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Review Article

A meta-analysis of fluconazole for the prevention of invasive fungal infection in preterm infants

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Abstract: Objective: The present aimed to evaluate the efficacy and safety of fluconazole for prophylactic use in preterm infants with very low birth weight (VLBW) by using an evidence-based methodology. Methods: A computerized literature search was conducted in PubMed, the Cochrane Database of Systematic Reviews, MEDLINE, EMBASE, the ISI Web of Knowledge databases, the Chinese Biomedical (CBM) database, China National Knowledge Infrastructure, the WanFang database, and the VIP Chinese science and technology journal database to find all the randomized controlled trials conducted between January 2000 and December 2019 that studied the prevention of invasive fungal infection (IFI) by fluconazole in preterm infants with VLBW. A meta-analysis was conducted using the RevMan 5.3 and GRADEprofiler 3.2.2 software. Results: A total of 14 studies (including 1,930 preterm infants with VLBW) were included. The meta-analysis found that the prophylactic use of fluconazole significantly reduced the incidence of IFI (RR = 0.39; 95% CI: 0.24-0.64, P < 0.05), overall mortality (RR = 0.77; 95% CI: 0.61-0.97, P < 0.05), and fungal colonization rate (RR = 0.32; 95% CI: 0.25-0.41, P < 0.05) in preterm infants with VLBW. There was no significant effect on some common complications and neurological development in preterm infants. The application of fluconazole would not lead to the development of fungal resistance in the short term and would have no significant adverse effects. Conclusion: The prophylactic use of fluconazole significantly reduced the incidence of IFI, overall mortality, and fungal colonization in preterm infants; however, the impact of prophylactic use of fluconazole on preterm infants needs to be evaluated in a large number of clinical studies because of the limited data.

Keywords: Fluconazole, prophylaxis, infant, very low birth weight, meta-analysis

Introduction

Invasive fungal infection (IFI) is the leading cause of death and the occurrence of long-term neurological sequelae in preterm infants with very low birth weight (VLBW) [1]. Greenberg reported that the incidence of IFI was 2-8% in preterm infants with a VLBW of less than 1500 g and 10-16% in preterm infants with an extremely low birth weight (ELBW) of less than 1000 g [2]. Manzoni et al. reported that prior to the prophylactic use of fluconazole, the colonization rate of Candida in preterm infants with VLBW could be up to 60% in the first month of life, and 20% of these children could develop IFI [3]. Infants with IFI are more likely to develop myocardial injury, renal and hepatic injury, intraventricular hemorrhage, retinopathy of prematurity, and chronic pulmonary disease [4]. Gestational age of less than 28 weeks, birth weight of less than 1000 g, and early abdominal surgery are high-risk factors for the development of IFI [5]. Other risk factors include the retention of central venous catheters, prolonged intubation for mechanical ventilation, prolonged use of broad-spectrum antibiotics, and use of glucocorticoids and H2 blockers [6]. As it is difficult to diagnose IFI early, the treatment is often started late. The use of fluconazole for the prevention of IFI in preterm infants with VLBW has become increasingly common and has achieved certain therapeutic effects. However, there is still controversy surrounding the dosage of fluconazole, as well as the safety and efficacy of different dosages. Therefore, the present study aimed to conduct a meta-analysis to further clarify the need for and the efficacy and safety of prophylactic use of fluconazole in preterm infants with VLBW by collecting the data from the published clinical random-

ized controlled trials (RCTs) on the use of fluconazole for the prevention of fungal infections in preterm infants with VLBW in China and abroad.

Materials and methods

The inclusion criteria

Study design: RCT of fluconazole for the prevention of IFI in very preterm infants and/or infants with VLBW.

Study objects: Infants with ELBW < 1500 g and/or extremely preterm infants with a gestational age < 32 weeks in whom fluconazole was started intravenously or orally within 1 week of birth.

Interventions: The intervention in the experimental group was fluconazole, and the intervention in the control group was an antifungal drug other than fluconazole or a placebo.

Outcomes: The primary outcomes were as follows: (1) incidence of IFI; (2) overall mortality; and (3) fungal colonization rate. The secondary outcomes were as follows: (1) incidence of bacterial sepsis; (2) incidence of some complications related to premature birth, such as bronchopulmonary dysplasia (BPD), periventricular leukomalacia (PVL), intraventricular hemorrhage (IVH) above grade III, patent ductus arteriosus (PDA) that needed surgical ligation, retinopathy of prematurity (ROP) above the threshold lesions, and neonatal necrotizing enterocolitis (NEC) (Bell grading phase 2 or 3); (3) outcome of the development of the nervous system; (4) influence on the drug resistance in fungus; and (5) some side effects associated with the administration of fluconazole, including hepatic and renal injury and an increase in direct bilirubin.

The exclusion criteria

The exclusion criteria were: (1) the research objects were not preterm infants; (2) studies that excluded the incidence of IFI and the overall mortality for the primary outcome measures; (3) research without a control group; and (4) articles of poor quality, repetitive reporting, too little information reported, and poorly described data.

Search strategy

Selection of databases: The databases included the Cochrane Central Register of Controlled Trials (CENTRAL), PubMed, EMBASE, MEDLINE, Cochrane Library, Google Academic Search, Chinese Biomedical Database (CBM), China National Knowledge Infrastructure (CNKI), WanFang database (WanFang), and VIP Chinese science and technology journal database (VIP). The search time was from the library's construction to December 31, 2019, and all RCTs in English and Chinese that studied the effect of prophylactic use of fluconazole on the incidence of IFI and mortality in preterm infants were collected.

Selection of search terms: The Chinese search terms were as follows: preterm infant, very low birth weight infant, extremely low birth weight infant, fluconazole, fungal infection, prevention, and treatment. The English search terms were as follows: fluconazole, antifungal, premature, very low birth weight, extremely low birth weight, prophylactic, fungus infection, and treatment.

