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Review

The role of combination antifungal therapy in the treatment of invasive aspergillosis: a systematic review

Musa A. Garbati [a], Faisal A. Alasmari [a,b], Mohammad A. Al-Tannir [c], Imad M. Tleyjeh [a,b,c,d,*]

[a] Division of Infectious Diseases, Department of Medicine, King Fahd Medical City, Riyadh, Saudi Arabia
[b] Division of Infectious Diseases, Department of Medicine, Mayo Clinic, Rochester, Minnesota, USA
[c] Research Centre, King Fahd Medical City, PO Box 59046, Riyadh, Saudi Arabia
[d] Division of Epidemiology, Department of Health Sciences Research, Mayo Clinic, Rochester, Minnesota, USA

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SUMMARY

Background: Because treatment outcomes of invasive aspergillosis (IA) remain suboptimal, clinicians have resorted to the use of combination antifungal therapy. We therefore sought to systematically review the evidence that addresses the role of combination antifungal therapy in the treatment of invasive aspergillosis.

Methods: We retrieved the literature from MEDLINE, EMBASE, Web of Science, Cochrane Controlled Trials Register, and Scopus from inception up to March 2011 for cohort and randomized controlled trial (RCT) studies that assessed the efficacy of combination antifungal therapy for IA and reported on clinical outcomes.

Results: Eight studies (one RCT and seven cohort studies) that enrolled a total of 1071 patients met our inclusion criteria. Six cohort studies examined the role of combination therapy for the primary treatment of IA and two for salvage therapy. Various antifungal combinations were used, mainly azoles with either an echinocandin or a polyene. Of the seven cohort studies, four reported adjusted effect estimates, one of which showed a better outcome with combination antifungal therapy and one a trend towards a better outcome, while the remaining two revealed that there was no added advantage of combination antifungal therapy over monotherapy or a better response with monotherapy, respectively. The randomized controlled trial revealed that the use of combination therapy was associated with a better outcome.

Conclusion: Cumulative evidence supporting the use of combination antifungal therapy in IA is conflicting and of moderate strength. Well-designed RCTs are required to adequately address the issue of the usefulness of this approach.

1. Introduction

Aspergillus species have emerged as important causes of life-threatening infections, especially in immunocompromised patients.[1–3] The causative agents of invasive disease, *A. fumigatus*, *A. flavus*, *A. terreus*, and *A. niger*, cause a spectrum of infections ranging from chronic necrotizing pneumonia to invasive pulmonary aspergillosis (IPA) and other syndromes of tissue invasion. The rising incidence of invasive aspergillosis (IA) has led to significant compromise in treatment outcomes, especially in patients with hematologic malignancies and in hematopoietic stem cell transplant recipients.[4–8] Recent data from Upton et al.[9] showed that the probability of survival at 90 days in patients with IA diagnosed between 2002 and 2004 was higher compared with those diagnosed between 1990 and 2001; 44% vs. 22%, respectively ($p < 0.0001$). As the most common mould infection, IA has been the 'ground zero' of antifungal combination contention.

Amphotericin B deoxycholate (D-AMB) was the standard therapy for IA for decades, though with suboptimal responses and attendant side effects, especially in severely immunosuppressed patients.[10–13] Few randomized controlled trials (RCTs) exist on the treatment of IA, the largest of which demonstrated that voriconazole was superior to D-AMB as primary treatment for IA.[14] This has led to the recommendation of voriconazole as first-line therapy for IA by the Infectious Diseases Society of America (IDSA) (class A–level I evidence).[15] In patients who fail initial therapy, or who are intolerant of the initial regimen, second-line drugs are often used to replace the failing or intolerant agent. Administering both drugs simultaneously has not been well supported[16] and there are limited clinical data to support

* Corresponding author. Tel.: +966 1 2889999 ext. 1299.
E-mail addresses: Tleyjeh.imad@mayo.edu, itleyjeh@kfmc.med.sa (I.M. Tleyjeh).

