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Aerosolized amphotericin B as prophylaxis for invasive pulmonary aspergillosis: a meta-analysis

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SUMMARY

Objectives: Invasive pulmonary aspergillosis (IPA) is associated with high mortality in high-risk (immunosuppressed) patients. Many studies have investigated whether prophylactic inhalation of amphotericin B (AMB) reduces the incidence of IPA, but no definitive conclusions have been reached. The present meta-analysis was performed to evaluate the efficacy of prophylactic inhalation of AMB for the prevention of IPA.

Methods: MEDLINE and other databases were searched for relevant articles published until December 2013. Randomized controlled trials that compared aerosolized AMB with placebo were included. Two reviewers independently assessed and extracted the data of all trials.

Results: Six animal studies and two clinical trials involving 768 high-risk patients were eligible. The animal studies showed lower overall mortality rates among animals that underwent aerosolized AMB prophylaxis (odds ratio (OR) 0.13, 95% confidence interval (CI) 0.08–0.21). Similarly, the clinical trials showed a lower incidence of IPA among patients who underwent aerosolized AMB prophylaxis (OR 0.42, 95% CI 0.22–0.79).

Conclusions: This analysis provides evidence supporting the notion that the prophylactic use of aerosolized AMB effectively reduces the incidence of IPA among high-risk patients.

1. Introduction

Invasive aspergillosis (IA) is an increasingly frequent cause of morbidity and mortality in immunosuppressed patients, especially those undergoing solid organ or hematopoietic stem cell transplantation and those with prolonged neutropenia.[1] Invasive pulmonary aspergillosis (IPA) is the most common form of IA. Despite the fact that new non-invasive laboratory methods have been developed to improve the diagnostic yield, including the Aspergillus galactomannan assay, the (1,3)-β-D-glucan assay, and PCR techniques, IPA remains associated with a high fatality rate. In one systematic review, 70% of 1941 patients with aspergillosis exhibited pulmonary involvement, and the case-fatality rate was >60% despite the administration of intensive antifungal therapy.[2] Therefore, prophylactic therapy is important in high-risk patients. However, there is no consensus on the optimal agent or administration route.

Amphotericin B (AMB) was the first commercially significant antifungal drug. It has a broad spectrum of activity against many different fungal species and has been the standard IA treatment for decades.[3] Although new agents such as voriconazole and itraconazole have been recommended for patients with IPA, AMB is still considered to be the primary therapeutic agent for some patients and is included in many prophylactic regimens for fungal infection.[4] One study showed that the prophylactic administration of intravenous AMB to patients undergoing bone marrow transplantation was associated with fewer fungal microorganisms and higher survival rates; however, significantly greater numbers Q3 of infusion-related side effects occurred.[5] Therefore, aerosolized AMB represents an attractive alternative for the prevention of IPA because the administration of drugs by inhalation ensures a high drug concentration in the respiratory tract and a lower incidence of side effects.

Since the 1990s, many studies have been conducted to elucidate the feasibility, tolerability, and effectiveness of aerosolized AMB for the prevention of Aspergillus infection.[6–11] A retrospective study of 99 patients who underwent heart transplantation with no prophylaxis and 120 patients who underwent prophylactic

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inhalation of AMB demonstrated a significant difference between the two groups; prophylaxis with AMB effectively prevented IPA.[12] Another retrospective study evaluated the impact of prophylactic AMB inhalation on IA in 611 recipients of allogeneic stem cell transplantation and examined the recipients' tolerance of the inhalation therapy. The incidence of IA was lower in the prophylactic AMB inhalation group than in the placebo group, and the inhalation therapy was well tolerated.[13] However, other studies have reached different conclusions. In another study that investigated the effectiveness of aerosolized AMB as prophylaxis against IPA, 28% of the patients developed proven or possible infections. Inhalation of AMB does not appear to be useful in preventing IPA in patients with granulocytopenia.[14]

The present meta-analysis was performed to assess the prophylactic effect of aerosolized AMB against IPA by examining the IPA-associated mortality among immunocompromised animals and the incidence of IPA among high-risk patients.