Search protocols: The literature search was performed in four steps: (1) the original papers were searched in Chinese and English databases such as CENTRAL, MEDLINE, EMBASE, CBM, CNKI, VIP, and the WanFang database, and the titles, abstracts, keywords, and subject terms were analyzed to determine the keywords for the literature search; (2) a database search was conducted using all the relevant subjects and keywords, and if the abstract initially met the inclusion criteria, the full text was further searched and read; (3) further manual and electronic database searches were performed through the references attached to the literature obtained; and (4) when the information concerning the trial report was not available or was missing, it was obtained by contacting the main author of the study by phone or email, supplemented by a manual search of the references included in the literature to maximize the inclusion.

Document extraction and quality evaluation: Relevant information was extracted by two independent researchers after reading the searched literature, and the methodological quality of the included RCTs was assessed according to the risk of bias assessment methods recommended by the Cochrane Assist Network. Subsequently, the extraction and analysis of the target literature according to the outcome indicators were performed using the

RevMan 5.3 software. The collated data were imported into the GRADEprofiler software, and the GRADE evidence quality grading system was adopted to evaluate the overall quality of the evidence. In the case of disagreement, a discussion took place with the intervention of a third evaluator.

Data processing

RevMan 5.3, Stata, and GRADE-profiler software were used for the data analysis. Heterogeneity between similar studies was first evaluated by P and I^2. A fixed effects model was used if the likelihood of heterogeneity between studies was small. In the case of the existence of heterogeneity between studies, the source of the heterogeneity was analyzed, and a random effects model was adopted. The test level for the meta-analysis was set at $\alpha = 0.05$.

Results

Results of literature search

Initially, 243 relevant documents were identified, of which 30 were in Chinese and 213 were in English. Subsequently, 129 papers that were republished and clearly did not meet the inclusion criteria were excluded, and 32 controlled clinical trials were included by reading the title and abstract. After reading the full text, 18 non-RCTs were excluded, and 14 RCTs were included [3, 7-19]. Among these studies, the largest number of cases was 361 and the smallest was 13. Overall, there were 1,930 cases, of which 1,038 were in the fluconazole group. Of these, fluconazole and a placebo were compared in 11 studies [3, 7-10, 14-19], fluconazole and mycophenolate mofetil were compared in 2 studies [11, 13], and fluconazole, mycophenolate mofetil, and a placebo were compared in 1 study [12]. The dosages of fluconazole were 3 mg/kg [3, 9, 12, 14, 16, 19], 4 mg/kg [13] and 6 mg/kg [3, 7, 8, 10, 11, 15, 17-19], respectively.

One study [16] compared the efficacy of two different fluconazole dosing regimens (regimen A: administration once every 3 days for weeks 1-2, once every 2 days for weeks 3-4, and daily for weeks 4-6; regimen B: administration twice weekly. The overall duration of treatment was 6 weeks, and fluconazole was used at a dosage of 3 mg/kg once). The efficacy of two different dosages of fluconazole (3 mg/kg and 6 mg/kg) was compared in another study [3]. The pediatric patients included in the studies generally received a 4-week (pediatric patients with VLBW) or 6-week (pediatric patients with ELBW) pharmacological or placebo intervention starting as early as one week after birth. Patients in each study were tested for homogeneity, and the differences were not statistically significant ($P > 0.05$). The general characteristics and baseline data of the included studies are presented in **Table 1**.

Bias risk assessment of the included studies

As shown in **Figures 1**, **2**, according to the risk of bias assessment methodology recommended by the Cochrane Assist Network, the 14 studies included in the meta-analysis were assessed for risk of bias in terms of seven aspects: the generation of random assignment methods, concealment of assignment schemes, blinding of subjects and researchers, blinding of data analysis and reporters, completeness of outcome data, selective reporting of findings, and presence of other sources of bias. The results indicated that the baselines were comparable, but all had different levels of bias. Among the 14 studies included, the method of random assignment generation was reported in detail in 11 studies [3, 7, 8, 10-13, 15, 16, 18, 19], and random grouping was described in only 3 studies [9, 14, 17]; however, they failed to describe the method of random assignment generation in detail. Assignment concealment was found in 10 studies [3, 7, 10-13, 15, 16, 18, 19], but there were unclear descriptions of the assignment concealment scheme in four studies [8, 9, 14, 17]. Blinding of subjects and researchers, data analysis, and reporters was implemented in 10 studies [3, 7, 8, 10, 14-19], but there were unclear descriptions of whether blinding was implemented or not in 2 studies [9, 12], and blinding was not implemented in 2 studies [11, 13]. Complete data were reported in all the included studies. The findings were reported unselectively in 12 studies [3, 7, 8, 10-16, 18, 19], and there was an unclear description of the presence or absence of selective reporting of findings in 2 studies [9, 17]. No other risk factors for bias were identified in 3 studies [3, 10, 19], but the presence of other risk factors for bias was not identified in 11 studies [7-9, 11-14, 15-18].

