-inferiority because this population will consist of the number of study participants that were determined to have probable or proven candidemia, based on the EORTC/MSG.[6]

Continuous variables will be presented as the median and interquartile range and will be compared using the Mann-Whitney test. Categorical variables will be presented as numbers and percentages that will compared using either the Chi-square test or Fisher's exact test.

3.16 Timeline and Resources

Our proposed study will take approximately two years, which will include subject recruitment, baseline assessment, and follow-up period. We anticipate beginning our study in January 2021 with subject recruitment, which will extend to June 2022, totalling eighteen months (months 0-18). The proposed timespan for recruitment will allow for our study to maximize recruitment and develop a study population that will hopefully be generalisable to the interested population. This will allow for six months of completing the assigned regimen, which will be administered for no less than four weeks, but no more than eight weeks (months 18-20).[7] If clearance of candidemia is achieved, the assigned antifungal therapy will continue for an additional two weeks per standard of care (month 20).[12] In order to abide by recommendations for the treatment of Candida endocarditis, patients will be given an appropriate step-down therapy.[7] Follow-up after step-down therapy will determine resurgence of candidemia and/or Candida endocarditis within a twelve-week period (months 21-24).[6]

This period of twenty-four months will not include time to IRB approval or the proposed data analysis. It is anticipated that approval for this study by the IRB will take approximately four months. Additionally, we predict that data analysis will take no more than five months.

The required resources for this study will largely be covered by the recruited study sites. The only foreseeable additional charge that will affect these institutions is recruitment of research assistants. The need for additional research assistants will be decided by each site.

References

1. Li JS, Sexton DJ, Mick N, et al. Proposed modifications to the Duke criteria for the diagnosis of infective endocarditis. *Clin Infect Dis.* 2000;30(4):633-638.
2. De Pauw B, Walsh TJ, Donnelly JP, et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. *Clin Infect Dis.* 2008;46(12):1813-1821.
3. Maertens JA, Raad, II, Marr KA, et al. Isavuconazole versus voriconazole for primary treatment of invasive mould disease caused by Aspergillus and other filamentous fungi (SECURE): a phase 3, randomised-controlled, non-inferiority trial. *Lancet.* 2016;387(10020):760-769.
4. Siciliano RF, Gualandro DM, Sejas ONE, et al. Outcomes in patients with fungal endocarditis: A multicenter observational cohort study. *Int J Infect Dis.* 2018;77:48-52.
5. Whitney LC, Bicanic T. Treatment principles for Candida and Cryptococcus. *Cold Spring Harb Perspect Med.* 2014;5(6).
6. Kuse ER, Chetchotisakd P, da Cunha CA, et al. Micafungin versus liposomal amphotericin B for candidaemia and invasive candidosis: a phase III randomised double-blind trial. *Lancet.* 2007;369(9572):1519-1527.
7. Pappas PG, Kauffman CA, Andes DR, et al. Clinical Practice Guideline for the Management of Candidiasis: 2016 Update by the Infectious Diseases Society of America. *Clin Infect Dis.* 2016;62(4):e1-50.
8. Arnold CJ, Johnson M, Bayer AS, et al. Candida infective endocarditis: an observational cohort study with a focus on therapy. *Antimicrob Agents Chemother.* 2015;59(4):2365-2373.
9. Fay MP, Follmann DA. Non-inferiority tests for anti-infective drugs using control group quantiles. *Clin Trials.* 2016;13(6):632-640.
10. Spellberg B, Talbot G. Recommended design features of future clinical trials of antibacterial agents for hospital-acquired bacterial pneumonia and ventilator-associated bacterial pneumonia. *Clin Infect Dis.* 2010;51 Suppl 1:S150-170.
11. Reboli AC, Rotstein C, Pappas PG, et al. Anidulafungin versus fluconazole for invasive candidiasis. *N Engl J Med.* 2007;356(24):2472-2482.
12. Mellinghoff SC, Cornely OA, Jung N. Essentials in Candida bloodstream infection. *Infection.* 2018;46(6):897-899.