2. Materials and methods

2.1. Search strategy

Two separate electronic searches were conducted to identify eligible studies. MEDLINE, Embase, the Chinese Biomedical Literature Database, and the Cochrane Library were searched for relevant articles published until December 25, 2013. The following search terms were used: "inhaled" or "inhalational" or "aerosol" or "aerosolized" or "nebulized" or "nebulization" and "amphotericin". No limitations were placed on language or year. The reference lists of related reviews and original papers were also checked for relevant trials.

2.2. Study selection

The following inclusion criteria were established before article collection. Animal studies were required to (1) be randomized controlled trials, (2) compare aerosolized AMB with placebo, (3) administer aerosolized AMB before exposure to *Aspergillus fumigatus* conidia, and (4) provide the number of animals sacrificed. Human studies were required to (1) be randomized controlled trials, (2) include adult patients (aged >18 years) scheduled to receive chemotherapy with an anticipated duration of neutropenia $<0.5 \times 10^9$ cells/l of ≥10 days, (3) compare aerosolized AMB with placebo, and (4) administer aerosolized AMB before any signs of proven or probable IPA. When an individual author published several articles involving the same patient population, only the most complete article was included. Studies that did not meet the above-described inclusion criteria were excluded from the meta-analysis.

2.3. Quality assessment

Clinical randomized controlled trials were assessed using the Jadad scale.[15] This scale is used to assess trials according to the following three questions: (1) Was the study described as randomized (i.e., did it use the terms 'randomly', 'random', or 'randomization')? (0–2 points); (2) Was the study described as double-blind? (0–2 points); (3) Was there a description of withdrawals and dropouts? (0–1 point). A study can receive a maximum Jadad score of 5 points.

2.4. Data extraction

Two reviewers (DX and WKS) independently carried out the data extraction and validity assessment, and any discrepancies were resolved by discussion. For the animal studies, a piloted data extraction form was used to collect information on the first author, year of publication, animal species, number of animals in each group, method of inducing immunosuppression, details of experimental drug and placebo treatments, follow-up duration, and final mortality rate. For the clinical trials, a data extraction form was used to collect information on the first author, year of publication, country of origin, Jadad score, number of patients in each group, and incidence of IPA.

2.5. Statistical analysis

The results of prophylaxis for dichotomous outcomes are expressed as odds ratios (ORs) with 95% confidence intervals (CIs) for both the animal studies and clinical trials. The I^2 statistic was used to determine the extent of inconsistency and thus assess the heterogeneity between trials. We considered an I^2-value of >50% and a p-value of <0.1 to indicate heterogeneity. A fixed-effects model was used to estimate the effects of aerosolized AMB. However, if significant heterogeneity was present, a random-effects model was used to generate a more conservative estimate.

Publication bias among the randomized controlled trials involving animals was examined by visual inspection of a funnel plot. Publication bias was suspected when the funnel plot was asymmetrical; in such cases, Egger's test was performed for further analysis of bias.

Sensitivity analyses were conducted by comparing the estimates derived from the random- and fixed-effects models. One study that used AMB inhalation powder (ABIP) as the prophylactic drug was excluded from the sensitivity analyses because this drug is not widely used.

Subgroup analyses of the animal studies were performed to explore important differences that might be expected to alter the magnitude of the prophylactic effect.

3. Results

3.1. Study selection and characteristics

Figure 1 shows the study selection process. In total, 1362 potentially relevant citations were identified from the electronic search, 1348 of which were determined to be non-relevant after reading the titles and abstracts. The remaining 14 studies underwent full review by the two above-mentioned independent reviewers. Eight of these 14 studies met the inclusion criteria and were subjected to the meta-analysis.[16–23] Six studies were initially thought to fulfill the inclusion criteria, but were excluded after detailed examination. One study was not a randomized controlled trial,[24] one evaluated the therapeutic rather than the prophylactic efficacy of aerosolized AMB,[25] one evaluated the beneficial effect of intravenous rather than aerosolized AMB,[26] one evaluated the beneficial effect of aerosolized AMB on the fungal burden rather than on mortality,[27] and two were duplicate publications.[10,28] Of the eight remaining eligible studies, six were animal randomized controlled trials[16–21] and two were human randomized controlled trials.[22,23]

In all six animal studies, a systemic steroid and/or cyclophosphamide was used to induce immunosuppression. The fungal inoculation and drug administration methods were described in detail. The various formulations of aerosolized AMB were AMB desoxycholate (AMB-d), liposomal AMB (L-AMB), AMB lipid complex (ABLC), AMB colloidal dispersion (ABCD), and ABIP. Table 1 lists the details of the six animal studies included in this meta-analysis.