Table 1. General characteristic of the included studies

Included study	Study object (Case)		Mode of administration and dosage	Intervention	Subject of intervention	Course of treatment	Outcome
	Fluconazole group	Control group					
Autmizguine J 2018 [7]	188	173	6 mg/kg Intravenous or through a gastric tube	Fluconazole and placebo	Preterm infants with BW < 750 g	6 weeks	Primary outcome: fungal resistance
Kirpal H 2016 [8]	38	37	6 mg/kg Intravenous, every other day for week 1 and daily for weeks 2-4 or until discharge.	Fluconazole and placebo	VLBW	4 weeks	Primary outcomes: (1) IFI (2) overall mortality Secondary outcomes : Transamine, NEC, bacterial sepsis
Jannatdoust A 2015 [9]	43	50	3 mg/kg, Intravenous; Once every 3 days for the first 2 weeks, once every 2 days for the second 2 weeks and once daily for the third 2 weeks	Fluconazole and placebo	BW < 1250 g and GA < 32 w	6 weeks	Primary outcome: (1) overall mortality
Benjamin DK 2014 [10]	188	173	6 mg/kg Twice weekly, Intravenous or through a gastric tube	Fluconazole and placebo	BW < 750 g	6 weeks	Primary outcomes: (1) IFI (2) overall mortality Secondary outcomes: transaminase, bacterial sepsis, IVH and PVL, BPD, PDA requiring surgery, ROP requiring surgery, NEC, neurodevelopmental outcomes at the corrected age of 18-22 months
Mersal A 2013 [11]	33	24	6 mg/kg, Intravenous, every 72 h during the first week of life and every 48 h during weeks 2-6; oral administration of mycophenolate mofetil (1 ml, 100000 IU, Q8H, 6 W)	Fluconazole and mycophenolate mofetil	GA < 30 w, and/ BW < 1200 g	6 weeks	Length of stray and expenditure
Aydemir C 2011 [12]	93	mycophenolate mofetil group: 94 placebo group: 91	3 mg/kg, Intravenous or through a gastric tube , once every three days, oral administration of mycophenolate mofetil (1 ml, 100000 IU, Q8H, 6 w)	Fluconazole mycophenolate mofetil Placebo	VLBW	1000 g-1500 g: 4 weeks ELBW: 6 weeks	Primary outcomes: (1) IFI (2) Overall mortality (3) Fungal colonization Secondary outcomes: Bacterial sepsis, NEC, ROP requiring surgical intervention, IVH of grade 3/4, BPD, fungal resistance
Violaris K 2010 [13]	38	42	4 mg/kg through gastric tube , once dailyl, 100000 IU, Q6H, orally	Fluconazole and mycophenolate mofeti	VLBW	From 3-7 d after birth until complete enteral nutrition	Secondary outcome: direct bilirubin
Kim CS 2010 [14]	28	27	3 mg/kg intravenous	Fluconazole and placebo	VLBW	4-6 weeks	Primary outcomes: (1) IFI (2) overall mortality (3) Fungal colonization
Manzoni P 2007 [3]	112+104	106	112 (6 mg/kg) 104 (3 mg/kg) Every 3 days for the first 2 weeks, then daily for a total of 6 weeks for ELBW and 4 weeks for VLBW, Intravenous or through a gastric tube	Fluconazole and placebo	VLBW	1000 g-1500 g: 4 weeks ELBW: 6 weeks	Primary outcomes: (1) IFI (2) overall mortality (3) Fungal colonization Secondary outcomes: transaminase, bacterial sepsis, ROP requiring surgical intervention, IVH, BPD, fungal resistance
Parikh TB 2007 [15]	60	60	6 mg/kg Once every 3 days for week 1 and once a day for weeks 2-4. Intravenous first, then oral with total enteral nutrition.	Fluconazole and placebo	VLBW	4 weeks	Primary outcomes: (1) IFI (2) overall mortality (3) Fungal colonization

Kaufman D 2005 [16]	41	40	3 mg/kg Mode A: Every 3 days * 2 weeks, then every 2 days * 2 weeks; every day * 2 weeks; Mode B: twice weekly, intravenous	Fluconazole and placebo	ELBW	6 weeks	Primary outcome: (1) IFI (2) overall mortality (3) Fungal colonization Secondary outcomes : bacterial sepsis; transaminase
Cabrera C 2002 [17]	7	6	6 mg/kg Week 1, every 3 days, then every 2 days	Fluconazole and placebo	BW < 1500 g, GA < 34 w	6 weeks	Primary outcomes: (1) IFI (2) overall mortality
Kicklighter SD 2001 [18]	53	50	6 mg/kg Every 72 h for the first week and every 24 h for weeks 2-4; intravenous or oral	Fluconazole and placebo	VLBW	4 weeks	Primary outcomes: (1) IFI (2) overall mortality (1) Fungal colonization Secondary outcomes: fungal resistance
Kaufman D 2001 [19]	50	50	3 mg/kg Every 3 days * 2 weeks; every 2 days * 2 weeks; every day * 2 weeks, intravenous	Fluconazole and placebo	ELBW	6 weeks	Primary outcomes: (1) IFI (2) overall mortality (3) Fungal colonization Secondary outcomes: bacterial sepsis, NEC, PDA requiring surgery; ROP requiring surgical intervention, PVL, fungal resistance, developmental outcomes of the nervous system

Note: IFI: Invasive fungal infection; BW: birth weight; GA: gestational age.

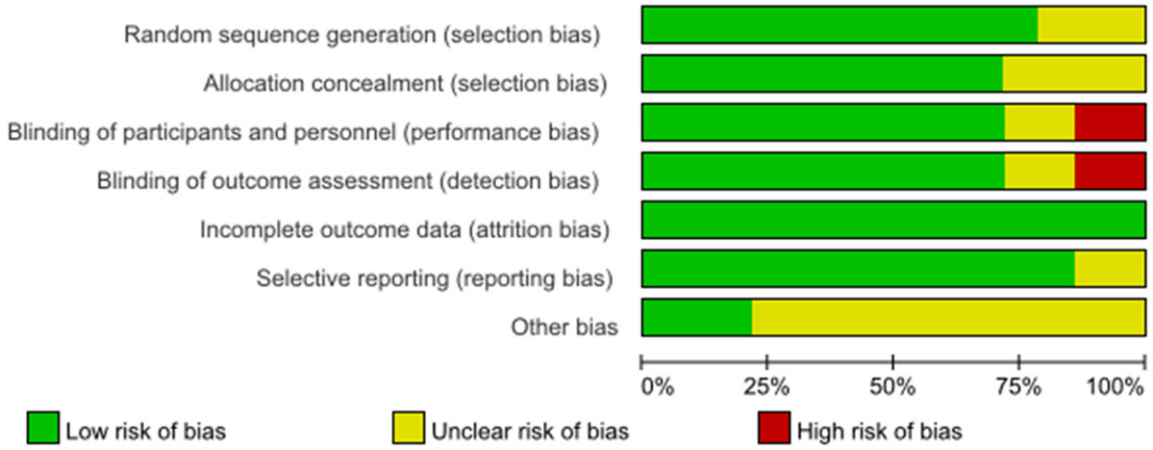

Figure 1. Risk of bias: the author's judgment on the percentage of projects that have a risk of bias in all included studies.