Chapter 4 – Conclusion

Candida endocarditis is a rare and deadly disease.[1] It has been shown to be an issue for both non-neutropenic and neutropenic patients.[2-4] Research regarding this infection is scarce, with the only data supporting the current standard of care being from five decades of successful treatment in case reports.[4,5] Furthermore, the evidence to show a possible alternative to the standard of care is from successful treatments in case reports as well. Gaps in knowledge around Candida endocarditis will continue if there are no prospective randomized control trials conducted. This proposed study aims to determine if the recommendations for the treatment of Candida endocarditis can be given evidence in a controlled study. If effective, recommendations by the IDSA and ESCMID will finally be able to support their strong recommendations with high-quality evidence instead of low-quality evidence.[4,6,7]

4.1 Study Advantages

If this study is approved, there are a plethora of benefits that will be provided by the gathered data. To date, this will be the first prospective randomised control trial to compare the standard of care for Candida endocarditis to a novel antifungal therapy. By providing evidence of non-inferiority, the primary outcome, and taking advantage of existing data of echinocandins, specifically micafungin, an alternative treatment can be provided to patients. Additionally, the existing literature today has primarily observed the use of caspofungin on Candida endocarditis. This study will broaden our knowledge on one of the lesser used echinocandins, in this specific context, micafungin.

Based on the results of the secondary outcomes, several benefits could be drawn from that data. Through analysis of the adverse effects, combined with the existing data from the literature review, we will be able to determine if micafungin is

not only an alternative to Candida endocarditis based on efficacy, but also if micafungin is safer and/or more tolerable for patients. Through the isolation of *Candida* species, we will also be able to characterise epidemiological differences between *Candida* species in different areas of the world. This will provide more information for practitioners so that empirical treatment can be more evidence-based.

4.2 Study Disadvantages

Unfortunately, like all other studies, our study will have its disadvantages. Despite having a prolonged period for recruitment, it is assumed that the majority of the patients will be non-neutropenic because Candida infections are showing growing incidence through the increased use of illicit intravenous drugs.[2-4] Similar to most mycological studies, we attempt to reduce this generalizability bias through stratification of study participants into groups of neutropenic patients and non-neutropenic patients. Additionally, we cannot generalize our results to patients with renal insufficiency, acute heart failure, underweight patients, or patients with poor glycaemic control because they were excluded through each of these factors being confounding and independent to mortality.

Furthermore, this study will not give any information or data regarding adjunctive surgery, such as optimal timing and necessity of the surgery. This study will only be examining pharmacological therapy. Finally, our study will be monitoring the efficacy of micafungin monotherapy. There have been studies, not involving echinocandins however, demonstrating superiority of combination antifungal therapy, but there is not enough information nor published case reports to suggest which antifungals may be best to pair with micafungin or other echinocandins.

4.3 Clinical and/or Public Health Significance

As incidence of Candida endocarditis increases, there is a definitive need for evidence-based medicine to guide optimal management of this deadly infection. Historically, amphotericin B deoxycholate has been used, but was limited in its use through significant renal toxicity.[8] Although there has been a development of a lesser nephrotoxic formulation, liposomal amphotericin B, it still has a high propensity to cause damage to the kidneys and is extremely expensive.[8] The data from this study would have the potential of being a landmark study through evidence of a less expensive, more tolerable, and possibly non-inferior alternative in treating this deadly disease—echinocandins.

4.4 Future Studies

Depending on the results from our proposed trial, there are a series of logical steps that can be taken in the treatment of this mycological heart infection. If non-inferiority is shown with micafungin, future studies can observe the efficacy of the other lesser used echinocandin, anidulafungin. Furthermore, since it was determined that combination therapy is superior to monotherapy in the pharmacological treatment of Candida endocarditis, future studies could investigate what antifungal drugs could be best combined with echinocandins.