In both of the human studies, randomization was performed using a computer-generated blocked list. Both studies included a description of the patients who withdrew from or dropped out of

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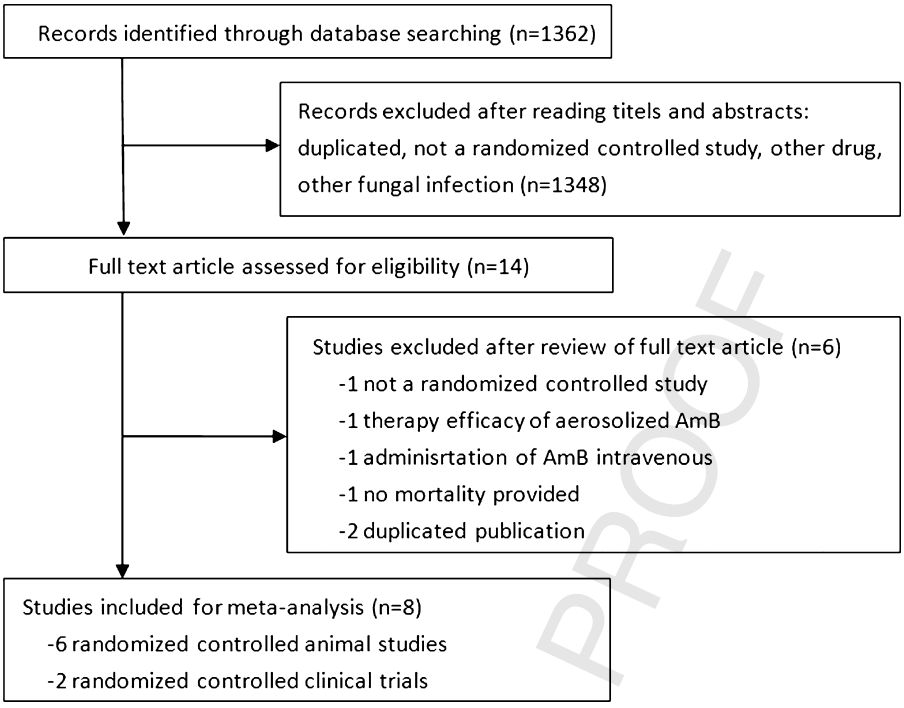

Figure 1. Flow diagram of study selection method
Q7 AMB: amphotericin B

the study, but only one trial was double-blind.[23] L-AMB was used in one trial and AMB-d in the other. The details of these two trials are given in Table 2. Both clinical trials mentioned the potential toxic effects of the therapy, and one described the toxic effects in detail.[22] For the patients who received aerosolized L-AMB, the median serum creatinine levels after the last inhalation were not greater than the baseline levels, but coughing was observed more frequently than at baseline.[23] About two-thirds of patients who received aerosolized AMB-d reported at least one unpleasant sensation such as coughing, a bad taste, nausea, or others.[22] No serious drug-related adverse events were reported.

3.2. Meta-analysis results

Six studies of immunosuppressed animals were eligible for inclusion in the meta-analysis. The overall mortality of animals treated with prophylactic inhalation of AMB was lower than that of animals treated with placebo. No heterogeneity was observed (I^2 = 7%, p = 0.36) and a fixed-effects model was used. The combined OR for all six eligible studies was 0.13 (95% CI 0.08–0.21; $p < 0.00001$) (Figure 2), indicating that the prophylactic use of aerosolized AMB was effective in immunocompromised animals. No significant difference (p = 0.28) was observed between the effectiveness of