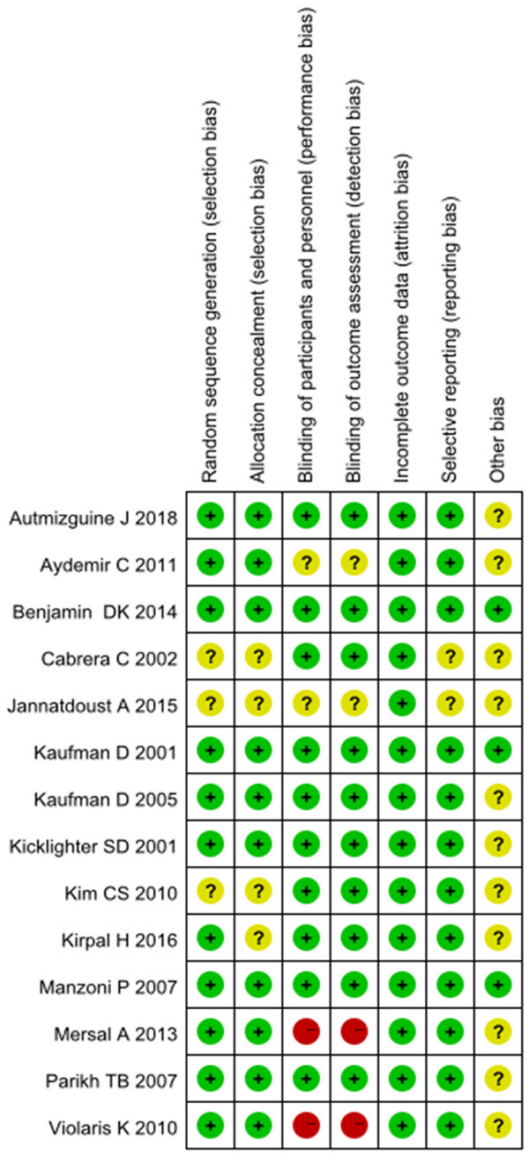

Figure 2. Risk of bias: the author's judgment on the risk of each bias in all included studies.

Among the studies, three studies [3, 10, 19] were at low risk of bias, two studies were at high risk of bias [11, 13], and nine studies [7-9, 12, 14-18] were at medium risk of bias.

Recommended classification of GRADE system

There were three primary outcomes in the present study: incidence of IFI, overall mortality, and fungal colonization rate. The GRADE system recommended a medium grade for incidence of IFI and a high grade for overall mortality and fungal colonization rate.

Results of meta-analysis

Primary outcome indexes

Effects of fluconazole on the incidence of IFI: Nine studies [3, 8, 10, 12, 14, 15, 17-19] (including 1,439 preterm infants) compared the effect of fluconazole on the incidence of IFI, with heterogeneity across studies ($P = 0.06$, I^2 46%), and combined analysis was performed using a random effects model. The results of the meta-analysis revealed that the incidence of IFI in the group using fluconazole prophylactically was significantly lower than that in the control group, and the difference was statistically significant (RR = 0.39; 95% CI: 0.24-0.62, $P < 0.05$), and the prophylactic use of fluconazole significantly reduced the incidence of IFI in preterm infants with VLBW (see **Figure 3**). The funnel plot was asymmetric (**Figure 4**), sug-