Additionally, based on the literature review performed, future studies could evaluate adjunctive surgery for Candida endocarditis. One future study that should be addressed would be the most appropriate time for adjunctive surgery after the initiation of pharmacological therapy. There is no recommendation regarding when adjunctive surgery should take place after initiation of antifungal therapy. Conversely, a future study should be performed to determine if adjunctive surgery is as mandatory

as it has been cited to be. Several case reports have already shown that optimal medical therapy could obviate the need for adjunctive surgery.

References

1. Steinbach WJ, Perfect JR, Cabell CH, et al. A meta-analysis of medical versus surgical therapy for Candida endocarditis. *J Infect.* 2005;51(3):230-247.
2. Kauffman CA. Complications of Candidemia in ICU Patients: Endophthalmitis, Osteomyelitis, Endocarditis. *Semin Respir Crit Care Med.* 2015;36(5):641-649.
3. Ammannaya GKK, Sripad N. Fungal endocarditis: what do we know in 2019? *Kardiol Pol.* 2019.
4. Pasha AK, Lee JZ, Low SW, Desai H, Lee KS, Al Mohajer M. Fungal Endocarditis: Update on Diagnosis and Management. *Am J Med.* 2016;129(10):1037-1043.
5. Mora-Duarte J, Betts R, Rotstein C, et al. Comparison of caspofungin and amphotericin B for invasive candidiasis. *N Engl J Med.* 2002;347(25):2020-2029.
6. Pappas PG, Kauffman CA, Andes DR, et al. Clinical Practice Guideline for the Management of Candidiasis: 2016 Update by the Infectious Diseases Society of America. *Clin Infect Dis.* 2016;62(4):e1-50.
7. Arnold CJ, Johnson M, Bayer AS, et al. Candida infective endocarditis: an observational cohort study with a focus on therapy. *Antimicrob Agents Chemother.* 2015;59(4):2365-2373.
8. Scudeller L, Viscoli C, Menichetti F, et al. An Italian consensus for invasive candidiasis management (ITALIC). *Infection.* 2014;42(2):263-279.

Appendices

Appendix A. Candida Endocarditis Data Collection

Subject Participant Number:

Age:

Sex:

Race:

Neutropenic Status:

Assigned Group:

Candida Endocarditis Status: *Please list prespecified criteria for diagnosis*

Species:

Initiation Date of Assigned Medication:

Date of Candidemia Clearance: *if applicable*

Date of Candida Endocarditis Clearance: *if applicable*

Adverse Effect & Grade: *Please refer to attached documentation for grading*

Appendix B. Sample HIC Consent Form

CONSENT FOR PARTICIPATION IN A RESEARCH PROJECT
200 FR.1

YALE UNIVERSITY SCHOOL OF MEDICINE

Study Title: Liposomal Amphotericin B and Flucytosine Versus Micafungin in Treatment of Candida Endocarditis
Principal Investigator: Dr. Matthew Grant, MD
Co-Investigator: Anton Matthew Yanker, PA-SII
Funding Source: Yale School of Medicine

Invitation to Participate and Description of Project

We are inviting you to participate in a research study designed to look at the efficacy of two different antifungal treatments in the setting of Candida Endocarditis. You have been asked to participate because you are highly suspected of being affected of Candida endocarditis. Approximately 200 individuals will be participating in the study.

In order to decide whether or not you wish to be a part of this research study, you should know enough about its risks and benefits to make an informed decision. This consent form gives you detailed information about the research study, which a member of the research team will also discuss with you. This discussion should take place over all aspects of this research study—its purpose, procedures that will be performed, any potential risks of the procedures, possible benefits, and possible alternative treatments. Once you understand the study, you will be asked if you wish to participate. If you agree, you will be asked to sign this form.

Description of Procedures

If you agree to participate in this study, you will be randomly assigned to receive either (a) liposomal amphotericin B with flucytosine, OR (b) micafungin with placebo.

In this study program, you will be asked to adhere to your assigned medication regimen at the prespecified frequency and dosage. The study nurses will be providing the medications to you at scheduled times, dosages, and frequencies. You will remain in the hospital for the duration of the treatment, which will last no longer than fifty-six days. Blood draws will be obtained daily to monitor candidemia levels and to gather information about standard laboratory information, such as complete blood counts and metabolic panels. Transthoracic and/or transoesophageal echocardiograms will be conducted as well to determine the presence of Candida endocarditis.