Table 1
Characteristics of the animal studies included in the meta-analysis

Author (year)	Animal	Method of inducing immunosuppression	Aspergillus inoculum dose	AMB administration dose and time prior to pulmonary inoculation	Time point of mortality measure
Schmitt (1988)	Rat	Steroid (100 mg/kg) administered 2 weeks before fungal inoculation and continued throughout the experiment	10^6 conidia	AMB-d: 1.6 mg/kg 2 days	21 days after fungal inoculation
Niki (1991)	Rat	Steroid (150 mg/kg) administered three times weekly, 2 weeks before and 1 week after fungal inoculation	10^6 conidia	AMB-d: 1.6 mg/kg 48 h	4 weeks after fungal inoculation
Allen (1994)	Mouse	Steroid (150 mg/kg) administered 1 day before until 1 day after fungal inoculation	1.4×10^6 1.5×10^7 1.3×10^8 conidia	L-AMB: 6.05 mg/kg AMB-d: 6.73 mg/kg 1, 2, and 3 days	9 days after fungal inoculation
Cicogna (1997)	Rat	Steroid (150 mg/kg) administered for 2 weeks until the day of fungal inoculation or steroid (150 mg/kg) administered for 2 weeks before fungal inoculation and continued throughout the experiment	10^6 conidia	ABLC: 0.4, 0.8, and 1.6 mg/kg AMB-d: 1.6 mg/kg ABLC: 1.6 mg/kg 2 days	14 days after fungal inoculation
Ruijgrok (2005)	Rat	Cyclophosphamide (90 mg/kg) administered 5 days before and cyclophosphamide (60 mg/kg) administered every 4 days after fungal inoculation	1.5×10^5 conidia	AMB-d: 2 mg/ml L-AMB: 4 mg/ml ABLC: 4 mg/ml 1, 2, and 6 weeks	12 days after fungal inoculation
Kirkpatrick (2012)	Guinea pig	Cyclophosphamide (250 mg/kg) and steroid (250 mg/kg) administered 2 days before and 3 days after fungal inoculation	1×10^8 conidia	ABIP: 0.05, 0.50, 4.00, and 10.00 mg/kg 24 h	11 days after fungal inoculation

AMB, amphotericin B; AMB-d, amphotericin B desoxycholate; L-AMB, liposomal amphotericin B; ABLC, amphotericin B lipid complex; ABIP, amphotericin B inhalation powder.

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Table 2
Characteristics of clinical trials included in the meta-analysis

Author (year)	Study period	Study region	Patients	Jadad quality score	Treatment group, IPA/total	Placebo group, IPA/total
Schwartz (1999)	1993/03–1996/04	Germany	Adult patients with hematological disease and neutropenia	3	10/227	11/155
Rijnders (2008)	2000/11–2006/01	Netherlands	Adult patients with hematological disease or solid tumors and neutropenia	5	6/139	18/132

IPA, invasive pulmonary aspergillosis.

AMB-d (OR 0.07, 95% CI 0.03–0.18; $p < 0.00001$) (Figure 3A) and that of lipid-associated AMB formulations (OR 0.06, 95% CI 0.03–0.14; $p < 0.00001$) (Figure 3B).

Two human trials involving 768 high-risk patients were eligible for inclusion in the meta-analysis. The incidence of IPA in patients who underwent administration of aerosolized AMB during neutropenic episodes was lower than that of patients who underwent administration of placebo (4.4% vs. 10.4%, respectively). No evidence of heterogeneity was observed ($I^2 = 21\%$, $p = 0.26$), and a fixed-effects model was used. Aerosolized AMB demonstrated a significant preventive advantage over placebo in terms of a lower incidence of IPA (OR 0.42, 95% CI 0.22–0.79; $p = 0.007$) (Figure 4).

3.3. Sensitivity analyses

Despite the absence of statistical heterogeneity, significant trial heterogeneity was present across the analyzed studies (different animal types, AMB formulations, AMB doses, etc.). Therefore, we performed a sensitivity analysis of the six animal studies by repeating the main computations using a random-effects model. The random-effects model did not significantly change the results of our meta-analysis. Similarly, other sensitivity analyses showed no changes in the results after exclusion of specific studies (Table 3). Therefore, the results of this meta-analysis are stable.

