

# TWICE WEEKLY FLUCONAZOLE PROPHYLAXIS FOR PREVENTION OF INVASIVE *CANDIDA* INFECTION IN HIGH-RISK INFANTS OF <1000 GRAMS BIRTH WEIGHT

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**Objectives** We tested the hypothesis that twice weekly prophylactic dosing of fluconazole prevents invasive candidiasis without promoting resistant *Candida* species in high-risk, preterm infants.

**Study design** We compared our previous dosing schedule (Group A) to a less frequent dosing schedule of twice a week (Group B) of fluconazole prophylaxis for up to 6 weeks in a prospective, randomized, double-blind clinical trial in preterm infants weighing <1000 grams at birth and with an endotracheal tube and/or central vascular catheter over a 24-month period. Weekly surveillance cultures were obtained on study patients.

**Results** *Candida* colonization was documented in 5 (12%) of 41 Group A and in 4 (10%) of 40 Group B infants. *Candida* sepsis developed in two (5%) of Group A and one (3%) of Group B infants (risk difference,  $-0.02$ ; 95% confidence interval,  $-0.14$ - $0.10$ ;  $P = .68$ ). All fungal isolates remained sensitive to fluconazole, and no drug side effects were documented.

**Conclusions** Twice weekly dosing of prophylactic fluconazole can decrease *Candida* colonization and invasive infection, cost, and patient exposure in high-risk, preterm infants weighing <1000 grams at birth. We speculate that lower and less frequent dosing may delay or prevent the emergence of antifungal resistance. (*J Pediatr* 2005;147:172-9)

We previously demonstrated the prevention of fungemia using fluconazole prophylaxis in high-risk, preterm infants.<sup>1</sup> Although all fungal isolates in the study remained sensitive to fluconazole, one concern with this prophylaxis strategy is the emergence of azole resistance. Multiple factors are involved in fungal resistance, including the intrinsic properties of the fungus, frequency of fluconazole exposure, length of treatment, and dosage.<sup>2-7</sup> As the number of doses and duration of fluconazole therapy increases, point mutations and overexpression of the *ERG11* gene have been reported.<sup>3-4</sup> Upregulation of the azole and fluconazole efflux pumps also may occur with increased and/or prolonged fluconazole exposure.<sup>2</sup>

In our previous study, we limited the total fluconazole dose and length of exposure by administering a dose of 3 mg/kg for no longer than 42 days.<sup>5</sup> Additionally, in some patients, prophylaxis was discontinued before 6 weeks if intravenous access was no longer required. The frequency of dosing in our previous study (every 72 hours during the first 2 weeks, every 48 hours for weeks 3 and 4, and every 24 hours thereafter for weeks 5 and 6) was based on pharmacologic studies in preterm infants.<sup>8-9</sup> For prophylaxis, a decreased and simplified dosing schedule may be as effective. Less frequent dosing has been an effective preventive strategy in other antifungal and antimicrobial prophylaxis studies,<sup>10,11</sup> and in other clinical settings, one dose of fluconazole has provided persistent therapeutic concentrations in mucosal secretions for more than 72 hours.<sup>12-15</sup>

The goal of this study is to examine the efficacy of twice weekly dosing on the prevention of *Candida* colonization and invasive infection, and the development of adverse drug events in high-risk, preterm infants weighing <1000 grams at birth. Because our earlier study demonstrated significant efficacy, our Institutional Review Board recommended against comparing twice weekly dosing with a placebo-control group. Therefore, this prospective study compares our previously studied prophylactic schedule described above with twice weekly dosing for a period of time not to exceed 6 weeks.

See editorial, p 135, and related articles, p 156, p 162, and p 166.