Throughout the entirety of the study, the investigators will ask you to document and/or report any adverse effects you are feeling that you believe may be a result of your study medication regimen.

A description of this study will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. (*See Clinical Trials Identifier Number* xxxxxxxxxx). This website will not include information that can identify you. The purpose of this database is to allow everyone to see information on what studies are being done, and what studies have already been done. At most, the website will include a summary of the results. You can search this website at any time.

You will be told of any significant new findings that are developed during the course of your participation in this study that may affect your willingness to continue to participate. Research results will not be returned to your clinician. If research results are published, your name and other personal information will not be disclosed or given.

Risks and Inconveniences

Liposomal amphotericin B has been used for several years and studied in a number of clinical trials for many fungal infections. The most common adverse effect is nephrotoxicity and infusion-related events. Flucytosine has also been used for several years and studied in a number of clinical trials. The most common adverse effect is myelotoxicity after prolonged usage. Therapeutic-drug monitoring will be conducted to watch for serious adverse effects; however, we would like you to report any adverse effects you may be experiencing.

Micafungin is another antifungal drug that has been used for several years and studied in a number of clinical trials. To our knowledge, the resulting adverse effects are minimal in severity.

Other risks from participating in the study include the breach of confidentiality about your health status and participation in the study. This is unlikely to happen, as all study investigators are trained and certified in research privacy, as well as HIPAA.

We will also ask you to have your blood drawn daily. The risks involved in venepuncture include, but are not limited to, momentary discomfort at the site of the blood draw, possible bruising, redness, and swelling around the site, bleeding at the site, feeling of lightheadedness when the blood is draw, and rarely, infection at the site of venepuncture.

Benefits

The potential benefit resulting from the study includes full treatment of candidemia and/or Candida endocarditis. This study may also provide better insights to treatment guidelines for this rare and deadly disease, which may lead to more treatment success in the future.

Economic Considerations

The medications will be provided to you free of charge. There are no other costs associated with your participation in the study. Parking will also be provided free of charge to visitors.

Treatment Alternatives/Alternatives

If you choose not to participate in this study, there are no alternative treatments available, except those that are already being administered by your treatment team, including pharmacotherapy (medications/drugs). You may choose not to participate.

Confidentiality and Privacy

Any identifiable information that is obtained in connection with this study will remain confidential and will be disclosed only with your permission or as required by U.S. or State law. Examples of information that we are legally required to disclose include abuse of a child, abuse of an elderly person, or certain reportable diseases. Information will be kept confidential by using only identification numbers on study forms, storing signs forms in locked cabinets, and password protecting data to be stored on a computer. When the results of the research are published or discussed in conferences, no information will be included that would reveal your identity unless your specific permission for this activity is obtained.

We understand that information about your health is personal and we are committed to protecting the privacy of that information. If you decide to be in this study, the researcher will get information that identifies your personal health information. This may include information that might directly identify you, such as name, address, telephone number, email address, and/or mobile phone number. This information will be de-identified at the earliest reasonable time after we receive it, meaning we will replace your identifying information with a code that does not directly identify you. The principal investigator will keep a link that identifies you and your coded information. This link will be kept secure and available only to the principal investigator, or selected members of the research team. Any information that can identify you will remain confidential. Information will be kept confidential by using only identification numbers on study forms, storing signed forms in locked cabinets, and password protecting data stored on a computer. The research team will only give this coded information to others to carry out this research study. The link to your personal information will be kept for five years. After five years, the link will be destroyed, and the data will become anonymous. The data will be kept in this anonymous form indefinitely.