3.4. Publication bias

The funnel plot of the animal studies was asymmetrical, suggesting possible publication bias (Figure 5). Egger's test was then performed to check for bias; the result was significant ($p = 0.01$), again suggesting possible publication bias.

4. Discussion

This meta-analysis has shown that aerosolized AMB can help to prevent IA in both immunocompromised animals and high-risk

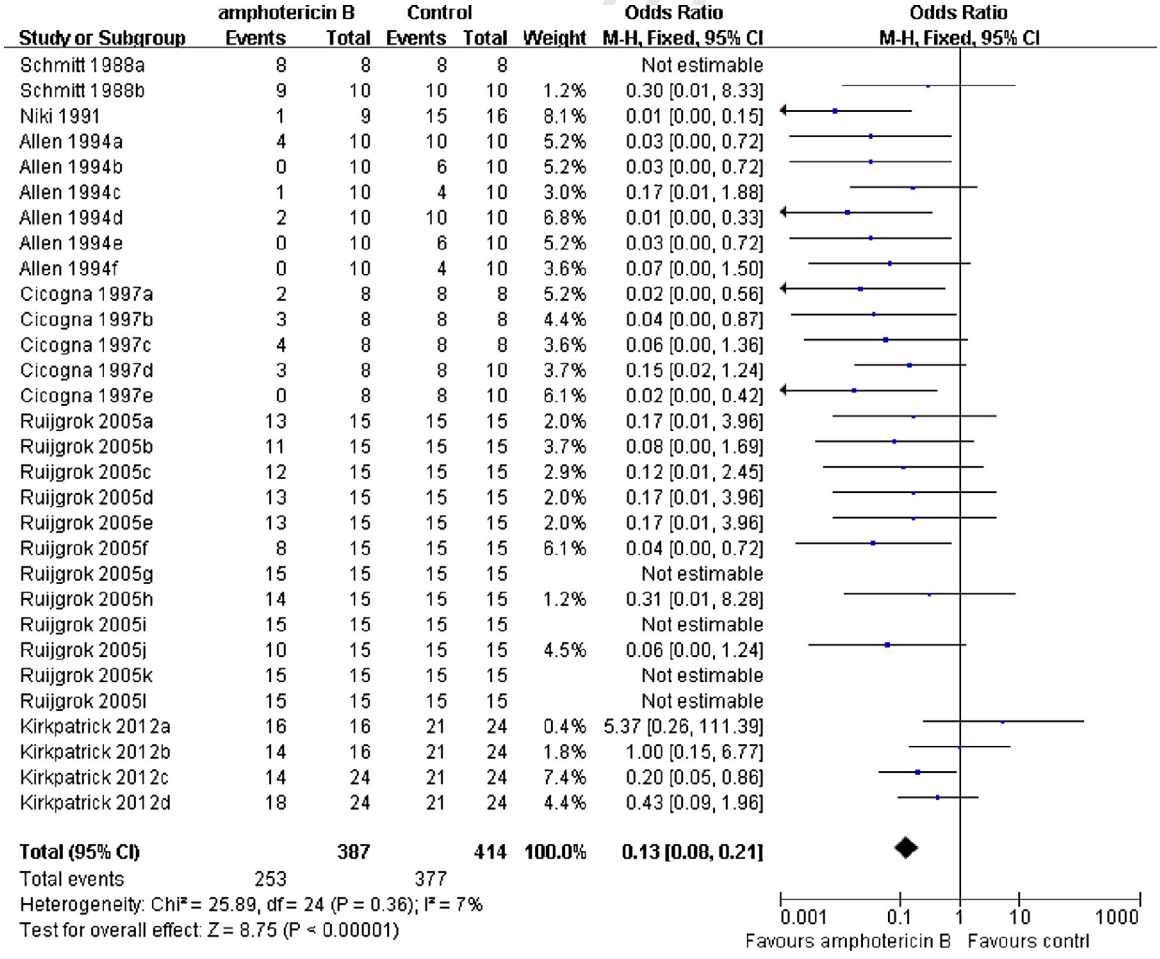

Figure 2. Forest plot showing effect of prophylactic aerosolized amphotericin B on mortality of immunosuppressed animals
M-H: Mantel–Haenszel analysis, CI: confidence interval