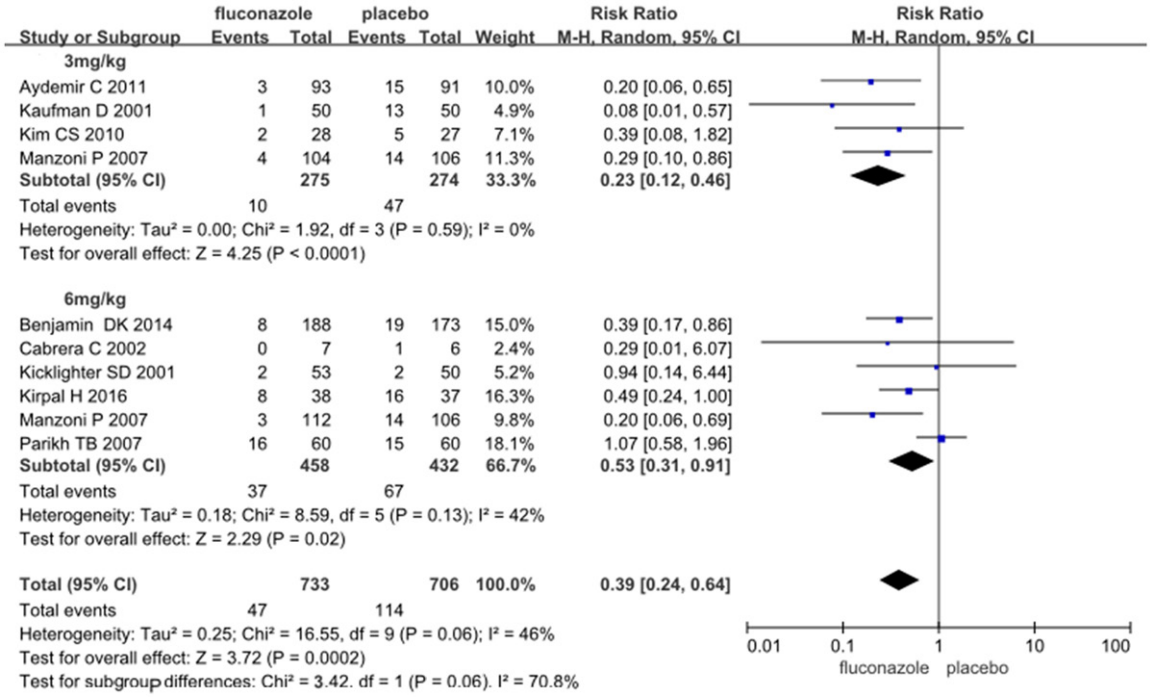

Figure 3. The effect of prophylactic fluconazole on the incidence of IFI.

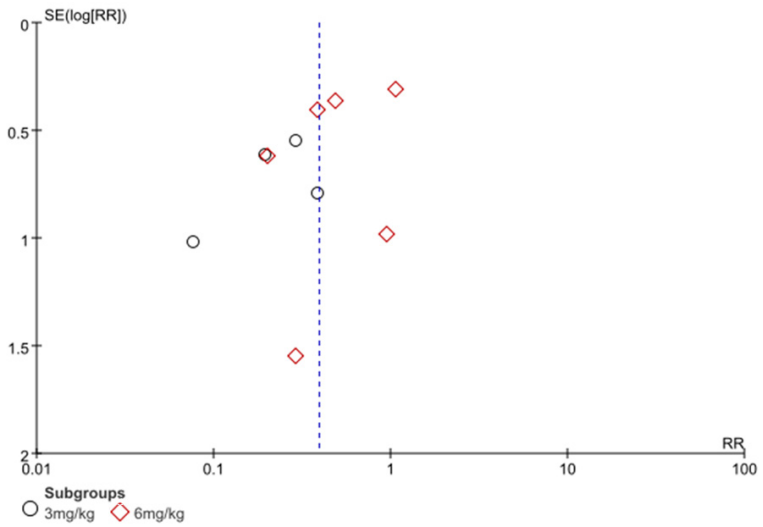

Figure 4. The funnel plot of the effect of prophylactic fluconazole on the incidence of IFI.

gesting the existence of publication bias, which might be related to the unpublished negative results. Subgroup analysis showed that the prophylactic use of fluconazole at both 3 mg/kg and 6 mg/kg significantly reduced the incidence of IFI in pediatric patients with VLBW, and the difference was statistically significant. Sensitivity analysis found that heterogeneity was altered with the exclusion of Parikh's study, and therefore this study was the source of the heterogeneity. In this study, the prevalence of IFI in the group using fluconazole prophylactically was 26.7%, which was significantly higher than in the other studies, and there was no difference in the prevalence of IFI between the fluconazole prophylaxis group and the control group.

Effects of fluconazole on overall mortality: The influence of fluconazole on mortality was compared in 10 studies [3, 8-10, 12, 14, 15, 17-19] (including 1,532 preterm infants), and the overall number of deaths was 236. There was homogeneity across studies (P = 0.82, I^2 = 0%), and fixed effects models were used for combined analysis. The results of the meta-analysis showed that the overall mortality in the group with prophylactic use of fluconazole was significantly lower than that in the control group, and the difference was statistically significant (RR = 0.77;

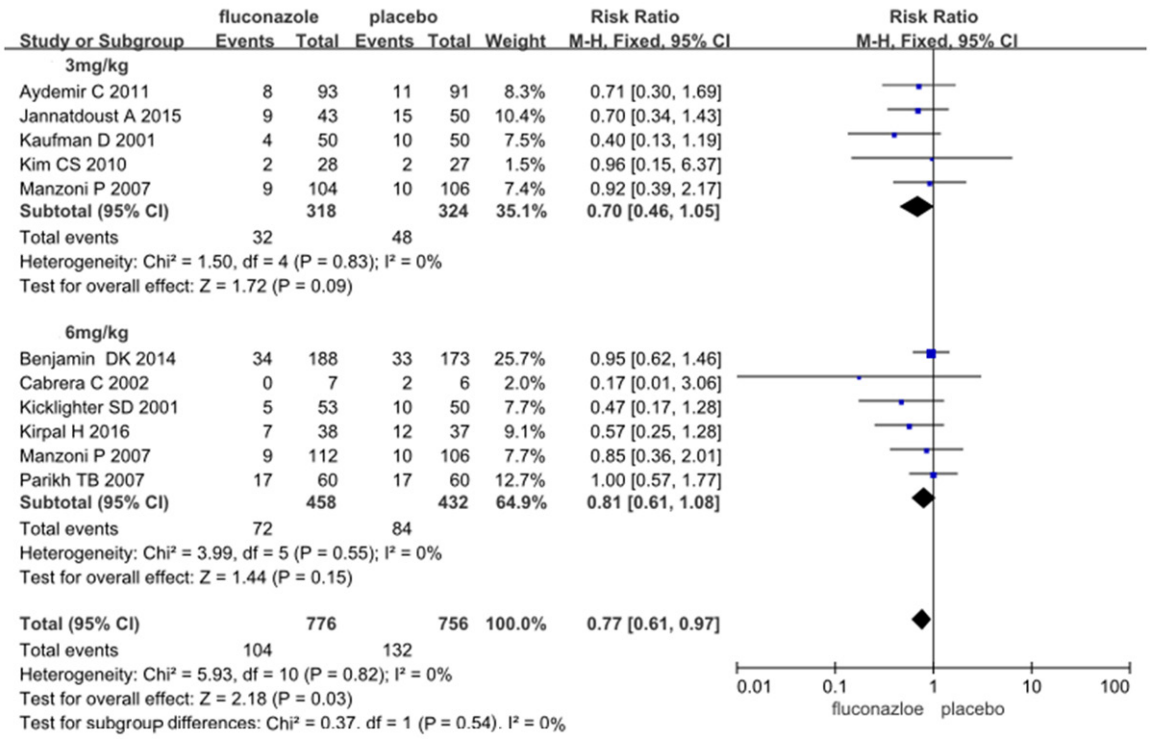

Figure 5. The effect of prophylactic fluconazole on the overall mortality.