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combination antifungal therapy over single-agent therapy for the primary treatment of IA.[17]

Combinations of azoles and echinocandins that target different cellular sites have been used in in vitro studies, animal models, case reports, and some studies in IA.[18–23] There is significant interest in combination antifungal therapy pairing an echinocandin with either an azole or amphotericin B formulation for the treatment of IA. Antifungal agents with distinct mechanisms of action offer the possibility of synergistic activity against invasive moulds when used in combination.[21,24–26] The possibility of antagonism is raised when some of these drugs are given in combination, particularly sequentially, with drugs blocking ergosterol synthesis given before the polyenes.[27–32] The most common reason for using or investigating combinations of antifungal agents is the hope of achieving synergy; however, detrimental effects, including attenuation of activity, increased resistance or toxicity, increased cost, and drug interactions, are hazards of combination therapy and must be carefully considered, along with the difficulty of interpreting much of the in vitro, animal, and clinical data in this arena. In the absence of a well-controlled, prospective clinical trial, the combination of antifungal drugs for primary therapy of IA is not routinely recommended by IDSA (class B–level II evidence), but may be considered in salvage situations.[15] The addition of a second antifungal agent to a first one that is failing or toxic is usually practiced out of understandable desperation. Nevertheless, limited in vitro, in vivo, and non-randomized clinical trial data suggest the benefit of some forms of combination therapy against IA.[17–23,33]

Given the dismal prognosis of patients with IA, various combinations of antifungal agents have been used to treat this ailment. However, the efficacy of this approach has not been indisputably documented. High cost, toxicity, and insufficient supporting evidence pose limitations to this approach. We therefore aimed to systematically review the available literature to critically evaluate and summarize the evidence for this approach in the treatment of IA.

2. Methods

2.1. Review question and study protocol

We aimed to systematically review the effectiveness of combination antifungal therapy in the treatment of IA in different patient populations. 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 of 2002[34] were used to review articles published before the 2008 revision. For the article published in 2010,[35] the revised definitions of 2008 were applied.[36] We included only those patients diagnosed with either proven or probable IA.

2.2. Eligibility criteria

We included cohort and RCT studies that assessed the efficacy of combination antifungal therapy and reported on clinical outcomes, without restriction to language. Two reviewers (MAG and FAA) independently assessed the eligibility and quality of studies addressing the role of combination antifungal therapy in the treatment of IA.

2.3. Search strategy

A senior experienced librarian searched MEDLINE, EMBASE, Web of Science, Cochrane Central Register of Controlled Trials, and Scopus from inception up to March 2011 for any relevant comparative studies (RCT and cohort studies) published in any language. The search was conducted using these key words: invasive SAME (aspergillosis OR aspergillus) AND (dual OR combination OR combined) AND (“anti-fungal” OR antifungal) AND Topic= (random OR blind OR trial OR cohort OR “case series” OR retrospective OR prospective OR evidence OR “meta-analysis”). We sought additional studies by reviewing the reference lists of eligible studies and relevant review articles. The complete search strategy is available on request from the authors. The search was extended to include all trials (completed and on-going) on combination antifungal therapy for IA from www.clinicaltrials.gov.

2.4. Screening and data extraction

Following completion of the search, all titles and abstracts were screened for possible inclusion in the review. Screening was based on the study being an RCT or a cohort study that was conducted on humans, and addressing our topic of interest. Studies were excluded only if the screener could be sure that it did not fulfill our inclusion criteria. Screening of titles and abstracts was conducted in duplicate by the two reviewers independently. Disagreement was resolved by subsequent discussion. A third author (IMT) resolved disagreements that could not be solved by consensus.

2.5. Quality assessment

The Newcastle–Ottawa quality assessment scale[37] for cohort studies is intended to assess for selection bias, comparability of the exposed and unexposed groups of each cohort, outcome assessment, and attrition bias. Two reviewers independently evaluated these components of the scale. There was 100% agreement ($\kappa = 1$). We assessed the quality of the RCT using the method of Jüni et al.[38]

3. Results

3.1. Search results

The initial search yielded 363 abstracts for screening, 328 of which were excluded: 114 were considered non-relevant, 149 were review articles or commentaries, 31 were animal or basic research, 30 studies had non-clinical outcomes, and 4 were guidelines. Thirty-five abstracts were considered for full review (Figure 1). Eight studies[9,13,20,23,35,39–41] (one RCT and seven cohorts) were eligible for final review (Table 1) with a total of 1071 patients with either proven or probable IA diagnosed according to the initial[34] and revised[36] definitions from the EORTC/MSG Consensus Group. The modified Newcastle–Ottawa quality assessment scale[37] was used to assess the methodological quality and the possibility of bias among the cohort studies. Table 2 shows that the included cohort studies were of good methodological quality and shared most of the characteristics examined.