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ALT	Alanine aminotransferase	MIC	Minimal inhibitory concentration
AST	Aspartate aminotransferase	NICU	Neonatal intensive care unit

## METHODS

### Study Design

We conducted a prospective, randomized, double-blind clinical trial to evaluate the efficacy of twice weekly intravenous fluconazole prophylaxis to prevent *Candida* colonization and invasive infection. Twice weekly fluconazole prophylaxis was compared with the dosing schedule used in our previous study (Table I). Invasive *Candida* infection was defined as isolation of *Candida* species from the blood (venipuncture), urine ( $\geq 10^5$  or more colony forming units/mL from sterile bladder catheterization or suprapubic aspiration), and/or cerebrospinal fluid. Clinical cultures were obtained by the neonatologist based on the clinical status of the infant.<sup>16</sup> All infants with a birth weight <1000 grams and <5 days of age admitted to our neonatal intensive care unit (NICU) were eligible for enrollment. Inclusion criteria were the presence of an endotracheal tube and/or central vascular catheter. Elevated serum hepatic enzymes >250 IU/L for aspartate aminotransferase (AST) or alanine aminotransferase (ALT) was the only exclusion criterion.

Using a computer-generated randomization table, the pharmacy randomly assigned infants, in a 1:1 ratio to Group A or Group B (Table I). Blinding of the groups was performed accordingly. For Group A, fluconazole was administered according to Schedule A and placebo (normal saline) was administered according to Schedule B. For Group B, fluconazole was administered according to Schedule B and placebo was administered according to Schedule A.

Data on the demographic and clinical characteristics of the infants were collected prospectively during their hospitalization and through review of the medical records. Microbiology culture results were collected during all episodes of fungal and bacterial infection of blood, urine, and cerebrospinal fluid during and after the 6-week treatment period.

Before enrollment, written informed consent was obtained from each infant's parent or guardian. The protocol was approved by the Human Investigation Committee of the University of Virginia. Investigators solicited research support from Pfizer. The study sponsor was not involved in the study design, collection of specimens, analysis or interpretation of data, decision to publish, or preparation of the manuscript.

### Drug Administration

Equal volumes of intravenous fluconazole and placebo were administered for up to 6 weeks utilizing a fluconazole dose of 3 mg/kg of body weight according to Schedule A and B (Table I). The administration of the study drugs (fluconazole and placebo) and the collection of surveillance cultures were discontinued before the end of the 6-week prophylaxis period if: (1) intravascular access was discontinued, (2) antifungal therapy was initiated for the treatment of a documented or presumed invasive fungal infection, (3) the infant was discharged from the hospital or transferred to another facility, or (4) the infant died. The study drug was not discontinued if empiric antifungal therapy was administered for  $\leq 48$  hours.

### Fungal Isolation and Identification

Fungal surveillance cultures of the skin (intertriginous, and, if present, moist areas), gastrointestinal tract (stool or rectum), and respiratory tract (tracheal secretions, if intubated, and nasopharyngeal sample, if not intubated) were obtained by two study personnel at study enrollment and then weekly during the 6-week prophylaxis period. Umbilical swab cultures also were performed at enrollment and week 1.

To determine if other neonates in the NICU who were not receiving fluconazole prophylaxis would become colonized and/or infected with azole-resistant fungi, we also evaluated all clinical fungal isolates from NICU patients who were not receiving the study drug during the 24-month study period. Additionally, 6 months before the study completion surveillance cultures of the skin, gastrointestinal tract, and respiratory tract were obtained from all nonstudy patients in the NICU at that time.

Stool and tracheal secretions were submitted in sterile containers, and skin and rectal specimens were obtained by swab (Culturette, Becton Dickinson Microbiology Systems, Sparks, Md). Each specimen was inoculated onto Inhibitory Mold Agar (Remel, Lenexa, Kan), and the plates were incubated at 30°C and read for 4 weeks or until positive.

Speciation of fungal isolates was performed using standard methods, an initial screen for germ-tube formation, exoenzyme production (*Candida albicans* screen, Carr-Scarborough Microbiologicals, Decatur, Ga), and a commercial auxanographic method (API 20 C AUX, BioMérieux, Hazelwood, Mo).<sup>17-19</sup>

### Fluconazole-Susceptibility Testing

Each yeast isolate was tested for susceptibility to fluconazole using a standardized microbroth dilution assay as described by the National Committee of Clinical Laboratory Standards document M27-A.<sup>20</sup> We reported the minimal inhibitory concentration (MIC) as the lowest concentration of drug resulting in a prominent decrease in growth at 48 hours as compared with the control.