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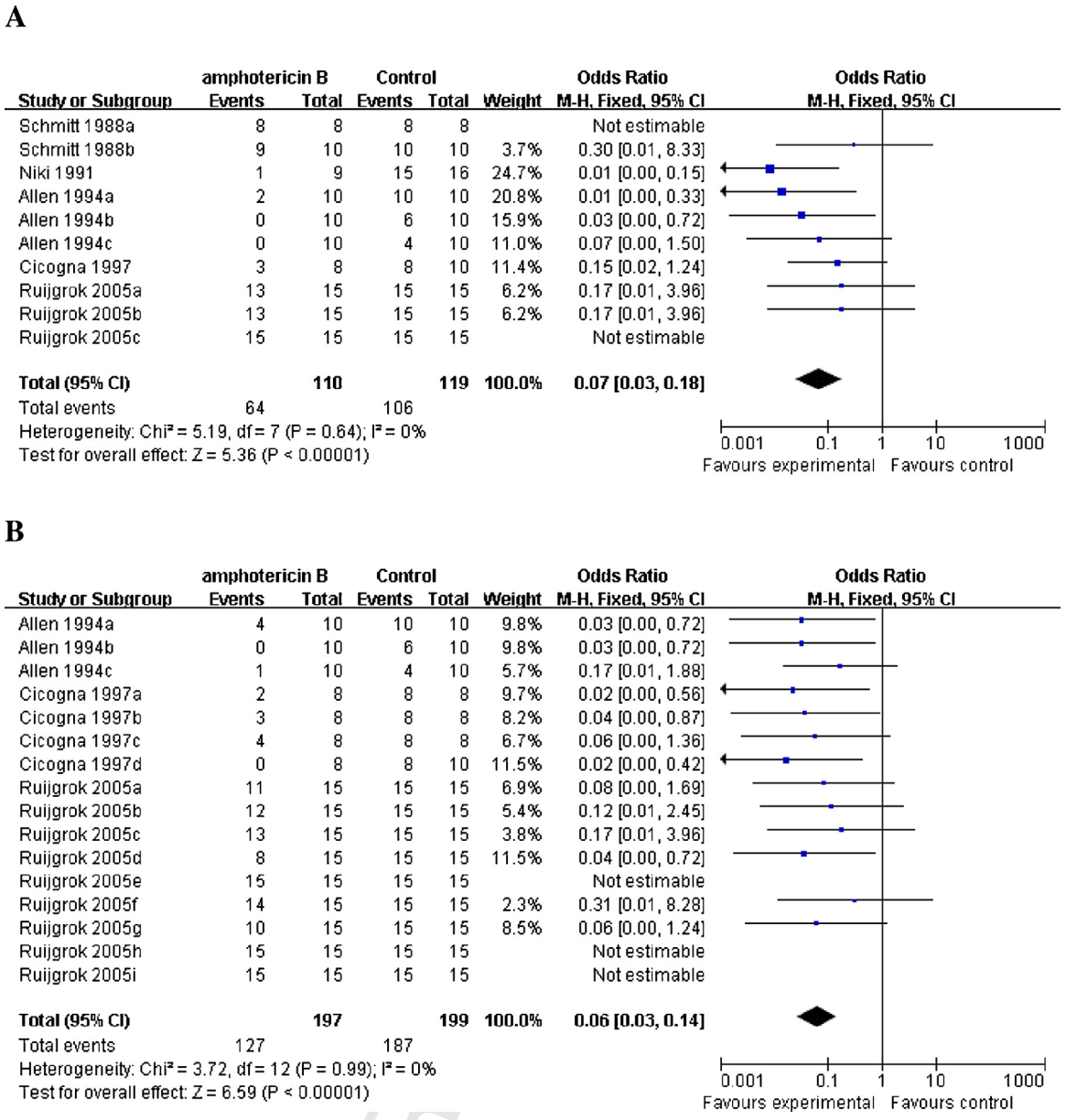

Figure 3. Forest plot showing effect of prophylactic aerosolized amphotericin B desoxycholate (A) and lipid-associated amphotericin B (B) on mortality of immunosuppressed animals.
M-H: Mantel–Haenszel analysis, CI: confidence interval