The search was extended to include all trials (completed, terminated, withdrawn, and on-going) on combination antifungal therapy for IA from www.clinicaltrials.gov (Table 3). This yielded nine trials, three of which have been completed; two out of these were non-comparative trials while the third (COMBISTRAT) was a randomized pilot study and has been included in our review.[40] On the other hand, two of the trials were terminated due to inadequate enrollment and one trial was withdrawn prior to enrollment. Moreover, three intervention trials comparing combination and monotherapy in patients with IA are currently ongoing (Table 3). The first compares the efficacy of a combination of micafungin and voriconazole versus voriconazole/placebo (NCT01207128) and the second anidulafungin and voriconazole versus voriconazole monotherapy (NCT00531479); both are prospective, randomized, double-blind international trials that

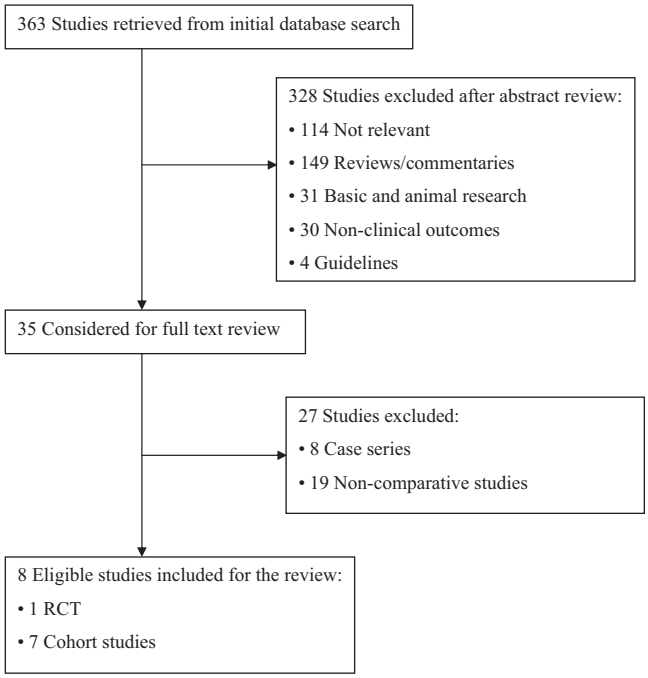

Figure 1. Flow chart of the study selection process.

are currently recruiting participants. Furthermore, the third, a randomized, open-label, single-center trial comparing the efficacy of anidulafungin and voriconazole versus voriconazole monotherapy in patients with IA aged 2 to 17 years (NCT01188759), is yet to start recruiting.

3.2. Characteristics of the included studies

A summary of the studies included in our systematic review is presented in Table 1. The sample sizes of the reviewed studies varied widely (21–405). The reviewed studies varied also in the antifungal drugs used in combination. Seven of the studies had a follow-up duration of 12 weeks, while the study duration was not mentioned in the eighth.[41] We compared the outcomes of the different antifungal regimens in different patient populations included in the review. Studies that used similar drugs were compared. Two groups of three studies compared voriconazole/caspofungin combinations[9,20,23] and amphotericin B/caspofungin,[35,39,40] respectively, while the remaining two studies compared the combinations of amphotericin B/itraconazole.[13,41]

The three studies[9,20,23] that used the combination of voriconazole and caspofungin compared it with either voriconazole or lipid formulation of amphotericin B as monotherapy. This combination was used in patients with hematologic malignancies and in solid organ transplant recipients. One study observed that the use of caspofungin and voriconazole combination therapy was associated with a significant reduction in mortality compared with monotherapy,[20] while another showed only a trend.[23] However, the study by Upton et al.,[9] did not show any significant difference between the combination and monotherapy groups. The most frequently observed adverse events in the combination group included transaminitis (22.5%), visual disturbances (15%), hallucinations (5%), and cutaneous rash (5%). These, however, did not lead to discontinuation of therapy.[23]