### Side Effects and Patient Safety

Hepatic (AST, ALT, direct bilirubin, alkaline phosphatase), hematologic (white blood count and differential, platelet count, hematocrit), and renal function (blood urea nitrogen, creatinine) were screened weekly while study infants were receiving the study drug. Infants were discontinued from receiving the study drug if AST and ALT levels were >250 IU/L.

### Statistical Analysis

**PRIMARY AND SECONDARY OUTCOMES.** The primary efficacy endpoints were the incidence of *Candida* colonization and invasive infection during study drug administration. The secondary outcomes were bacterial infection of blood, urine, or cerebrospinal fluid; patent ductus arteriosus ligation; threshold retinopathy of prematurity requiring laser ablation; periventricular leukomalacia; and mortality.

**Table I. Study dosing regimens****Schedule A**

Day of Study*	Dosing
1-14	every 72 hours (Day 1, 4, 7, 10, 13)
15-28	every 48 hours (Day 15, 17, 19, 21, 23, 25, 27)
29-42	every 24 hours

**Schedule B**

Day of Study*	Dosing
1-42	Twice a week (Day 1, 4, 8, 11, 15, 18, 22, 25, 29, 32, 36, 39)

**Group A<sup>†</sup>**

Study Drug	Dosing
Fluconazole prophylaxis	Schedule A
Placebo (saline)	Schedule B

**Group B<sup>†</sup>**

Study Drug	Dosing
Fluconazole prophylaxis	Schedule B
Placebo (saline)	Schedule A

\*Day of enrollment is Day of Study 1.

†This was a blinded study in which the pharmacy prepared two study drugs (fluconazole and placebo) for each patient.

**SAMPLE SIZE SELECTION.** This study was powered a priori with regard to the comparison of the risk of fungal colonization between Groups A and B. With a total sample size of 82 infants, 41 randomized to each of the two interventions, this study was designed to have at least an 80% chance of rejecting the null hypothesis of equal risk of fungal colonization if the true risk or probability differed by a value of at least 0.29 between the two study populations.

The power analysis was conducted under the assumption that the type-1 error rate of the statistical test for comparing the risk of colonization between the two study populations would be  $\leq 0.05$ , and also under the assumption that the true probability of experiencing fungal colonization during the intervention period would be 0.22 for Group A (an estimate of the risk that we derived from data from our previous study using the same dosing schedule). We further assumed as a one-sided alternative hypothesis that the infants who received the twice weekly dosing of fluconazole (Group B), which would be a lower total dose of fluconazole than administered to Group A, would be at greater risk of experiencing fungal colonization during the intervention.

**STATISTICAL METHODS.** The cumulative incidence data of primary and secondary outcomes during study drug administration within Groups A and B were analyzed by the exact methods of Agresti and Min.<sup>21</sup> For each outcome variable, the frequency data were analyzed as binomial random variables, and risk differences were evaluated using a two-sided binomial exact-test.<sup>22</sup> The same method was used to compare the frequency data of Groups A and B with that of the placebo group from our previous study. The exact-tests and the 95%

confidence intervals were computed with StatXact 5.0 (Cytel Software Corporation, Cambridge, Mass).

## RESULTS

### Study Participants

Forty-one infants were randomized to Group A and 40 infants were randomized to Group B over the 24-month period between July 23, 2001, and July 4, 2003. Infants were born during the study period at our institution or transferred from another facility. Ninety-eight patients were born weighing <1000 grams. Nine of these patients did not require a central vascular catheter or endotracheal tube and thus were ineligible for study enrollment. A total of 89 infants met enrollment criteria during the study period. Four patients died before consent being obtained; for two infants consent was not obtained during the first 5 days of life; for one infant there was no parent or guardian available for informed consent; and for one, an interpreter was not available to obtain informed consent. Group B had 40 patients because one patient was randomized to this group twice but did receive the correct drug dosing and was analyzed only one time.