95% CI: 0.61-0.97, P < 0.05), and the prophylactic use of fluconazole significantly reduced the overall mortality in preterm infants with VLBW (see **Figure 5**). The funnel plot was symmetric (**Figure 4**), suggesting there was no publication bias. However, the subgroup analysis showed that neither 3 mg/kg (RR = 0.70; 95% CI: 0.46-1.05, P > 0.05) nor 6 mg/kg (RR = 0.81; 95% CI: 0.61-1.08, P > 0.05) of fluconazole alone could reduce the mortality in pediatric patients with VLBW, which might be attributed to the small sample size in the subgroup analysis. The sensitivity analysis revealed that the combined effects were all still statistically significant, and the direction of the results of the forest plot did not change when 10 studies were excluded separately.

Effects of fluconazole on fungal colonization: Six studies [3, 12, 14, 15, 18, 19] (including 884 preterm infants) compared the effect of fluconazole on the fungal colonization in preterm infants. There was homogeneity (P = 0.96, I^2 = 0%) among the studies, and a fixed effects model was adopted for combined analysis. The results of the meta-analysis suggested that the fungal colonization rate in the group using fluconazole prophylactically was significantly lower than that in the control group, and the difference was statistically significant (RR = 0.32; 95% CI: 0.25-0.41, P < 0.05). Therefore, the prophylactic use of fluconazole significantly reduced the fungal colonization in preterm infants (see **Figure 6**).

Other outcome indexes

Effects of fluconazole on the incidence of bacterial sepsis: Five studies [3, 8, 10, 12, 19] (including 1,042 preterm infants) compared the incidence of bacterial sepsis between the two groups of patients, which were homogeneous (P = 0.82, I^2 = 0%), and combined analysis was performed using a fixed effects model. The meta-analysis revealed that there was no significant difference in the incidence of bacterial sepsis between the two groups (RR = 0.97; 95% CI: 0.84-1.11, P > 0.05), and therefore the prophylactic use of fluconazole had no effect on the incidence of bacterial sepsis in the two groups of patients (see **Figure 7**).

Effects of fluconazole on other common complications in preterm infants: The effects of fluconazole on a number of other common complications in preterm infants were also compared

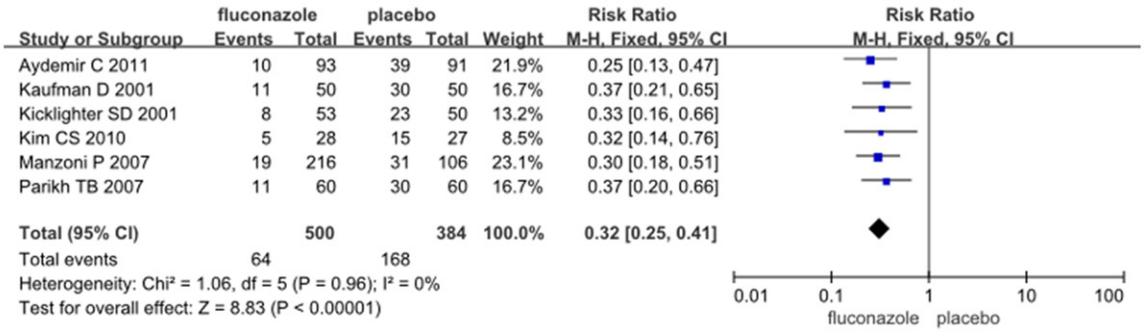

Figure 6. The effect of prophylactic fluconazole on the fungal colonization.

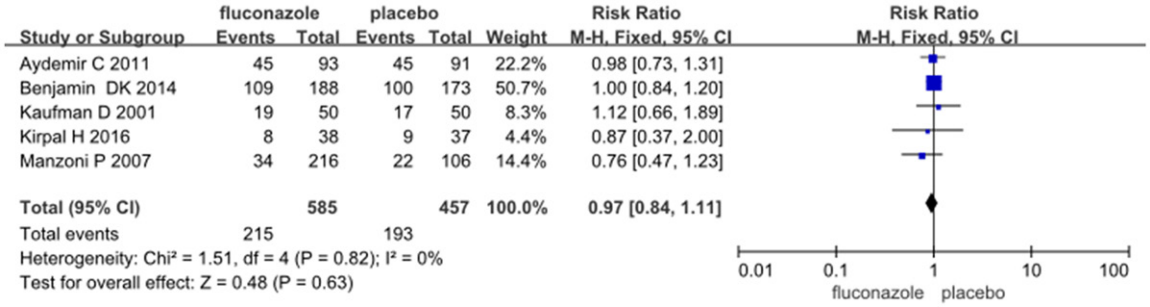

Figure 7. Effects of fluconazole on the incidence of bacterial sepsis.

in the present meta-analysis, including NEC (5 studies [3, 8, 10, 12, 19] with 1,042 preterm infants [RR = 0.88; 95% CI: 0.60-1.30]), ROP for the threshold lesions (4 studies [3, 10, 12, 19] with 867 preterm infants [RR = 0.92; 95% CI: 0.65-1.30]), BPD (3 studies [3, 10, 12] with 1,042 preterm infants [RR = 0.88; 95% CI: 0.60-1.30]), PDA requiring surgical intervention (2 studies [10, 19] with 461 preterm infants [RR = 0.93; 95% CI: 0.65-1.31]), and PVL or IVH above grade III (4 studies [3, 10, 12, 19] with 967 preterm infants [RR = 0.92; 95% CI: 0.70-1.23]). The results suggested that the prophylactic use of fluconazole had no significant effect on the occurrence of common complications in preterm infants.