Among those treated with amphotericin B (as deoxycholate or lipid formulation) either alone or in combination with itraconazole,[13,41] no significant difference was observed in their response rates, even though no adjusted analyses were provided. Mild increases in total bilirubin and transaminases were noticed in the combination groups, while none in the monotherapy group.[41]

In a cohort of patients with hematologic malignancy and IA,[39] multiple regression analysis showed that the combination of a high-dose lipid formulation of amphotericin B (HD-LPD/AMB) and caspofungin was associated with a significantly lower likelihood of a favorable response than posaconazole salvage therapy (odds ratio 0.25, 95% confidence interval 0.07–0.91; $p = 0.03$). The study also showed that posaconazole was better tolerated than the combination, with significantly lower rates of renal and hepatic toxicity ($p \leq 0.02$).

In the study by Mihu et al.,[35] a favorable response was achieved in 21% of patients treated with a combination of a lipid formulation of amphotericin B (L-AMB) and echinocandins at the end of 12 weeks of salvage therapy vs. 28% of patients treated with echinocandins and 9% of those treated with L-AMB ($p = 0.04$, unadjusted analysis). Overall toxicity in the combination group was 31% vs. 6% in the echinocandin group and 26% in the L-AMB group ($p = 0.03$).

In the RCT,[40] 30 patients (21 men and nine women) with hematologic malignancies were prospectively randomized in an open trial (COMBISTRAT) to receive either a combination of a standard dose of liposomal amphotericin B (3 mg/kg/day) and caspofungin, or monotherapy with a high-dose amphotericin B (10 mg/kg/day). At the end of treatment, there were significantly more favorable overall responses (partial or complete responses; $p = 0.028$) in the combination group (10 of 15 patients; 67%) compared with the high-dose monotherapy group (four of 15 patients; 27%). Survival rates at 12 weeks after inclusion were 100% and 80%, respectively, among those on combination and monotherapy.

4. Discussion

Our systematic review identified eight comparative studies (one RCT and seven cohorts) published in any language up to March 2011 that assessed the efficacy of combination antifungal therapy for the treatment of IA among various hosts. Six studies[9,13,23,39–41] examined the role of combination therapy for primary IA and two for salvage therapy.[20,35] Of the seven cohort studies, four reported adjusted effect estimates,[9,20,23,39] one of which showed a better outcome with combination antifungal therapy[20] and one a trend towards a better outcome,[23] while the remaining two revealed that there was no added advantage of combination antifungal therapy over monotherapy[9] or a better response with monotherapy.[39] In one pilot RCT,[40] combination therapy was associated with a better outcome. Different antifungal combinations were used where caspofungin was added to either voriconazole or amphotericin B products. Given the heterogeneity of the treatment regimens, patient populations, endpoints, and conflicting results, the cumulative evidence is not strong enough to support routine combination therapy for primary IA. Our findings are consistent with the recent IDSA guidelines, which stated that there are limited clinical data to support combination antifungal therapy over single-agent therapy for the primary treatment of IA.

An international multicenter, randomized, double-blind, controlled trial (NCT00531479) comparing the safety and efficacy of anidulafungin plus voriconazole versus voriconazole monotherapy for the treatment of IA is currently recruiting. Another single-center, randomized, double-blind, placebo-controlled trial (NCT01207128) of voriconazole and micafungin versus voriconazole monotherapy (plus placebo) in IA is also recruiting. A single-center study of the efficacy of voriconazole and anidulafungin combination versus voriconazole monotherapy for IA in pediatric subjects aged 2 to 17 years is yet to start recruiting patients at the time of writing this