Enrollment demographics and risk factors for fungal infection between the two study groups are listed in Table II. There were more outborn patients in Group A and more focal intestinal perforations in Group B patients. There were no other significant differences between the study groups. All infants were placed in a humidified isolette for skin care during the first 7 days of life. Topical petrolatum ointment (Aquaphor®, Beiersdorf, Norwalk, CT) was used to decrease insensible water loss during the first week of life until June 2003, and then only used in two patients in each group thereafter if there was significant skin breakdown until the skin area healed.

At baseline, two (5%) Group A and two (5%) in Group B infants were colonized (risk difference, 0.001; 95% confidence interval,  $-0.12$ - $0.14$ ;  $P = 1.00$ ). These infants with baseline fungal colonization were enrolled later than those who were not colonized at enrollment (day of life  $3 \pm 0.8$  vs  $1.6 \pm 1.4$ ;  $P = .04$ ).

The mean ( $\pm$ SD) length of study drug administration and surveillance cultures for all study participants was 4.4 ( $\pm 2.2$ ) weeks (Group A,  $4.4 \pm 2.2$  vs Group B,  $4.3 \pm 2.2$  weeks;  $P = .87$ ), and patients were followed for a mean of 11.5 ( $\pm 6.7$ ) weeks (Group A,  $10.5 \pm 6.1$  vs Group B,  $12.3 \pm 7.1$  weeks,  $P = .26$ ). There were 774 fluconazole doses administered to Group A and 332 to Group B. For one patient in Group B, the initial dose of fluconazole was missed, and the first dose was given on Study Day 4. This patient subsequently developed fungal colonization and sepsis. On six other occasions, one fluconazole dose was missed; on two occasions, one fluconazole dose was given a day early; and on one occasion, for one patient in each group, surveillance cultures were missed. One patient in Group A and two patients in Group B received empiric antifungal therapy with amphotericin B for 48 hours, and none of these patients had isolation of fungus from clinical cultures during their hospitalizations.

**Table II. Patient demographic data, risk factors, and secondary outcomes\***

	Group A [N = 41 (%)]	Group B [N = 40 (%)]	P Value
<b>STUDY POPULATION</b>			
Birth weight (grams)	691 ± 122	704 ± 164	0.67
Gestational age (weeks)	24.9 ± 1.6	25.8 ± 2.2	0.07
Male	24 (59)	22 (55)	0.81
Maternal nonwhite race	10 (24)	11 (28)	0.81
Maternal Hispanic or Latino ethnicity	3 (7)	1 (3)	0.40
Antenatal steroids	27 (66)	32 (80)	0.24
Antenatal antibiotics	22 (54)	27 (68)	0.22
ROM ≥24 hours	3 (7)	7 (18)	0.22
Born outside study hospital	18 (44)	8 (20)	0.03
Vaginal delivery	16 (39)	18 (45)	0.65
Apgar score			
1 minute	3 ± 2	4 ± 2	0.45
5 minute	6 ± 2	5 ± 2	0.32
Age at enrollment (days)	1.9 ± 1.4	1.4 ± 1.3	0.09
<b>RISK FACTORS<sup>†</sup></b>			
<b>Management Choices<sup>†</sup></b>			
Parenteral Nutrition	39 (95)	37 (93)	0.70
Granulocyte-colony stimulating factor	1 (2)	5 (13)	0.10
Corticosteroid	25 (61)	19 (48)	0.27
Exposure (days) <sup>†</sup>	5 (1-28)	3.5 (1-41)	0.73
Topical Petrolatum Ointment	20 (49)	12 (30)	0.10
<b>Antimicrobial</b>			
Ampicillin, Gentamicin	38 (93)	37 (95)	1.00
Nafcillin	21 (51)	20 (50)	0.93
Vancomycin	21 (51)	18 (45)	0.60
Metronidazole	2 (5)	7 (18)	0.08
Third-generation Cephalosporin	22 (54)	18 (45)	0.46
Exposure (days) <sup>†</sup>	8 (1-17)	5 (1-16)	0.71
<b>Vascular Access (no.)</b>			
Umbilical Catheters	40 (98)	39 (98)	1.00
Central Venous Catheter	38 (93)	36 (90)	0.93
Peripheral Arterial Catheter	14 (34)	14 (35)	1.00
<b>Endotracheal Intubation</b>			
Necrotizing Enterocolitis <sup>†</sup>	4 (10)	3 (8)	0.80
Focal Intestinal Perforation <sup>†</sup>	0 (0)	4 (10)	0.04
Abdominal Surgery <sup>†</sup>	2 (5)	6 (15)	0.13
Major Surgery <sup>†‡</sup>	10 (24)	15 (38)	0.24
<b>SERUM HEPATIC ENZYMES AFTER PROPHYLAXIS</b>			
Aspartate aminotransferase (mean peak, IU/L)	23 (11-114)	23 (9-133)	0.26
Alanine aminotransferase (mean peak, IU/L)	8 (3-30)	10 (5-175)	0.09
<b>SECONDARY OUTCOMES<sup>‡</sup></b>			
Bacterial septicemia <sup>†</sup>	12 (29)	12 (30)	1.00
Patent ductus arteriosus ligation <sup>†</sup>	8 (20)	8 (20)	1.00
Threshold Retinopathy of Prematurity <sup>§</sup>	15 (37)	19 (48)	0.35
Periventricular Leukomalacia <sup>§</sup>	7 (17)	5 (13)	0.60
Mortality <sup>§</sup>	6 (15)	6 (15)	1.00