Effects of fluconazole on the development of the nervous system: The effect of prophylactic use of fluconazole on the neurodevelopmental outcomes in preterm infants was reported in two studies [10, 19]. Kaufman et al. [19] reported that there were no significant differences in the prevalence of developmental delay (modified Gesell score) and neuropsychological developmental disorders among infants with a mean age of 16 months. Furthermore, there were no statistically significant differences in the follow-up assessments (Vineland Adaptive Behavior Scale-II and the Child Health Questionnaire completed by the parents) up to the age of 8-10 years in 45% of survivors. Another study [10] showed no significant difference in the neurodevelopmental deficits between the fluconazole prophylaxis group and the placebo control group at a corrected gestational age of 18-22 months (31% [95% CI: 21-41%] in the fluconazole group; 27% [95% CI: 18-37%] in the placebo control group). Moreover, the differences in the composite scores for language, cognitive, and motor development and the proportion of infants with a Bayley-III cognitive composite score < 70 were not statistically significant (18% [95% CI: 10-26%] in the fluconazole group; 14% [95% CI: 6-15%] in the placebo control group), and there was no statistical difference in the proportion of patients with cerebral palsy, blindness, or deafness between the two groups.

Effects of fluconazole on the drug resistance of fungus: The drug resistance of fungus to fluconazole was reported in five studies [3, 7, 12, 18, 19]. Kaufman et al. [19] failed to find any statis-

tically significant change in the minimum inhibitory concentration (MIC) of fluconazole against Candida albicans during the 30-month study period. Furthermore Kicklighter [18] did not find any statistically significant difference between the two groups in the MIC of fluconazole against Candida albicans during the fluconazole treatment or during the four weeks after the discontinuation of fluconazole. Similar results were reported by Manzoni et al. [3] and Aydemir [12], in whose studies the sensitivity of Candida albicans to fluconazole isolated between groups remained unchanged during the study period. The results of Autmizguine et al.'s study [7] revealed that although the mean MIC was significantly higher in the fluconazole prophylaxis group than in the control group on days 29-49 of the study, neither drug resistance of Candida albicans to fluconazole nor infections of invasive drug-resistant Candida albicans occurred.

Side effects of fluconazole: The main side effect of fluconazole was hepatic dysfunction. Three [3, 8, 10] of the included studies compared the possible elevation of transaminases caused by fluconazole treatment, and one [13] compared the elevation of direct bilirubin. The results showed that prophylactic fluconazole did not significantly result in elevation of transaminases or the incidence of bacterial sepsis (RR = 1.41; 95% CI: 0.44-4.52, P > 0.05) and direct bilirubin when compared with the controls (RR = 1.63; 95% CI: 0.52-5.13, P > 0.05), and the difference between the two groups was not statistically significant. No pediatric patients withdrew from the study because of the side effects of fluconazole.

Publication bias

Stata 13.0 software was used to perform Begg's test and Egger's test on the 14 included studies, and the publication bias test was performed on each subgroup of the included studies for the primary outcomes. Results of the publication bias test conducted for the three primary outcomes showed than in the case of the effect of fluconazole on the incidence of IFI, the funnel plot was asymmetric, Begg's test result was Pr > |z| = 0.029, and Egger's test result was P = 0.023, both lower than 0.05, suggesting that the results of the meta-analysis were unstable. The risk of bias was high and heavily weighted (18.1%) in the study conducted by Parikh [15], and there was no statistical difference in the combined effect with the exclusion of this study. Therefore, the effect of fluconazole on the incidence of IFI needs to be validated by further high-quality studies. The results of the meta-analysis of the other two primary outcomes were stable. In the case of the influence of fluconazole on overall mortality, Begg's test result was Pr > |z| = 0.283, and Egger's test result was P = 0.177, both higher than 0.05. Furthermore, in the case of the effect of fluconazole on fungal colonization rate, Begg's test result was Pr > |z| = 0.707, and Egger's test result was P = 0.911, both higher than 0.05. Therefore, there was no publication bias.

Discussion

Candidemia is the most common fungal infection in the neonatal intensive care unit (NICU). Candida can colonize, invade, and spread without any clinical manifestations [20], and it often progresses to infectious shock, meningitis, and even renal failure, increasing the short-term and long-term mortality [21]. The US Neonatal Research Network study of 1,317 pediatric patients with IFI with a birth weight < 1000 g showed a significant increase in the odds of neurodevelopmental disorders at a corrected age of 18 months (OR, 1.83; 95% CI: 1.01-3.33) and a significant increase in mortality (OR, 4.76; 95% CI: 2.24-10.14) [22]. Prevention of IFI not only reduces mortality, but also reduces the occurrence of neurocognitive and neurosensory sequelae in survivors. The Infectious Diseases Society of America states that in NICUs where the incidence of IFI is greater than 10%, fluconazole is recommended as prophylaxis for preterm infants with VLBW [23]. The results of the meta-analysis showed that prophylactic use of fluconazole reduced the incidence of IFI and the overall mortality in preterm infants with VLBW, reduced the fungal colonization with no significant side effects, and showed no significant increase in the fungal resistance to fluconazole, which was consistent with the findings of Austin [24].

Although there are evidence and recommendations supporting the use of fluconazole for the prevention of IFI in preterm infants with VLBW, there is controversy surrounding the dosage, frequency of use, indications, and safety of fluconazole administration.