Table 1
Characteristics of included studies

Reference	Study population	Sample size	Female, *n* (%)	Age range or mean (years)	Study design	Indication for combination therapy	Treatment category		Follow-up duration (weeks)	Outcome of combination vs. monotherapy	Adjusted effect estimates
							Combination	Monotherapy			
Popp et al., 1999[41]	Hematologic malignancy	21	8 (38)	44.95	Cohort	Primary	AMB 1 mg/kg/day + itraconazole 400 mg/day caps or suspension	AMB 1 mg/kg/day	NR	Favorable response	No adjusted analysis; univariate analysis: 82% vs. 50%, $p = 0.12$
Marr et al., 2004[20]	Hematologic malignancy	47	29 (61.7)	16–66	Cohort	Salvage	Voriconazole 6 mg/kg q12h IV for 1 day, then 4 mg/kg q12h ± caspofungin 70 mg IV for 1 day, then 50 mg/day	AMB 1 mg/kg/day and LFABs 5 mg/kg/day for those with pre-existing renal disease or intolerance to AMB	12	Mortality	HR 0.28, 95% CI 0.1–0.92
Kontoyiannis et al., 2005[13]	Hematologic malignancy	179	112 (62.6)	30–66	Cohort	Primary	LipoAMB 5 mg/kg/day + itraconazole 200 mg IV/PO bid, then 200 mg qd	LipoAMB 5 mg/kg/day	12	Favorable response	No adjusted analysis; univariate analysis: 0% vs. 10%, p = not significant
Singh et al., 2006[23]	Organ transplant recipients	87	36 (41.4)	19–68	Cohort	Primary	Voriconazole 6 mg/kg q12h IV for 1 day, then 4 mg/kg q12h + caspofungin 70 mg IV on day 1, then 50 mg/day	LipoAMB 5.2 mg/kg/day	12	Mortality	HR 0.58, 95% CI 0.3–1.14, $p = 0.11$
Upton et al., 2007[9]	Bone marrow transplant recipients	405	165 (40.7)	42.2	Cohort	Primary	Voriconazole ± caspofungin	Voriconazole (before 1996: AMB 0.5 mg/kg/day; after 1996: LipoAMB 5 mg/kg/day)	12	Mortality	HR 2.3, 95% CI 0.6–9.4, $p = 0.23$
Raad et al., 2008[39]	Hematologic malignancy	143	24 (16.8)	43.7	Cohort	Primary	HD-LPD/AMB ≥7.5 mg/kg/day ± caspofungin 70 mg on day 1 and 50–100 mg/day	Posaconazole (salvage) 400 mg PO q12h as outpatient or 200 mg by NG tube q6h or 800 mg q24h	12	Favorable response	OR 0.25, 95% CI 0.07–0.91, $p = 0.03$
Caillot et al., 2007[40]	Hematologic malignancy	30	9 (30)	16–75	RCT	Primary	LipoAMB 3 mg/kg/day IV + caspofungin 70 mg IV for 1 day, then 50 mg/day	LipoAMB 10 mg/kg/day	12	Favorable response	4/15 (27%) vs. 10/15 (67%), $p = 0.028$
Mihu et al., 2010[35]	Hematologic malignancy	141	54 (38.3)	9–79	Cohort	Salvage	L-AMB + echinocandin (90% caspofungin) (no dosage)	L-AMB (no dosage) or echinocandin (89% caspofungin) (no dosage)	12	Mortality	No adjusted estimates; 62% combination vs. 61% echinocandins vs. 67% L-AMB, $p = 0.78$
			39 (44)	9–80						Favorable response	No adjusted estimates; 21% combination vs. 28% echinocandins vs. 9% L-AMB, $p = 0.04$

AMB, conventional amphotericin B; bid, twice daily; 95% CI, 95% confidence interval; HD-LPD/AMB, high-dose lipid formulation of amphotericin B; HR, hazard ratio; IV, intravenous; L-AMB, lipid formulation of amphotericin B; LFABs, amphotericin B lipid complex or liposomal amphotericin B; LipoAMB, liposomal amphotericin B; NR, not reported; OR, odds ratio; NG tube, nasogastric tube; PO, per os (oral); q6 h, every 6 h; q12 h, every 12 h; q24 h, every 24 h; qd, four times daily; RCT, randomized controlled trial.

Table 2
Modified Newcastle–Ottawa quality assessment scale for cohort studies included in the review

Reference	Selection[a]				Comparability[b]	Outcome[c]		
	Representativeness of the exposed cohort	Selection of the non-exposed cohort	Ascertainment of exposure	Incident disease		Assessment of outcome	Length of follow-up	Adequacy of follow-up
Popp et al.,1999[41]	A	A	A	A	A	B	NR	NR
Marr et al., 2004[20]	A	A	A	A	A	B	A	A
Kontoyiannis et al., 2005[13]	A	A	A	A	A	B	A	A
Singh et al., 2006[23]	A	A	A	A	A	B	A	A
Upton et al., 2007[9]	A	A	A	A	A	B	A	A
Raad et al., 2008[39]	A	A	A	A	A	B	A	A
Mihu et al., 2010[35]	A	A	A	A	A	B	A	A