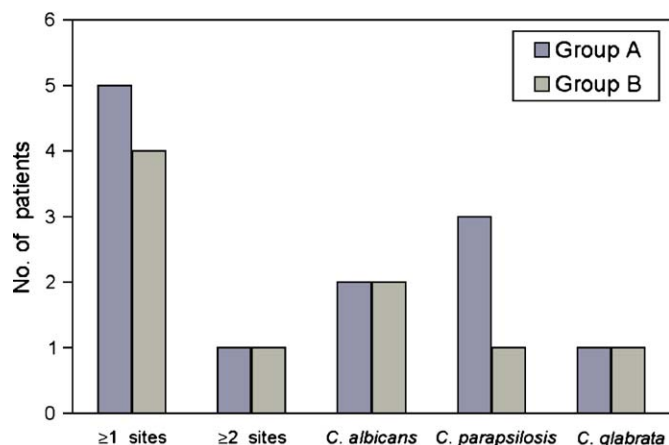
\*Plus-minus values are mean ± SD. Median (range). ROM denotes rupture of membranes. Granulocyte-colony stimulating factor given for neutropenia (<500/mm<sup>3</sup>). All maternal testing for human immunodeficiency virus was negative in both groups.

†During 6-week treatment period.

‡Focal intestinal perforation: isolated perforation without the presence of pneumatosis. Major surgery: patent ductus arteriosus ligation, abdominal surgery, or ventricular-peritoneal shunt placement. Threshold retinopathy of prematurity requiring laser ablation; periventricular leukomalacia detected by ultrasonography.

§During entire hospitalization.





**Figure 1.** *Candida* colonization during the 6-week period of prophylaxis in groups A (N = 41) and B (N = 40). There is no significant difference between study groups. Available in color online at [www.us.elsevierhealth.com/jpeds](http://www.us.elsevierhealth.com/jpeds).

## Fungal Colonization

Fungal colonization at one or more sites during the 6-week treatment period was present in five (12%) Group A and four (10%) Group B patients (risk difference 0.02; 95% confidence interval, -0.18-0.14;  $P = .83$ ) (Figure 1). Fungal colonization at multiple sites (two or more sites) occurred in one (2%) Group A and one (3%) Group B infant ( $P = 1.00$ ). Skin and gastrointestinal tract were the most common sites of fungal colonization in both groups, whereas respiratory and umbilical fungal colonization occurred in one patient in each group during the study period. Application of topical petrolatum ointment was not more common in colonized compared with noncolonized infants. After the second week, only *C parapsilosis* skin colonization occurred in one infant in each group. There was no *Candida* colonization after the fourth week of the prophylaxis period in either group.

