ORIGINAL ARTICLE

A Multicenter, Randomized Trial of Prophylactic Fluconazole in Preterm Neonates

Paolo Manzoni, M.D., Ilaria Stolfi, M.D., Lorenza Pugni, M.D., Lidia Decembrino, M.D., Cristiana Magnani, M.D., Gennaro Vetrano, M.D., Elisabetta Tridapalli, M.D., Giuseppina Corona, M.D., Chiara Giovannozzi, M.D., Daniele Farina, M.D., Riccardo Arisio, M.D., Franco Merletti, M.D., Ph.D., Milena Maule, M.D., Fabio Mosca, M.D., Ph.D., Roberto Pedicino, M.D., Mauro Stronati, M.D., Michael Mostert, M.D., and Giovanna Gomirato, M.D., for the Italian Task Force for the Study and Prevention of Neonatal Fungal Infections and the Italian Society of Neonatology

ABSTRACT

BACKGROUND

Invasive candida infections are a major cause of morbidity and mortality in preterm infants. We performed a multicenter, randomized, double-blind, placebo-controlled trial of fluconazole for the prevention of fungal colonization and infection in very-low-birth-weight neonates.

METHODS

During a 15-month period, all neonates weighing less than 1500 g at birth from eight tertiary Italian neonatal intensive care units (322 infants) were randomly assigned to receive either fluconazole (at a dose of either 6 mg or 3 mg per kilogram of body weight) or placebo from birth until day 30 of life (day 45 for neonates weighing <1000 g at birth). We performed weekly surveillance cultures and systematic fungal susceptibility testing.

RESULTS

Among infants receiving fluconazole, fungal colonization occurred in 9.8% in the 6-mg group and 7.7% in the 3-mg group, as compared with 29.2% in the placebo group ($P<0.001$ for both fluconazole groups vs. the placebo group). The incidence of invasive fungal infection was 2.7% in the 6-mg group and 3.8% in the 3-mg group, as compared with 13.2% in the placebo group ($P=0.005$ for the 6-mg group and $P=0.02$ for the 3-mg group vs. the placebo group). The use of fluconazole did not modify the relationship between colonization and the subsequent development of invasive fungal infection. Overall mortality was similar among groups, as was the incidence of cholestasis. No evidence for the emergence of resistant candida species was observed, but the study did not have substantial power to detect such an effect.

CONCLUSIONS

Prophylactic fluconazole reduces the incidence of colonization and invasive candida infection in neonates weighing less than 1500 g at birth. The benefit of treating candida colonization is unclear. (Current Controlled Trials number, ISRCTN85753869).

From Sant'Anna Hospital, Turin (P.M., C.G., D.F., G.G.); Policlinico Umberto I, Rome (I.S., R.P.); Mangiagalli Hospital IRCCS, University of Milan, Milan (L.P., F. Mosca); San Matteo Hospital, Pavia (L.D., M.S.); Arcispedale, Reggio Emilia (C.M.); Fatebenefratelli Hospital, Benevento (G.V.); University of Bologna, Bologna (E.T.); University of Messina, Messina (G.C.); and University of Turin, Turin (R.A., M. Maule, F. Merletti, M. Mostert) — all in Italy. Address reprint requests to Dr. Manzoni at the Neonatology and Hospital Neonatal Intensive Care Unit, Sant'Anna Hospital, Corso Spezia 60, 10126 Turin, Italy, or at paolomanzoni@hotmail.com.

N Engl J Med 2007;356:2483-95.

Despite efforts to improve the outcomes of preterm infants, systemic fungal diseases caused mainly by candida species are an important complication of the care of neonates on the threshold of viability. Candida species colonize up to 60% of very-low-birth-weight neonates (those weighing less than 1500 g) during their first month in the neonatal intensive care unit (NICU). Such colonization may progress to invasive fungal infection in up to 20% of these infants.[1-5] Nonspecific clinical features, poor sensitivity of diagnostic tests, and late recognition mean that at the time of diagnosis invasive fungal infection is often advanced. Such infections increase the rate of death from all causes (28%, vs. 7% for infants without such infections[3]) and death that is attributable to fungal infection (up to 44% of deaths).[1-5] Early diagnosis and successful treatment have not been shown to prevent prolonged stays in the NICU, high costs, or neurodevelopmental impairment.[6-8]

