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Combination Antifungal Therapy: A Review of Current Data

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Abstract

The incidence of invasive fungal infections has been on the rise, particularly in transplant recipients and in patients with hematological malignancies and other forms of immunosuppression. There is a mismatch between the rate of antifungal resistance and the development of new antifungal agents. Based on this, the idea of combining antifungals in the treatment of invasive fungal infections appears tempting for many clinicians, particularly after many *in vitro* studies showed synergism between many antifungal agents. Several randomized controlled trials have been published regarding the efficacy and safety of combination of antifungals, but the high cost, the limited number of cases and the multitude of confounding factors lead in some instances to weak and sometimes contradictory results. The lack of consensus in many clinical scenarios raises the importance of the need for more studies about combination antifungal therapies and should incite infectious disease societies to develop specific recommendations for the clinicians to follow while approaching patients with invasive fungal infections.

Keywords: Combination antifungals; Invasive infection; Synergism; Salvage therapy

Introduction

Invasive fungal infections incidence is on the rise in the era of transplant medicine. Beside organ recipients, other high risk patients are susceptible to invasive fungal infections, including acquired immunodeficiency syndrome (AIDS) patients, patients with congenital immunodeficiency syndromes and those with hematological malignancies.

On average, there are about 30,000 transplants performed each year in the United States [1]. This number is expected to increase because over 121,000 people in need for an organ are on the United States government waiting list [1]. With this, comes many new and old immunosuppressant medications and sometimes profound state of immunocompromise in thousands of hospitalized patients.

Of the fungal infections seen in immunosuppressed patients, invasive aspergillosis, fusarium infection, mucormycosis and invasive candida infection might be the most common ones, with a high mortality and a significant incidence of failure of therapy.

The evidence for using amphotericin with 5-flucytosine for cryptococcal infection to reduce mortality has been effectively proven [2], but beside this single indication for combination antifungal therapy, literature lacks well-controlled clinical trials to evaluate the adequacy, timing and risks for such combination in other invasive fungal infections.

The need for dual antifungal therapy stems from the ongoing risk of invasive fungal infection and the significant mortality related to fungal infections in immunosuppressed patients.

In an analysis of lethal infectious complications in hematopoietic stem cell transplant patients, it has been found that infections are related to 11% of deaths. Of these infections, 28% are fungal, with an ongoing risk beyond the initial period of neutropenia [3].

In solid organ transplant recipients, the increasingly potent immunosuppressive agents have dramatically reduced the incidence of rejections at the expense of increased susceptibility to malignancies and opportunistic infections. The greatest risk has been reported to include aspergillus infection of the tracheal anastomosis after lung transplantation [4] and candida infection after pancreas or liver transplantation [5]. Some fungal infections are recipient derived and include aspergillus species infection with marijuana use [6], *Cryptococcus neoformans* infection with pigeon contact, and endemic fungi (*Histoplasma capsulatum*, *Coccidioides immitis*, and *Paracoccidioides brasiliensis*).

In an epidemiological study of invasive fungal infections in solid organ transplant recipients, 515 invasive fungal infections were prospectively identified in 429 adults followed from 2004 to 2007, with most of these infections caused by Candida sp. (59%) [7]. While survival is improved compared to historical reports, invasive fungal infections still constitute a challenge for transplanted patients and healthcare providers [8].

Despite the need for effective and reliable approach for initial and salvage therapy for invasive fungal infections, the Infection Disease Society of America (IDSA) still recommends monotherapy for the majority of these infections.

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Antifungals Combination Therapy

Current guidelines

For invasive aspergillosis, IDSA practice guidelines suggested use of amphotericin B (AmB) and its lipid derivative for initial and salvage treatment of invasive aspergillosis when voriconazole cannot be administered [9]. What is remarkable in these guidelines is that echinocandins are listed as effective in salvage therapy either alone or in combination against invasive aspergillosis. IDSA recognized that the combination of polyenes or azoles with echinocandins suggests additive or synergistic effect in some preclinical studies, but expressed uncertainty as how to interpret these findings [9].

IDSA notes few indications for combination antifungal (CAF) agents for treatment of candida infection including use of AmB with or without flucytosine for initial therapy for candida native valve endocarditis, candida CNS infection, azole-resistant *Candida glabrata*, ascending pyelonephritis and fluconazole-resistant candida endophthalmitis [10]. Guidelines also advise for the need to add intravitreal injection of either AmB or voriconazol to the systemic antifungal agent used in fluconazole-resistant *Candida endophthalmitis* when macular involvement is present or when the infection is associated with vitritis [10].