NR, not reported.
[a] Selection: (1) *Representativeness of the exposed cohort*: A, truly representative of the average patient; B, somewhat representative of the average patient; C, selected group; D, no description of the derivation of the cohort. (2) *Selection of the non-exposed cohort*: A, drawn from the same community as the exposed cohort; B, drawn from a different source; C, no description of the derivation of the non-exposed cohort. (3) *Ascertainment of exposure*: A, secure record (e.g., surgical record); B, structured interview; C, written self-report; D, no description. (4) *Incident disease*, for demonstration that the outcome of interest was not present at start of study: A, yes; B, no.
[b] Comparability: For comparability of cohorts on the basis of the design or analysis: A, study controls for co-morbidities; B, study controls for any additional factor (e.g., age and severity of illness); C, not done.
[c] Outcome: (1) *Assessment of outcome:* A, independent blind assessment; B, record linkage; C, self-report; D, no description. (2) *Length of follow-up*, was follow-up long enough for outcomes to occur?: A, yes; B, no. (3) *Adequacy of follow-up*: A, complete follow-up and all subjects accounted for; B, subjects lost to follow-up but unlikely to introduce bias because a small number were lost (i.e., >90% were available for follow-up) or a description was provided of those lost; C, follow-up rate 90% or lower and no description of those lost; D, no statement.

Table 3
List of studies on combination antifungal therapy and their status from www.clinicaltrials.gov

Identifier	Status	Study	Condition	Intervention (drugs)	
				Monotherapy	Combination
NCT00334412	Completed	COMBISTRAT: AmBisome® in combination with caspofungin for the treatment of invasive aspergillosis	Invasive aspergillosis	AmBisome®	AmBisome® + caspofungin
NCT00047827	Terminated	Trial of micafungin (FK463) in combination with liposomal amphotericin B (AmBisome®) for aspergillosis	Aspergillosis	Non-comparative	Micafungin + LPD-amphotericin B
NCT00076869	Completed	MK0991 in combination with standard antifungal agent(s) for the treatment of salvage invasive aspergillosis	Aspergillosis	Non-comparative	MK0991; caspofungin, amphotericin B or liposomal amphotericin B and/or azole
NCT00531479	Recruiting	Anidulafungin plus voriconazole versus voriconazole for the treatment of invasive aspergillosis	Aspergillosis	Voriconazole	Voriconazole + anidulafungin
NCT00620074	Terminated; has results	Study to test the combination of voriconazole and anidulafungin in patients who have, or are thought to have, invasive aspergillosis and who are unable to take a common antifungal therapy (polyene)	Aspergillosis	Voriconazole	Voriconazole + anidulafungin
NCT00423163	Withdrawn	A study to evaluate the effectiveness of voriconazole + micafungin versus voriconazole alone for invasive aspergillosis	Aspergillosis/blood aspergillosis/invasive	Voriconazole	Voriconazole + micafungin
NCT00037206	Completed	A safety and effectiveness study of intravenous anidulafungin with AmBisome® for treatment of IA	Aspergillosis	Non-comparative	Anidulafungin + AmBisome®
NCT01207128	Recruiting	Trial of combination antifungal therapy (Vori + Mica vs. Vori + Placebo) in invasive aspergillosis	Invasive aspergillosis	Voriconazole + placebo	Voriconazole + micafungin
NCT01188759	Not yet recruiting	Voriconazole and anidulafungin combination for invasive aspergillosis in pediatric subjects	Invasive aspergillosis	Voriconazole	Voriconazole + anidulafungin

LPD-amphotericin B, lipid formulation of amphotericin B; IA, invasive aspergillosis; Vori, voriconazole; Mica, micafungin.

manuscript. Once these studies are completed, it is hoped that the role of combination antifungal therapy will become clearer.

In conclusion, cumulative evidence supporting the use of combination antifungal therapy in IA is conflicting and of moderate strength. Well-designed controlled, randomized, multicenter clinical trials are required to adequately address the issue of the usefulness of this approach. Data from ongoing RCTs to address this controversy are patiently awaited.

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