Very-low-birth-weight neonates are at risk for invasive fungal infection because of their immature immune system and the invasive supportive care they require.[9-14] Prophylaxis with antifungal drugs is established in certain select high-risk patients, such as adult and pediatric patients with hematologic cancers and immunity defects.[15-19] Among such patients, prophylactic fluconazole has decreased candida-related complications.[20,21] After a single-center, randomized trial suggested similar efficacy in high-risk, extremely-low-birth-weight neonates (those with birth weights below 1000 g),[22] some NICUs began using fluconazole for routine prophylaxis.[23-26] Even so, expert opinion[27-30] and Cochrane reviews[31,32] do not recommend this practice because of the paucity of data regarding safety and resistance and the absence of adequately powered, multicenter trials. This study presents the results of such a multicenter, prospective, randomized, double-blind, placebo-controlled evaluation of fluconazole prophylaxis in very-low-birth-weight infants in the NICU.

METHODS

PATIENTS

From May 1, 2004, to July 31, 2005, we enrolled neonates at eight tertiary Italian NICUs in the study, which was approved by the ethics committee at each hospital. Parents or guardians provided written informed consent. Pfizer Italia supported the study with a grant and supplied both fluconazole and placebo; the sponsor was not involved in the study design, in the enrollment of patients, or in the collection, analysis, interpretation of the data or preparation of the manuscript. All authors vouch for the completeness and accuracy of the data presented.

The primary objective was to evaluate the effectiveness of fluconazole at doses of 3 mg and 6 mg per kilogram of body weight in the prevention of candida colonization and infection. Secondary objectives were assessment of the incidence of gram-positive and gram-negative sepsis, necrotizing enterocolitis, ligation of patent ductus arteriosus, threshold retinopathy of prematurity requiring surgery, severe intraventricular hemorrhage, bronchopulmonary dysplasia, and alteration of liver function at baseline and at the end of prophylaxis.

Very-low-birth-weight neonates who were admitted to the NICU before day 3 of life were eligible for enrollment. Exclusion criteria were a lack of parental consent and liver failure (levels of aspartate aminotransferase and alanine aminotransferase that were three times the upper limit of the range of normal values). The pharmacy at each center used computer-generated randomization lists to form three groups in a 1:1:1 ratio and prepared the daily drug doses. Infants received either 6 mg or 3 mg of fluconazole (Diflucan, Pfizer Italia) per kilogram of body weight every third day for the first 2 weeks and then every other day; the placebo group received 1 ml of normal saline on the same schedule.[20,21] Extremely-low-birth-weight infants received prophylaxis for 6 weeks; neonates weighing 1000 to 1500 g received prophylaxis for 4 weeks,[20,21,25] unless they were discharged earlier or required systemic antifungal therapy for proven or presumed invasive fungal infection. Administration of the study drug began on day 3 with one daily dose intravenously, if a catheter was present, or through an orogastric tube.

Weekly surveillance of liver function (levels of serum aspartate aminotransferase, alanine aminotransferase, γ-glutamyltransferase, and bilirubin) was performed for the duration of administration of the study drug. Drug interactions with flucon-

azole were screened. Invasive fungal infection was defined as a positive culture from blood (a peripheral site), urine (collected by sterile suprapubic puncture or bladder catheterization, with growth of ≥10,000 organisms per milliliter), or cerebrospinal fluid. Diagnosis relied on guidelines from international consensus documents[33,34] and recommendations of the Italian Neonatology Society's Fungal Infections Task Force.[35] Invasive fungal infections were treated with intravenous liposomal amphotericin B at the recommended doses. Investigators remained unaware of study-group assignments during such treatment.