## Invasive Fungal Infection

Fungal bloodstream infection occurred in two (5%) Group A and one (2.5%) Group B patients (risk difference, 0.02; 95% confidence interval, -0.14-0.10;  $P = .68$ ) (Figure 2 available online only at [www.us.elsevierhealth.com/jpeds](http://www.us.elsevierhealth.com/jpeds)). None of the infants with systemic fungal infection died. The three cases of fungemia were due to *C albicans* (1), *C parapsilosis* (1), and *C glabrata* (1) and occurred on day of life 13, 19, and 19, respectively. In the infant with *C albicans* fungemia, an atrial thrombus was documented by ultrasonography 2 days before the documented fungemia. Severe fungal dermatitis preceded *C parapsilosis* fungemia in the second patient. *C glabrata* was isolated from both the blood and urine simultaneously with an intestinal perforation in the third patient. There were no episodes of fungal meningitis. These infections were not clustered temporally during this study period. Systemic fungal infection was treated in all cases with intravenous amphotericin B and central venous catheter removal. After the 6-week prophylaxis period, the infant who had developed invasive infection with *C glabrata* developed a *C glabrata* urinary tract

**Table III. MIC of *Candida* isolates from study infants during 24-month study period\***

Species	<i>Candida albicans</i>	<i>C parapsilosis</i>
Study infants (N = 81)		
Number of surveillance isolates	4	11
MIC ( $\mu\text{g/mL}$ )		
Group A (no.)	0.125-0.50 (2)	0.5-4.0 (8)
Group B (no.)	0.125-0.25 (2)	0.5 (3)
1 <sup>st</sup> 12 months of study (no.)	0.125-0.50 (3)	0.5-2.0 (4)
Final 12 months of study (no.)	0.125 (1)	0.5-4.0 (7)
Nonstudy infants (N = 14)		
Number of surveillance isolates	2	1
MIC ( $\mu\text{g/mL}$ )	0.25-0.50	1.0

\*Fungal isolates obtained from 571 surveillance cultures in group A and 515 in group B during the prophylaxis period.

infection on day of life 102. In one Group B patient, *Malassezia furfur* was isolated from a blood culture drawn from a central venous catheter, but no fungus was detected from any peripheral blood cultures, and the infant was treated and survived to discharge.

All three infants with invasive fungal infection had fungal colonization documented at study enrollment or during the prophylaxis period. Fungal colonization was identified at baseline in one infected patient and at two or more sites in the other two infected patients. Patients with any prior fungal colonization (risk difference, 0.88; 95% confidence interval, 0.01-0.96;  $P = .01$ ) and colonization at two or more sites (risk difference, 0.66; 95% confidence interval, 0.09-0.99;  $P < .001$ ) were more likely to develop invasive fungal infection. Colonization with a specific fungal species or at a specific site was not associated with an increased risk for invasive fungal infection.

## Minimal Inhibitory Concentration

**STUDY INFANTS.** Fungal isolates from study infants did not demonstrate the development of fluconazole resistance. Sensitivity to fluconazole measured by MIC did not change significantly for patients in either group during the 6-week prophylaxis period or over the 24-month study period (Table III). The MIC values of the three blood isolates, *C albicans* (0.125  $\mu\text{g/mL}$ ), *C parapsilosis* (2.0  $\mu\text{g/mL}$ ), and *C glabrata* (8  $\mu\text{g/mL}$ ) were not significantly different from the MIC values of the surveillance isolates of infants who did not develop invasive infection.