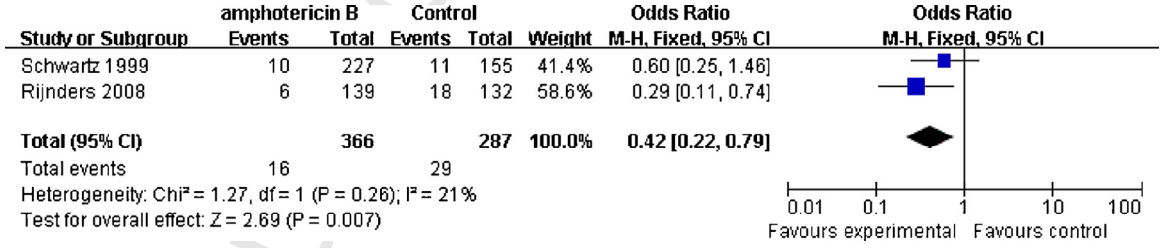

Figure 4. Forest plot showing effect of prophylactic aerosolized amphotericin B on incidence of invasive pulmonary aspergillosis in high-risk patients
M-H: Mantel–Haenszel analysis, CI: confidence interval

patients. Such high-risk patients include recipients of hematopoietic stem cell or solid organ transplantation, patients with malignancies undergoing intensive chemotherapy, and patients with other causes of immunosuppression. Asymptomatic patients with Aspergillus galactomannan in the bronchoalveolar lavage fluid or serum may especially benefit from the prophylactic use of aerosolized AMB.

Aerosolized AMB is relatively safe. To the best of our knowledge, no serious drug-related adverse events have been reported in association with its prophylactic use. In one study, patients who

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Table 3
Sensitivity analyses for the primary outcome

	Number of studies	RR (95% CI)	*p*-Value
Random-effects model	6	0.13 (0.07–0.22)	<0.00001
Exclusion of one study that used ABIP	5	0.06 (0.03–0.12)	<0.00001

RR, risk ratio; 95% CI, 95% confidence interval; ABIP, amphotericin B inhalation powder.

underwent administration of aerosolized AMB as antifungal prophylaxis considered the inhalations to be unpleasant mostly because of a bad taste or the development of coughing. However, the inhalation therapy was feasible and safe, and no severe side effects occurred.[29] Monforte et al. found that nebulized L-AMB for prophylactic treatment of Aspergillus infection exhibits neither significant systemic absorption nor adverse effects on respiratory function.[30] They also found that nebulized L-AMB does not change the lipid content of pulmonary surfactant. This agent safely and effectively prevents *Aspergillus spp* infection in lung transplant recipients.[31]

Different AMB formulations may have different clinical effects because each has a distinct pharmacological profile. Drew et al. concluded that patients who received AMB-d are more likely to experience adverse events.[32] However, our subgroup analysis of animal studies showed no significant difference between AMB-d and lipid-associated AMB formulations. This result is consistent with that obtained in a previous observational study in which 104 consecutive patients who underwent prophylaxis with aerosolized L-AMB were compared with 49 historical control subjects who received aerosolized AMB-d. The two groups exhibited similar rates of Aspergillus infection and side effects such as transitory breathing difficulty, nausea, and bronchospasm.[33] One systematic review and meta-analysis showed no difference between the adverse events associated with inhaled AMB-d and those associated with lipid formulations of inhaled AMB.[34] A worldwide survey on antifungal prophylaxis in patients undergoing lung transplantation also revealed that inhaled lipid formulations of AMB are effective and being used with increased frequency.[35] Therefore, lipid formulations may be more effective than AMB-d in preventing IPA.

New formulations of aerosolized AMB have recently been developed. Lipid nanoemulsions may serve as successful nanocarriers for the delivery of AMB to the peripheral airways.[36] Nonionic surfactant vesicles that deliver AMB to the lungs reportedly enhance pulmonary delivery while minimizing systemic exposure and toxicity.[37] Further studies comparing the effects of these various formulations on the prevention of IA are also needed.