Clinically, the dosage and frequency of fluconazole prophylactic administration vary. The commonly used prophylactic dosages include 3 mg/kg per dose and 6 mg/kg per dose, with individual studies using 4 mg/kg per dose. It remains unclear whether there is a difference in the therapeutic effects in terms of dosage and frequency of administration. The results of the present meta-analysis showed that both 3 mg/kg and 6 mg/kg per dose significantly reduced the incidence of IFI and the overall mortality. The vast majority of the included studies only compared different dosages of fluconazole with a placebo, whereas Manzoni et al. directly compared the effect of two different dosages of fluconazole on the incidence of IFI. However, the authors failed to find a difference in the effect of the two different dosages of fluconazole on the incidence of IFI because of the small sample size of the included studies and the low incidence of IFI [3]. In contrast, a network meta-analysis conducted by Leonart et al. included 11 RCTs composed of 1,578 preterm infants with fluconazole dosages of 3 mg/kg and 6 mg/kg. They found that both 3 mg/kg and 6 mg/kg of fluconazole were statistically significantly better than the placebo in reducing the incidence of IFI and the mortality due to IFI, but there was no statistically significant difference between the two dosages [25]. The effects of two different forms of fluconazole administration on the incidence of IFI were compared by Kaufman. Fluconazole was used at 3 mg/kg per dose for a total duration of 6 weeks. In group A, fluconazole was administered every 3 days in weeks 1-2, every 2 days in weeks 3-4, and daily in weeks 4-6 for a total of 774 doses of fluconazole, while in group B, fluconazole was administered twice a week for a total of 332 doses. The results revealed that in the high-risk preterm infants with a birth weight of less than 1000 g, both modes of administration significantly reduced the Candida colonization and invasive Candida infection, but the twice-weekly administration mode significantly reduced the multiple exposures to fluconazole and delayed or prevented the development of fungal resistance [16]. The results of a meta-analysis conducted by Jessica et al. also confirmed that prophylactic use of fluconazole at 3 mg/kg or 6 mg/kg twice weekly in pediatric patients with VLBW significantly reduced the incidence of IFI and Candida colonization and was safe and effective [26].

The widespread use of systemic antifungals may lead to the emergence of fungal resistance to drugs. The study conducted by Zhang et al. showed that the universal prophylactic use of fluconazole in very preterm infant wards increased the fungal resistance to fluconazole [27]. In a meta-analysis of fluconazole prophylaxis trials in immunosuppressed adults, Brion et al. found an increased risk of fungal colonization that was partially or completely resistant to fluconazole, but without the occurrence of invasive infections [28]. Sarvikivi et al. found that near-smooth Candida albicans was less sensitive to fluconazole in NICUs where 6-12 mg/kg of fluconazole per day was routinely used for fungal prophylaxis for 12 years [29]. It was reported in the literature that reducing the dosage, frequency, and duration of fluconazole administration could reduce the development of fungal resistance to drugs. Studies based on the pharmacokinetics of preterm infants have shown that 3 mg/kg and 6 mg/kg of fluconazole administered twice weekly may be sufficient to achieve an MIC of 2 mg/L and 4 mg/L for Candida albicans, respectively [30], which are much higher than the MIC90 (≤1 mg/L) for Candida albicans and Candida subsmoothus [7]. Among the studies included in the present meta-analysis, five studies reported fungal resistance to fluconazole, and all the results suggested that the prophylactic dose of fluconazole did not result in the occurrence of fungal resistance during the follow-up period, which might be due to the low frequency of fluconazole administration in the included studies and the fact that the maximum follow-up period of the included studies was only 30 months, which was not sufficient to monitor the significant changes in the fungal resistance profile, and the development of fungal resistance might take longer [29]. Therefore, although fluconazole has shown good antifungal effects, we should still use it with caution to avoid the development of drug resistance.

The common side effect of fluconazole is mainly hepatotoxicity, including mild and transient elevations of transaminases, bilirubin, and cholestasis. A single-center clinical study found that the incidence of cholestasis in 163 preterm infants in the fluconazole prophylaxis group was similar to that in 99 infants in the control group [31]. None of the studies included in the present meta-analysis reported

significant drug-related adverse events, and no pediatric patients were withdrawn from the study because of unacceptable adverse drug reactions. However, fluconazole can also cause rare and serious adverse reactions, such as toxic epidermal necrolysis and Stevens-Johnson syndrome. Prophylactic use of fluconazole increases the exposure to fluconazole and the risk of associated complications in neonates. Furthermore, the widespread use of prophylactic fluconazole has increased the drug interactions, such as interactions with theophylline and thiazide diuretics, increasing the risk of theophylline toxicity and renal impairment [32].

Prophylactic use of fluconazole might reduce the incidence of IFI in pediatric patients with VLBW and ELBW. However, the generalization of the results of the present meta-analysis was limited by the fact that the average incidence of IFI in the placebo controls in the studies included in the meta-analysis was 16% (4-43%), which was much higher than the incidence of IFI in pediatric patients with VLBW in other large cohort studies (1-5%) [33]. Moreover, data on the effects of fluconazole on the long-term neurological development were limited, with only one prospective study conducted by Kaufman evaluating the pediatric patients aged 8-10 years who were admitted to the NICU at birth for prematurity and treated with fluconazole for IFI prevention, and no long-term neurological developmental deficits or quality-of-life effects associated with fluconazole were identified [34]. Considering that the widespread use of fluconazole prophylaxis may lead to the development of fungal resistance, and limiting the prophylaxis to pediatric patients with high-risk factors may help delay the development of fungal resistance, fluconazole prophylaxis is recommended for NICUs with a high incidence of IFIs, while in the NICUs with a low incidence of IFIs, the body weight, presence of a central venous catheter, and use of high-risk factors for the development of IFIs, such as the triple cephalosporin and carbapenemycin antibiotics, should be taken into account when the prophylactic use of fluconazole is considered.

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