FUNGAL ISOLATION AND IDENTIFICATION

The following cultures were obtained: at baseline and then weekly, we performed surveillance cultures of the ear canal (at birth only), stool, gastric aspirate, and nasopharynx or endotracheal secretions while infants were receiving fluconazole or placebo; cultures were obtained from surgical devices after removal; and clinical cultures were obtained from sites indicated by the physician (e.g., skin and respiratory secretions). Baseline colonization was defined as the isolation of fungi from the ear canal at birth or from any site during days 1 and 2 of life.

Specimens were incubated on chromogen culture plates (Albicans ID, BioMérieux) to identify *Candida albicans* colonies as blue stains after 48 hours at 37°C. Colonies were speciated through a miniaturized system of biochemical tests (Vitec Yeast, BioMérieux). Isolates were tested for sensitivity to fluconazole with standardized microbroth dilution assays (ATB-Fungus-2-Int, BioMérieux) according to recommendations of the National Committee for Clinical Laboratory Standards (NCCLS).[36] The interpretative breakpoint of fluconazole resistance was defined as at least 64 μg per milliliter. A breakpoint of 16 to 32 μg per milliliter was considered to be indicative of dose-responsive susceptibility.[36]

STATISTICAL ANALYSIS

The following variables were analyzed: incidence of colonization (at least one site), invasive fungal infection, death from all causes before discharge from the hospital, candida-related deaths (death within 3 days after the last positive culture from any site in the absence of other causes or isolation of candida species at autopsy), the presence of natively fluconazole-resistant species, secondary outcomes (including those potentially related to fluconazole), the rate of progression of colonization to invasive fungal infection, and patterns of the sensitivity of isolates to fluconazole. Infants receiving either 6 mg or 3 mg of fluconazole per kilogram were compared separately with those receiving placebo. Since there were no significant differences between the 6-mg group and the 3-mg group, a post hoc analysis was performed comparing the placebo group with both fluconazole groups combined. Proportions and continuous variables were compared with the use of Fisher's exact two-tailed test and the t-test, respectively. Risk ratios and 95% confidence intervals were calculated to compare cumulative between-group incidences with the use of Stata software. A multivariate logistic-regression analysis was performed with adjustment for important risk factors possibly associated with invasive fungal infection. The Wald test was used to assess the significance of the estimated coefficients.

The number of patients needed for each group was estimated in 82 infants for colonization on the basis of a two-sided type I error rate of 0.05 or less and a power of 90% to detect an absolute difference of at least 66% (a decrease from 30% to 10%) in the cumulative incidence of fungal colonization between infants in the fluconazole groups and those in the placebo group, given a pretrial incidence of 30%. The number needed for each group was also estimated in 89 infants for invasive fungal infection on the basis of a two-sided type I error rate of 0.05 or less and a power of 80% to detect an absolute difference of at least 80% (a decrease from 15% to 3%) in the cumulative incidence of invasive fungal infection between infants in the fluconazole groups and those in the placebo group, given a pretrial incidence of 15%. A total of 118 infants would have been needed to reach a power of 90%. Given the low incidence of invasive fungal infection in the two fluconazole groups, the study was underpowered to detect significant differences for this outcome between these groups. Assuming an incidence of 4% in the 3-mg group, 1141 infants would have been needed to reach a power of 80% to detect an absolute difference of 50%, and 424 infants would have been needed for a difference of 75%. Power

calculations were performed with the use of S-plus software, version 2000 (MathSoft).

RESULTS

PATIENTS

A total of 363 very-low-birth-weight neonates survived for at least 3 days and were potentially eligible for the study. Of these infants, 27 were ineligible owing to liver failure at baseline (3 patients), a lack of consent (12 patients), and missing or incorrect randomization (12 patients). Another 14 infants were removed from the analysis owing to incomplete data (3 in the 6-mg group, 3 in the 3-mg group, and 4 in the placebo group), incorrect drug administration (2 in the 6-mg group and 1 in the 3-mg group), or accidental administration of five doses of fluconazole instead of placebo (1 in the placebo group). A total of 322 neonates were randomly assigned, 112 to the 6-mg group, 104 to the 3-mg group, and 106 to the placebo group (Fig. 1).