European Society of Clinical Microbiology and Infectious Disease and European Confederation of Medical Mycology joint clinical guidelines for management of mucormycosis recommend surgical debridement and AmB or its lipid derivative for treatment of mucormycosis [11]. Posaconazole is strongly recommended for salvage treatment of mucormycosis. AmB combined with posaconazole or caspofungin is supported with moderate strength to be administered with intent to cure for refractory disease or in case of intolerance to prior antifungal therapy [11]. The same guidelines recommend monotherapy with voriconazole or lipid AmB monotherapy (combined with surgical debridement, reversal of immunosuppressive state, and removal of venous catheters) or posaconazole as salvage therapy for fusarium infection [11].

Synergism and antagonism of antifungals

Many *in vitro* studies on antifungals showed that combinations can broaden the coverage, increase the fungicidal effect and decrease risk of development of resistance. Combined agents can have synergistic activity with decreased toxicity.

On the other hand, many studies have suggested that different concentrations of each drug combination can be associated with results that range from antagonism to synergy. Host factors greatly affect efficacy of an antifungal agent in the clinical setting and cannot always be simulated in *in vitro* studies or animal models. Issues of toxicities or decrease in efficacy can only be apparent when the combinations are studied in humans [12].

Studying multiple dose combinations in clinical setting is difficult considering the expense of clinical trials and limited number of research candidates. Probably, the combination of time and cost is currently the major factor limiting the number and power of the randomized clinical trials available on combination antifungals [13].

Many mechanisms for synergy between antifungals have been proposed. Terbinafine and azoles for instance, work on the same biochemical pathway by inhibiting ergosterol biosynthesis and thus impairing the function of the fungal cell membranes. This interaction was studied by Barchiesis et al *in vitro* against *Candida albicans* [14]. In this study, the synergistic activity was noted in more than 40% of the terbinafine-fluconazol and terbinafine-itraconazole combinations, without significant drop in this proportion on day 2 of incubation. Another remarkable finding was the absence of antagonism and the drop in minimal inhibitory concentration (MIC) of one or both medications when used in combination even when the definition of synergy was not reached.

Similar findings were reported when terbinafine-azole combination was studied *in vitro* against *C. neoformans* [15] and dermatophytes [16].

C. glabrata infection with low susceptibility to fluconazole might also benefit from terbinafine added to fluconazole, voriconazole or itraconazole [17].

AmB and azoles damage the cell membrane and allow increase uptake of other agents like flucytosine [18, 19]. It also allows agents like rifampin and quinolones to easily penetrate the fungal cell membranes and reach their target fungal DNA [20, 21].

Another mechanism of synergy includes simultaneous inhibition of different fungal cell targets, giving synergism between echinocandins (cell wall active) and AmB [22].

The antagonism amongst antifungals is one of the major elements that dictate the choice of any combination that might be useful in invasive fungal infections treatment. Clinicians can consider salvage treatment with combination antifungals in few situations but have to be aware that a wrong combination can decrease the fungicidal effect and sometimes increase the toxicity.

Similarly to synergism, antagonism mechanisms are diverse. The antagonism might be due to a direct action of both agents on the same site, decreasing the availability of one another or modification of a target in the fungal cell secondary to exposure to an antifungal, making the fungus less susceptible to another antifungal [23, 24]. These two mechanisms appear in the interaction between the azoles and AmB, where azoles prevent the synthesis of ergosterol, making the AmB that usually binds the ergosterol in the cell membrane inactive.

AmB is also believed to have partial antagonism with flucytosine by changing the cell membrane function [25], but this aspect of the interaction of amphotericin and flucytosine is still not well clarified in the *in vitro* studies, knowing that these two agents are used in the clinical setting in combination to treat cryptococcal infection (Table 1) [14-19, 24-26].