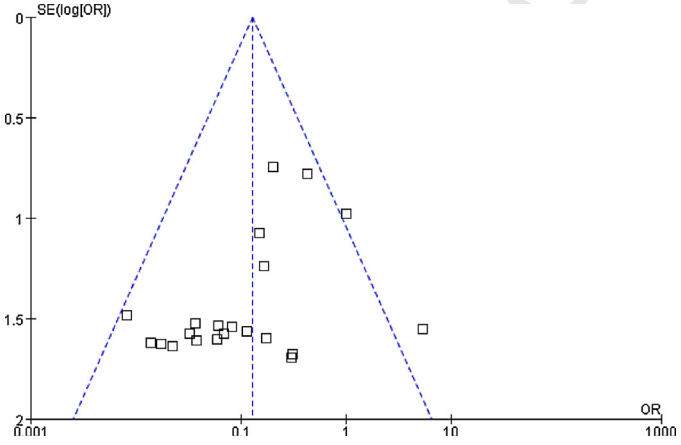

Figure 5. Funnel plot showing absence of small negative studies suggestive of small publication bias.

New antifungal agents in the azole group have been developed in recent years; they are also prescribed as prophylaxis against IPA. Neoh et al.[38] conducted a retrospective cohort study to explore the effect of prophylactic voriconazole in lung transplant recipients. They concluded that preemptive voriconazole treatment resulted in a lower incidence of IA and a lower IA-related mortality rate. Another retrospective study suggested that the routine use of prophylactic voriconazole against Aspergillus infection in lung transplant recipients did not appear to be warranted.[39] Additionally, single-agent itraconazole treatment in heart or lung transplant recipients did not affect the incidence of fungal infection as compared with a control group.[40] To the best of our knowledge, no definitive guidelines on the prophylactic use of azole agents in IPA have been established, and few studies comparing aerosolized AMB and azole agents for the prevention of IPA have been performed. One study assessed the efficacy of an inhaled aqueous solution of voriconazole as prophylaxis against IPA in a murine model.[41] Rodents with IPA that underwent treatment with inhaled voriconazole demonstrated significantly higher survival than did control rodents and those treated with AMB. However, AMB-d was administered intraperitoneally. Therefore it remains unknown whether inhaled voriconazole is superior to aerosolized AMB. Clinical trials comparing azoles and inhaled AMB are essential to shed light on the question of which agent and administration method is optimal for IPA prophylaxis. Meanwhile, cost should be taken into consideration when comparing different antifungal agents.

Some limitations of this meta-analysis should be noted. First, because the analysis was limited to the published scientific literature, the potential impact of publication bias cannot be ignored. Publication bias is a known threat to the validity of all forms of meta-analysis. Journals tend to accept positive results, while negative results are often rejected or not even submitted by authors. Second, none of the animal studies in the present meta-analysis explicitly described the blinding or allocation concealment methods used. Future studies should clearly explain the details of their blinding and allocation concealment methods. Third, all placebo groups in the present studies were used more than once to compare the effects of the different interventions. Both the placebo and experimental subgroups in some eligible studies exhibited a 100% mortality rate. However, this high mortality rate was not included in the overall estimate of this meta-analysis, making the combined OR appear to be much stronger. Fourth, the number of eligible clinical trials was small. Thus, the relatively small number of participants might not allow for a reliable conclusion. Fifth, of the two clinical studies, one did not mention the performance of an intention-to-treat analysis and was conducted in an unblinded fashion,[22] which may have resulted in high performance, measurement, and selection biases. Finally, a cost-effectiveness analysis was not performed; this may have given rise to a potential preference for the use of aerosolized AMB as prophylaxis for fungal infections in an era when many other antifungal drugs are available.

In conclusion, aerosolized AMB effectively reduces the incidence of IPA in high-risk patients and has proved to be useful clinically when used as prophylaxis. However, its effects should be confirmed in large sample-size, multicenter, randomized controlled trials. Analysis of cost-effectiveness and adverse effects between various aerosolized AMB formulations and newer antifungal agents should be included in future clinical trials.

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Conflict of interest: The authors declare no conflicts of interest. Ethical approval was not required.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijid.2014.11.004.

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