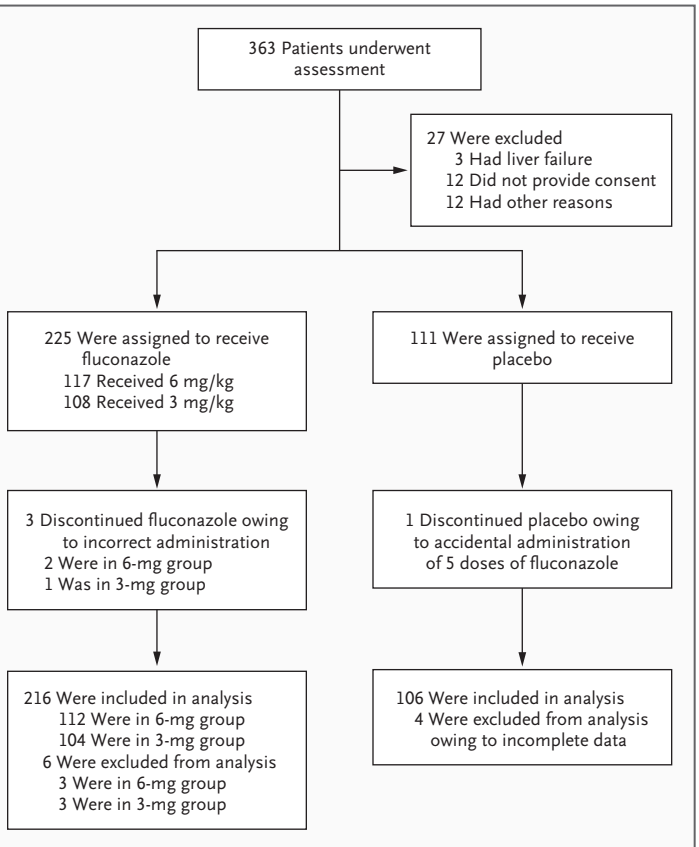

Figure 1. Enrollment and Outcomes.

Four infants (1.2%) who were incorrectly assigned to the 6-mg group by investigators in one NICU received a dose of 6 mg per kilogram. Analyses that were repeated without the data from these infants provided no significant changes in the estimates. A total of 32.2% of fluconazole doses were administered orally, and 90.1% of infants received both oral and intravenous administration. There were no intergroup differences in the route of administration. Demographic and neonatal characteristics and major risk factors for fungal infections are listed in Tables 1 and 2. There were no significant baseline differences in most risk factors for colonization and invasive fungal infection between groups. However, according to univariate analysis, the use of corticosteroids and oxygen was significantly increased in the groups receiving fluconazole, as compared with those receiving placebo (for corticosteroids, $P<0.001$ for the 6-mg group and $P=0.02$ for the 3-mg group; for oxygen, $P=0.05$ for the 3-mg group).

FUNGAL COLONIZATION AND INVASIVE FUNGAL INFECTION

Data regarding fungal colonization, invasive fungal infection, and distribution of fungal species and colonization sites and infection are shown in Tables 3 and 4. Colonization occurred less frequently in the 6-mg group (9.8%) and the 3-mg group (7.7%) than in the placebo group (29.2%; $P<0.001$ for both comparisons). Also less frequent was invasive fungal infection (2.7% in the 6-mg group and 3.8% in the 3-mg group, as compared with 13.2% in the placebo group; $P=0.005$ and $P=0.02$, respectively). The 21 episodes of invasive fungal infection were caused by *C. albicans* (16 patients), *C. parapsilosis* (2), *C. glabrata* (2), *C. tropicalis* (1), and *C. guilliermondii* (1). One neonate was infected with both *C. parapsilosis* and *C. glabrata.* Invasive fungal infections occurred in 2 of 7 neonates with baseline colonization (28.6%) in the fluconazole groups combined and in 4 of 14 neonates (28.6%) in the placebo group.