The information about your health that will be collected in this study includes:

- Research study records
- Records about phone calls made as part of this research
- Records about your study visits

Information about your health, which might identify your child, may be used or given to:

- The U.S. Department of Health and Human Services (DHHS) agencies
- Representatives from Yale University, the Yale Human Research Protection Program, and the Yale Human Investigation Committee (the committee that reviews, approves, and monitors research on human subjects), who are responsible for ensuring research compliance. These individuals are required to keep all information confidential.
- Those individuals at Yale who are responsible for the financial oversight of research, including billings and payments.
- The Principal Investigator, Dr. Matthew Grant
- Co-Investigators and other investigators
- Study Coordinator and members of the research team

By signing this form, you authorize the use and/or disclosure of the information described above for this research study. The purpose for the uses and disclosures you are authorizing is to ensure that the information relating to this research is available to all parties who many need it for research purposes.

All healthcare providers subject to the Health Insurance Portability and Accountability Act (HIPAA) are required to protect the privacy of your information. The research staff at the Yale School of Medicine are required to comply with HIPAA and to ensure the confidentiality of you or your child's information.

If you choose to participate in this study, the investigators will check your electronic medical record at Yale via EPIC to make sure you qualify. Any access to your electronic medical record will be done consistent with HIPAA regulations.

Some of the individuals or agencies listed above may not be subject to HIPAA, and therefore, may not be required to provide the same confidentiality protection. They could use or disclose your information in ways not mentioned in this form. However, to better protect your health information, agreements are in place with these individuals and/or companies that require that they keep your information confidential.

You have the right to review and copy your health information in your medical record in accordance with institutional medical records policies. This authorization to use and disclose your health information collected during your participation in this study will never expire.

Voluntary Participation and Withdrawal

You are free to choose not to participate in this study. Your healthcare outside the study, the payment for your healthcare, and your healthcare benefits will not be affected if you do not agree to participate. However, you will not be able to enrol in this research study and will not receive study procedures as a study participant if you

do not allow use of your information as part of this study. You do not give up any of your legal rights by signing this form.

Withdrawing from the Study

If you do not become a subject, you are free to stop and withdraw from this study at any time during its course.

To withdraw from the study, you can call a member of the research time at any time and tell him or her that you no longer wish to participate. This will cancel any future appointments.

The researchers may withdraw you from participating in the research, if necessary. This will only occur if you do not adhere to the assigned treatment.

If you choose not to participate, or if you withdraw, it will not harm your relationship with your treatment team, or with the Yale School of Medicine and Yale New Haven Hospital.

Withdrawing Your Authorization to Use and Disclose Your Health Information

You may withdraw or take away permission to use and disclose your health information at any time. You do this by calling or sending written notice to the Principal Investigator, Dr. Matthew Grant.

When you withdraw your permission, no new health information identifying you will be gathered after that date. Information that has already been gathered may still be used and given to others until the end of the research study, as necessary to ensure the integrity of the study and/or study oversight.

You do not give up any of your legal rights by signing this form.

Questions

We have used technical and/or legal terms in this form. Please feel free to ask about anything you do not understand and to consider this research and the permission form carefully—as long as you feel necessary—before you make a decision.

Authorization

I have read, or someone has read to me, this form and have decided to participate in the project described above. Its general purpose, the specifics of my involvement, possible hazards, and possible inconveniences have been explained to my satisfaction. My signature indicates that I, _____, have received a copy of this consent form.

Name of Subject: _____

Signature: _____

Relationship: _____

Date: _____

_____ _____

Signature of Person Obtaining Consent Date

If you have any further questions about this project, or if you have a research-related problem, you may contact the Principal Investigator, Dr. Matthew Grant at (xxx) xxx-xxxx.