What we learned from combination antifungals in cryptococcal infection

The idea of combining antifungals started more than 40 years ago with many case reports suggesting successful combination of flucytosine and AmB targeting cryptococcal meningitis

Table 1. Suggested Mechanisms of Synergism and Antagonism [14-19, 24-26]

Combination of antifungals	Synergism (S) or antagonism (A)	Mechanism	Reference
Terbinafine + azole	S	Inhibition of ergosterol biosynthesis	Barchiesi et al [14] (*C. albicans*) Guerra et al [15] (*C. neoformans*) Ashley and Johnson [16] (Dermatophytes) Perea et al [17] (*C. glabrata*)
AmB or azole + flucytosine	S	Cell wall damage (AmB or azole) and increase uptake of flucytosine	Yamamoto et al [18] (pulmonary cryptococcosis) Polak [19] (septicemic candidiasis)
AmB + azole	A	Modification of a target in the fungal cell (prevention of ergosterol synthesis by the azole)	Sugar and Liu [24] (invasive candidiasis) Baddley et al [26] (invasive candidiasis)
AmB + flucytosine	A	Changing of the cell membrane function	Shadomy et al [25] (*C. neoformans* and *C. tropicalis*)

Note that AmB combined with flucytosine had been reported to have synergy both *in vitro* and *in vivo*, but in some *in vitro* studies, partial antagonism was reported. This aspect of the interaction of Amphotericin and flucytosine is still not well clarified.

[27]. Back then, a prospective clinical trial by Bennett et al compared AmB treatment of cryptococcal meningitis in non-HIV-infected patients with a regimen containing both flucytosine and AmB. The combination regimen showed higher cure rate and fewer relapses than the monotherapy with AmB, and more interestingly, the combination regimen showed less nephrotoxicity than the monotherapy regimen [28].

Since then, many reports confirmed these suggested benefits, but the duration of the induction-combination therapy remained debatable (treatment of cryptococcal meningitis with combination of AmB and flucytosine for 4 as compared with 6 weeks). The IDSA 2010 guidelines recommended 2 weeks of combination of AmB and flucytosine for most cases of cryptococcal meningitis, but also suggested at least 4 weeks of the same combination for the induction therapy in non-HIV-infected, non-transplants hosts, and at least 6 weeks for those with cerebral cyptococcoma [29].

In the combination of flucytosine with AmB, the main concern is the impaired glomerular filtration induced by the AmB that decreases elimination of the flucytosine and increases its level, thus, increasing not only its antimycotic activity [30], but also its immunosuppressive and hepatotoxic effect [31].

Candidiasis treatment with combination antifungals

Most clinical experience of combination antifungals therapy has been obtained in the treatment of cryptococcal infection. For candidiasis, IDSA has mentioned few indications for CAF including native valve endocarditis, candida CNS infection, azole-resistant *C. glabrata*, ascending pyelonephritis and fluconazole-resistant candida endophthalmitis.

Despite the growing interest of CAF in invasive candida infections, clinicians should be aware of the antagonism and the high toxicity profiles of some of these combinations.

The combination of azole and AmB demonstrated antagonism that was explained by alteration of the ergosterol target by the azoles, reducing AmB activity [26]. This outcome was rejected by many other studies showing either indifferent effect [32] or additive therapeutic benefit [33].

A randomized, blinded clinical trial compared fluconazole alone versus fluconazole combined with AmB in treatment of candidemia (other than *C. krusei*). Non-neutropenic subjects studied showed higher rate and more rapid fungemia clearance when treated with the combination of azole and AmB.

Echinocandin with azole has been another well-known antifungal combination in the treatment of invasive candida infection. No clinical trials on this combination have been reported, but posaconazole combined with caspofungin or micafungin has been studied *in vitro* and in animals [34]. Chen et al demonstrated that posaconazole exhibits *in vitro* and *in vivo* synergy with caspofungin against *C. albicans*, including echinocandin-resistant isolates [35]. This finding was confirmed by a multilaboratory study conducted on *C. albicans*, *C. glabrata* and *Candida parapsillosis*, showing that a candida isolate with known resistance to azole or echinocandin can be susceptible to the two drugs combined, and more interestingly, demonstrating the absence of antagonism when posaconazole is combined with an echinocandin [36].

Monoclonal antibodies with antifungal effect have been also studied in combination with AmB. In a randomized controlled trial, combination of AmB with mycograb, a human recombinant monoclonal antibody against heat shock protein 90, revealed synergy against invasive candida, with a decrease in candida attributable deaths [37].

In conclusion, combination antifungal treatment in invasive candida infections is limited to only few indications. The combination of azole with AmB needs more clinical trial prior to its generalization and routine application in invasive candida treatment. For resistant and invasive infections, clinicians should do a thorough search of drug-drug interaction before adding a second antifungal to the treatment protocol. Wrong combinations can lead to antagonism and worsening of side effects with negative clinical repercussions on a patient that is already immunosuppressed and in critical condition.