**NONSTUDY NICU INFANTS.** Surveillance cultures were obtained 6 months before the study completion from 14 nonstudy infants in our NICU. Three (21%) of the 14 infants demonstrated fungal colonization, and all isolates were sensitive to fluconazole. Two infants had colonization with *C albicans* (MIC 0.25-0.5  $\mu\text{g/mL}$ ), and one infant was colonized with *C parapsilosis* (MIC 1.0  $\mu\text{g/mL}$ ). In addition,

*Candida* bloodstream infection occurred in five nonstudy infants who did not meet enrollment criteria during the 24-month study period. All *C. albicans* bloodstream isolates from two infants (MIC 0.125–0.25 µg/mL) and *C. parapsilosis* bloodstream isolates from three infants (MIC 0.5–1.0 µg/mL) were sensitive to fluconazole.

## Secondary Outcomes

The frequencies of the secondary outcomes are described in Table II. There was no significant difference in morbidity or mortality between the groups (six deaths in each group,  $P = 1.00$ ). Bacteremia was documented in 12 (29%) Group A and 12 (30%) Group B infants.

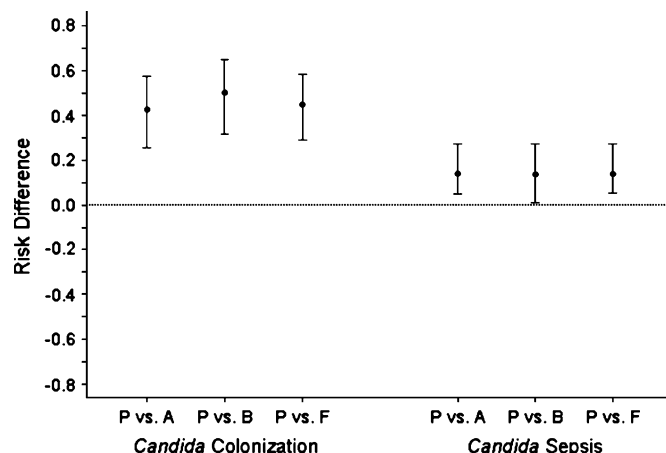
## Adverse Events

No adverse effects of the fluconazole therapy were documented, and no patient was withdrawn from the study. There was no measurable adverse effect of fluconazole therapy on hepatic, renal, or hematologic function (Table II). Serum aminotransferases were elevated (>2 SD above the mean) in two patients in Group A (range of peak, 65–114 IU/L) and four patients in Group B (range of peak, 73–175 IU/L) during the prophylaxis period. No patient was withdrawn from the study because of elevated serum hepatic enzymes or bilirubin, and levels returned to normal values in all patients who survived to discharge.

## DISCUSSION

This pilot study demonstrates that twice weekly dosing of fluconazole prophylaxis is similar in efficacy compared with the more frequent dosing of our earlier study in high-risk, preterm infants. The incidence of *Candida* colonization and invasive infection was similar for both regimens, and neither was associated with the emergence of resistant *Candida* or other fungal species. Twice weekly dosing, when compared with our previous study regimen, reduces the total number of fluconazole doses per patient from 26 to 12 doses in the highest-risk infants who require prophylaxis for 6 weeks. The reduced number of fluconazole doses decreases cost and lessens exposure to fluconazole.

In both our study periods covering 54 noncontinuous months, fungal resistance was not detected in our NICU. Recently, fungal resistance has been reported in a preterm animal intensive care unit.<sup>22</sup> As part of studies of chronic lung disease in animals, preterm baboons were intubated, had instrumentation with central vascular catheters, and received antibiotics and parenteral nutrition. Fungemia frequently occurred in these preterm animals with a high mortality rate. This prompted investigators to administer intravenous fluconazole prophylaxis using treatment doses of 6 mg/kg. After 43 months of prophylaxis, an outbreak of fungemia with resistant *C. parapsilosis* isolates occurred in the same month.<sup>22</sup> Factors that may have lead to resistance include the higher fluconazole dose, continuous prophylaxis of all animals in the small intensive care unit, and infection control practices.