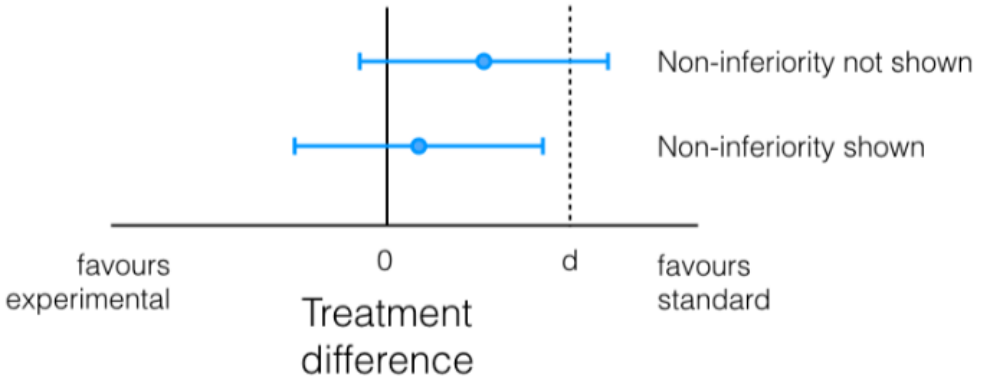
After signing this form, if you have any questions about your privacy rights, please contact the Yale Privacy Officer at (xxx) xxx-xxxx. If you would like to talk to someone other than the researchers to discuss problems, concerns, and/or questions you may have regarding the research, or to discuss your rights as a research subject, you may contact the Yale Human Investigator Committee at (xxx) xxx-xxxx.

Appendix C. Sample Size Calculation

Significance level (alpha)	5%
Power (1-beta)	80%
Percentage 'success' in control group	55 %
Percentage 'success' in experimental group	64 %
Non-inferiority limit, d	10 %

Calculate sample size

Sample size required per group	**82**
Total sample size required	**164**



Bibliography

Agnelli C, Guinea J, Valerio M, Escribano P, Bouza E, Munoz P. Infectious endocarditis caused by Candida glabrata: Evidence of in vivo development of echinocandin resistance. *Revista Espanola de Quimioterapia.* 2019;32(4):395-397.

Ahuja T, Fong K, Louie E. Combination antifungal therapy for treatment of Candida parapsilosis prosthetic valve endocarditis and utility of T2Candida Panel(R): A case series. *IDCases.* 2019;15:e00525.

Alothman AF, Al-Musawi T, Al-Abdely HM, et al. Clinical practice guidelines for the management of invasive Candida infections in adults in the Middle East region: Expert panel recommendations. *Journal of Infection and Public Health.* 2014;7(1):6-19.

Ammannaya GKK, Sripad N. Fungal endocarditis: what do we know in 2019? *Kardiol Pol.* 2019.

Arnold CJ, Johnson M, Bayer AS, et al. Candida infective endocarditis: an observational cohort study with a focus on therapy. *Antimicrob Agents Chemother.* 2015;59(4):2365-2373.

Baddley JW, Benjamin DK, Jr., Patel M, et al. Candida infective endocarditis. *Eur J Clin Microbiol Infect Dis.* 2008;27(7):519-529.

Bandyopadhyay S, Tiwary PK, Mondal S, Puthran S. Pacemaker lead Candida endocarditis: Is medical treatment possible? *Indian Heart J.* 2015;67 Suppl 3:S100-102.

Basch E, Reeve BB, Mitchell SA, et al. Development of the National Cancer Institute's patient-reported outcomes version of the common terminology criteria for adverse events (PRO-CTCAE). *J Natl Cancer Inst.* 2014;106(9).

Bauer BK, Schulze AB, Loher A, Reinke F, Eckardt L. Candida tropicalis defibrillator endocarditis: A case report and review of current literature. *Med Mycol Case Rep.* 2019;25:1-9.

Benjamin DK, Jr., Miro JM, Hoen B, et al. Candida endocarditis: contemporary cases from the International Collaboration of Infectious Endocarditis Merged Database (ICE-mD). *Scand J Infect Dis.* 2004;36(6-7):453-455.

Checchia TE, Moura LZ, Colatusso C, Veiga S, Fortes JA, Tuon FF. Heart transplantation and Candida endocarditis. *Transpl Infect Dis.* 2016;18(3):483-484.

De Pauw B, Walsh TJ, Donnelly JP, et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. *Clin Infect Dis.* 2008;46(12):1813-1821.

Durante-Mangoni E, Nappi G. Giant endocarditis vegetation on a pace-maker lead. *Intern Emerg Med.* 2010;5(3):251-252.