Mucormycosis treatment with combination antifungals

Combination of AmB with caspofungin or posaconazole is

Table 2. Commonly Used CAF Therapy [9-11, 33-35, 41, 43]

	Commonly used CAF therapy	
Invasive candidiasis	AmB + flucytosine	Pappas et al [10], 2016 IDSA guidelines*
	AmB + azole	Rex et al [33]
	Echinocandin + azole	Cui et al [34] Chen et al [35]
Invasive aspergillosis	Azole or AmB + echinocandin	Patterson et al [9], 2016 IDSA guidelines **Panackal et al [41]
	Voriconazole + anidulafungin	Marr et al [43]
Mucormycosis	AmB + posaconazole or caspofungin	Cornely et al [11]***

*Amphotericin B with or without flucytosine for initial therapy for candida native valve endocarditis, candida CNS infection, azole-resistant *Candida glabrata*, ascending pyelonephritis and fluconazole-resistant candida endophthalmitis. **Salvage therapy with echinocandin either alone or in combination against invasive aspergillosis. Uncertainty of the CAF still exists. ***Intent to cure therapy for refractory disease or in case of intolerance to prior antifungal therapy.

recommended by the European Society of Clinical Microbiology and Infectious Disease for refractory disease caused by mucormycosis.

The combination polyene-caspofungin has been reported by retrospective studies to show improved outcomes, with higher success rate and better survival compared to monotherapy with AmB or its lipid formulation [38]. However, prospective clinical trials studying this combination are still lacking.

Currently, isavuconazole (ISAV), a triazole active against mucormycosis emerged and was found similar in efficacy to AmB. The VITAL clinical trial suggested that ISAV can be used for treatment of mucomycosis and is well tolerated [39]. No clinical trials or retrospective studies compared ISAV to the combination of AmB and posaconazole or caspofungin.

Treatment with immunostimulating agents has also been described in mucormycosis. In an immunosuppressed, polytrauma case in Brussel, Belgium, and after failure of AmB and posaconazole combination for intractable abdominal mucomycosis, combination interferon G and nivolumab was successfully used, suggesting that combination immunotherapy might be helpful in fungal sepsis treatment [40].

In summary, combination antifungals of AmB and caspofungin or posaconazole are a recommended treatment strategy for refractory mucormycosis. The options for the clinician are still very limited in case of intolerance or contraindication to AmB. More data are still needed regarding use of ISAV. The future might also show emergence of combination immunotherapy for treatment of this rare, aggressive fungal infection.

Aspergillosis treatment with combination antifungals

The indication currently accepted by IDSA for combination antifungals in invasive aspergillosis is in salvage therapy. This recommendation is endorsed by a meta-analysis published in 2014 showing that the CAF improves 12-week survival compared to monotherapy [41].

When using voriconazole, clinicians should be aware that it requires at least 5 days before achieving a steady state. Additionally, voriconazole metabolism is influenced significantly by concomitant medications [42]. This pharmacokinetic concept makes the combination of voriconazole with another antifungal, especially in severe disease and immunocompromised host, at least for an overlap period prudent and promising.

Studies on the combination antifungals for invasive aspergillosis are still limited and conflicting. A randomized controlled trial done on patients with hematopoietic stem cell transplants or hematopoietic malignancies showed that combination of voriconazole with anidulafungin leads to a higher survival compared to voriconazole monotherapy [43], whereas Raad et al demonstrated that combination of voriconazole with caspofungin does not result in better outcomes compared with voriconazole alone, as primary or salvage therapy, in hematological malignancy patients [44].

In its latest guidelines on invasive aspergillosis, IDSA reminded the clinicians of the uncertainty of combinations of echinocandins with azole or polyene. These combinations showed conflicting results in the data currently available in the literature. Infectious disease specialists should be careful when prescribing such combinations because antagonism *in vivo* has not been completely refuted.

In summary, use of CAF in invasive aspergillosis is under investigations, and so far has been verified in salvage therapy. Physicians should be aware of the discordant result of many combinations when studied *in vivo* versus *in vitro*, and preferably refer to the most recent guidelines mentioned above until more concrete evidence on the use of CAF is available (Table 2) [9-11, 33-35, 41, 43].

Author Contributions

All authors certify that they participated sufficiently in the intellectual content, the analysis of data. Each author has reviewed this final version of the manuscript and approves it for publication.

Conflicts of Interest

The authors declare no conflicts of interest related to this manuscript.

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