**Figure 3.** Efficacy of fluconazole prophylaxis in high-risk, preterm infants <1000 grams at birth. The incidence of *Candida* colonization and bloodstream infection of the placebo-treated group (P) in our previous study<sup>1</sup> was compared with the different fluconazole prophylaxis dosing schedules as well as all patients who received fluconazole prophylaxis in both studies. From left to right, the placebo-treated group (P) was compared with: (1) patients who received Schedule A (A) dosing of fluconazole (the dosing in the fluconazole group from our previous study and Group A in this study); (2) Schedule B or twice a week dosing, which was Group B in this study (B); and (3) all patients who received fluconazole prophylaxis in both studies (F). Placebo-treated patients were more likely to develop *Candida* colonization and bloodstream infection compared with each dosing schedule and all fluconazole-treated patients.

The duration of prophylaxis in our studies of up to 6 weeks was chosen based on the time period when the majority of neonatal fungal infections occur and the evidence that fungal infection after 6 weeks of life is associated with lower mortality.<sup>1,23,24</sup> We also discontinued prophylaxis earlier if intravenous access was no longer required, therefore, exposure was restricted to the period of extreme prematurity and that period when the additional risk factors for fungemia were present (eg, use of central vascular catheter, parenteral nutrition, broad-spectrum antibiotics).<sup>25–27</sup> We speculate that lower fluconazole doses and selecting only those infants at highest risk for invasive fungal infection may delay or prevent the emergence of resistance.

A limitation of this study is the sample size and power. Based on the low incidence of candidemia in patients receiving fluconazole prophylaxis, the number of patients needed to demonstrate equivalence between these two groups would be 2140. Three infants developed fungemia despite fluconazole prophylaxis. For one patient, this may have been the result of missing the initial dose of antifungal prophylaxis. No adverse patient effects or fungal resistance have been demonstrated in this, our previous study,<sup>1</sup> and another prophylaxis study that included larger infants,<sup>28</sup> but the number of patients studied is insufficient to detect adverse effects that may occur very infrequently. For example, cisapride, which is no longer marketed in the United States, would be contraindicated with fluconazole because of an association with arrhythmia.

We have analyzed the results of this study and compared them with the results of our previous study<sup>1</sup> and its placebo-treated group (Figure 3). In both studies, high-risk, preterm infants weighing <1000 grams at birth who had an endotracheal tube and/or central vascular catheter were enrolled. Seventy-five percent of these high-risk infants were  $\leq 26$  weeks gestation at birth. The incidence of fungemia in the patients who received twice a week dosing (1 of 40) was lower compared with the placebo-treated patients (8 of 50) (risk difference 0.14; 95% confidence interval, 0.01, 0.27;  $P = .04$ ). Fungemia also was lower in all the patients who received fluconazole dosing according to Schedule A (fluconazole-treated patients in our previous study and Group A in this study) (3 of 91) compared with the placebo-treated patients (8 of 50) (risk difference 0.14; 95% confidence interval, 0.05–0.27;  $P = .002$ ). Overall, candidemia occurred in 2.3% (3 of 131) of all patients receiving fluconazole prophylaxis in both studies compared with 16% (8 of 50) of patients receiving placebo (risk difference 0.14; 95% confidence interval, 0.05, 0.22;  $P < .001$ ).

Most preterm infants are not colonized at birth, and thus there is a unique opportunity in this high-risk patient population to either prevent or limit fungal colonization and subsequent invasive infection by using prophylactic therapy soon after birth. Infants who were colonized at baseline were enrolled later, which may imply a possible benefit of initiating prophylaxis on the first day of life. Because of the high mortality rate (up to 40%) associated with fungemia in these high-risk, extremely low birth weight infants,<sup>1,23,27</sup> antifungal prophylaxis offers the potential to improve outcomes and decrease mortality.<sup>29</sup> Further study is needed utilizing a much larger study cohort to evaluate the effect of fluconazole prophylaxis on mortality. In addition, a multicenter clinical trial would yield more data on efficacy, safety, and azole resistance. In conclusion, twice weekly dosing of fluconazole prophylaxis can decrease *Candida* colonization and invasive infection, cost, and drug exposure in high-risk, preterm infants.

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