Fay MP, Follmann DA. Non-inferiority tests for anti-infective drugs using control group quantiles. *Clin Trials.* 2016;13(6):632-640.

Gardiner BJ, Slavin MA, Korman TM, Stuart RL. Hampered by historical paradigms - echinocandins and the treatment of Candida endocarditis. *Mycoses.* 2014;57(5):316-319.

Glavis-Bloom J, Vasher S, Marmor M, et al. Candida and cardiovascular implantable electronic devices: a case of lead and native aortic valve endocarditis and literature review. *Mycoses.* 2015;58(11):637-641.

Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet.* 2017;390(10100):1211-1259.

Glockner A. Recurrent candidaemia and pacemaker wire infection with Candida albicans. *Mycoses.* 2011;54 Suppl 4:20-23.

Gould FK, Denning DW, Elliott TS, et al. Guidelines for the diagnosis and antibiotic treatment of endocarditis in adults: a report of the Working Party of the British Society for Antimicrobial Chemotherapy. *J Antimicrob Chemother.* 2012;67(2):269-289.

Grosset M, Desnos-Ollivier M, Godet C, Kauffmann-Lacroix C, Cazenave-Roblot F. Recurrent episodes of Candidemia due to Candida glabrata, Candida tropicalis and Candida albicans with acquired echinocandin resistance. *Medical Mycology Case Reports.* 2016;14:20-23.

Jagernauth S, Patel A, Baig K, De Souza A. Fungal endocarditis of the eustachian valve in carcinoid heart disease: a case report. *J Heart Valve Dis.* 2007;16(6):631-633.

Kauffman CA. Complications of Candidemia in ICU Patients: Endophthalmitis, Osteomyelitis, Endocarditis. *Semin Respir Crit Care Med.* 2015;36(5):641-649.

Kluetz PG, Chingos DT, Basch EM, Mitchell SA. Patient-Reported Outcomes in Cancer Clinical Trials: Measuring Symptomatic Adverse Events With the National Cancer Institute's Patient-Reported Outcomes Version of the Common Terminology Criteria for Adverse Events (PRO-CTCAE). *Am Soc Clin Oncol Educ Book.* 2016;35:67-73.

Koehler P, Tacke D, Cornely OA. Our 2014 approach to candidaemia. *Mycoses.* 2014;57(10):581-583.

Kubota K, Soma K, Uehara M, et al. Combined Surgical and Medical Therapy for Candida Prosthetic Endocarditis in a Patient with Repaired Tetralogy of Fallot. *Int Heart J.* 2018;59(4):877-880.

Kuhn DM, George T, Chandra J, Mukherjee PK, Ghannoum MA. Antifungal susceptibility of Candida biofilms: unique efficacy of amphotericin B lipid formulations and echinocandins. *Antimicrob Agents Chemother.* 2002;46(6):1773-1780.

Kuse ER, Chetchotisakd P, da Cunha CA, et al. Micafungin versus liposomal amphotericin B for candidaemia and invasive candidosis: a phase III randomised double-blind trial. *Lancet.* 2007;369(9572):1519-1527.

Lefort A, Chartier L, Sendid B, et al. Diagnosis, management and outcome of Candida endocarditis. *Clin Microbiol Infect.* 2012;18(4):E99-E109.

Lemke A, Kiderlen AF, Kayser O. Amphotericin B. *Appl Microbiol Biotechnol.* 2005;68(2):151-162.

Li JS, Sexton DJ, Mick N, et al. Proposed modifications to the Duke criteria for the diagnosis of infective endocarditis. *Clin Infect Dis.* 2000;30(4):633-638.

Linden PK. Amphotericin B lipid complex for the treatment of invasive fungal infections. *Expert Opin Pharmacother.* 2003;4(11):2099-2110.

Maertens JA, Raad, II, Marr KA, et al. Isavuconazole versus voriconazole for primary treatment of invasive mould disease caused by Aspergillus and other filamentous fungi (SECURE): a phase 3, randomised-controlled, non-inferiority trial. *Lancet.* 2016;387(10020):760-769.

Mellinghoff SC, Cornely OA, Jung N. Essentials in Candida bloodstream infection. *Infection.* 2018;46(6):897-899.
Mora-Duarte J, Betts R, Rotstein C, et al. Comparison of caspofungin and amphotericin B for invasive candidiasis. *N Engl J Med.* 2002;347(25):2020-2029.
Morioka H, Tokuda Y, Oshima H, et al. Fungal endocarditis after transcatheter aortic valve replacement (TAVR): Case report and review of literature. *J Infect Chemother.* 2019;25(3):215-217.
Pappas PG, Kauffman CA, Andes DR, et al. Clinical Practice Guideline for the Management of Candidiasis: 2016 Update by the Infectious Diseases Society of America. *Clin Infect Dis.* 2016;62(4):e1-50.
Pasha AK, Lee JZ, Low SW, Desai H, Lee KS, Al Mohajer M. Fungal Endocarditis: Update on Diagnosis and Management. *Am J Med.* 2016;129(10):1037-1043.
Patil A, Majumdar S. Echinocandins in antifungal pharmacotherapy. *J Pharm Pharmacol.* 2017;69(12):1635-1660.
Pipa S, Dias C, Ribeiro J, Gregorio T. Fungal endocarditis of native valves. *BMJ Case Rep.* 2018;11(1).
Ramage G, Robertson SN, Williams C. Strength in numbers: Antifungal strategies against fungal biofilms. *International Journal of Antimicrobial Agents.* 2014;43(2):114-120.
Reboli AC, Rotstein C, Pappas PG, et al. Anidulafungin versus fluconazole for invasive candidiasis. *N Engl J Med.* 2007;356(24):2472-2482.
Rivoisy C, Vena A, Schaeffer L, et al. Prosthetic Valve Candida spp. Endocarditis: New Insights Into Long-term Prognosis-The ESCAPE Study. *Clin Infect Dis.* 2018;66(6):825-832.
Scudeller L, Viscoli C, Menichetti F, et al. An Italian consensus for invasive candidiasis management (ITALIC). *Infection.* 2014;42(2):263-279.
Shorr AF, Wu C, Kothari S. Outcomes with micafungin in patients with candidaemia or invasive candidiasis due to Candida glabrata and Candida krusei. *J Antimicrob Chemother.* 2011;66(2):375-380.
Siciliano RF, Gualandro DM, Sejas ONE, et al. Outcomes in patients with fungal endocarditis: A multicenter observational cohort study. *Int J Infect Dis.* 2018;77:48-52.
Spellberg B, Talbot G. Recommended design features of future clinical trials of antibacterial agents for hospital-acquired bacterial pneumonia and ventilator-associated bacterial pneumonia. *Clin Infect Dis.* 2010;51 Suppl 1:S150-170.
Steinbach WJ, Perfect JR, Cabell CH, et al. A meta-analysis of medical versus surgical therapy for Candida endocarditis. *J Infect.* 2005;51(3):230-247.
Stone NR, Bicanic T, Salim R, Hope W. Liposomal Amphotericin B (AmBisome((R))): A Review of the Pharmacokinetics, Pharmacodynamics, Clinical Experience and Future Directions. *Drugs.* 2016;76(4):485-500.
Tacke D, Koehler P, Cornely OA. Fungal endocarditis. *Curr Opin Infect Dis.* 2013;26(6):501-507.
Tattevin P, Revest M, Lefort A, Michelet C, Lortholary O. Fungal endocarditis: Current challenges. *International Journal of Antimicrobial Agents.* 2014;44(4):290-294.
U.S. Department of Health and Human Services NIoH, National Cancer Institute. Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0. 2017.

Victorio GB, Bourdon LMB, Benavides LG, et al. Antifungal activity of caspofungin in experimental infective endocarditis caused by Candida albicans. *Mem Inst Oswaldo Cruz.* 2017;112(5):370-375.
Whitney LC, Bicanic T. Treatment principles for Candida and Cryptococcus. *Cold Spring Harb Perspect Med.* 2014;5(6).