Fluconazole did not have an effect on the association between colonization and subsequent progression to invasive fungal infection, which occurred in 27.3% of infants in the 6-mg group and 50.0% in the 3-mg group, as compared with 45.2% in the placebo group ($P=0.47$ and $P=1.0$, respectively). Excluding the neonates with colonization at baseline, the adjusted rate of progression

Table 1. Characteristics of the Patients.*

Variable	Fluconazole		Placebo (N=106)	P Value†	
	6 mg (N=112)	3 mg (N=104)		6 mg of Fluconazole vs. Placebo	3 mg of Fluconazole vs. Placebo
M/F sex — no.	57/55	52/52	55/51	0.89	0.89
White race — %‡	88	86	90	0.83	0.41
Birth weight — g				0.14	0.09
Mean	1065±280	1060±245	1120±270		
Range	380–1500	360–1480	480–1500		
Gestational age — wk				0.09	0.14
Mean	28.9±2.3	28.9±2.8	29.4±2.0		
Range	25.0–35.0	24.0–35.0	24.0–34.0		
Born at another facility — %	17	20	23	0.31	0.74
Vaginal delivery — %	35	38	40	0.49	0.89
Mean Apgar score at 5 min§	6±2	6±3	6±2	1.00	1.00
Mother had preeclampsia — %	26	28	27	0.88	1.00
Use of medication — %					
Antenatal corticosteroids	72	75	70	0.77	0.44
Antenatal antibiotics	80	85	82	0.86	0.71
Surfactant (at least once)	81	85	76	0.41	0.16

* Plus–minus values are means ±SD.
† P values were calculated with the use of Fisher's exact two-tailed test for comparing proportions and the t-test for comparing continuous variables (e.g., birth weight).
‡ Race was determined by the investigators. Percentage refers to both parents.
§ The Apgar score ranges from 0 to 10, with higher scores indicating better functioning.

from colonization to invasive fungal infection was 33.0% in the fluconazole groups combined and 50.0% in the placebo group (P=0.30). In a post hoc comparison of the fluconazole groups combined versus the placebo group, prophylaxis significantly reduced colonization and invasive fungal infection (P<0.001 and P=0.001, respectively), without modifying the association between colonization and subsequent invasive fungal infection (P=0.77). Fluconazole at either dose decreased colonization and invasive fungal infection in extremely-low-birth-weight infants and in those weighing 1000 to 1500 g (P=0.001 for colonization in both groups; P=0.02 and P=0.03 for invasive fungal infection, respectively), as well as in neonates weighing 750 to 1500 g (P=0.007 for invasive fungal infection and P<0.001 for colonization). A nonsignificant decrease in invasive fungal infection was observed in infants weighing less than 750 g (16.7% vs. 2.2%, P=0.07), as well as in those weighing 750 to 1000 g (12.0% vs. 3.7%, P=0.17). However, our study was underpowered for a cluster analysis of these two subgroups.

MORTALITY

Overall mortality was similar in the three groups (8.0% in the 6-mg group [P=0.81] and 8.7% in the 3-mg group [P=1.0], as compared with 9.4% in the placebo group). In the fluconazole groups, no deaths were attributable to candida infection, as compared with two deaths (1.9%) in the placebo group (P=0.23 for the 6-mg group and P=0.50 for the 3-mg group) (Table 3).

ISOLATES NATIVELY RESISTANT TO FLUCONAZOLE

There were no significant between-group differences in the incidence of colonization and infection with *C. krusei*, *C. glabrata*, and *C. guilliermondii*. Overall, three isolates from these species were seen in both of the fluconazole groups (*C. krusei*, *C. glabrata*, and *C. guilliermondii*) and two in the placebo group (both *C. glabrata*). *C. glabrata* caused two

Table 2. Major Risk Factors for Invasive Fungal Infection and Secondary End Points.*

Variable	Fluconazole		Placebo (N=106)	P Value†	
	6 mg (N=112)	3 mg (N=104)		6 mg of Fluconazole vs. Placebo	3 mg of Fluconazole vs. Placebo
Risk factor					
Third-generation cephalosporins — day				0.28	0.50
Mean	7.1±6.0	7.4±6.0	8.0±7.0		
95% CI	1.0–19.0	1.0–21.0	1.0–17.0		
Central venous catheter — day				0.13	0.31
Mean	16.8±13.0	15.9±12.0	14.2±12.0		
95% CI	19.0–26.0	18.0–24.0	14.0–25.0		
Total parenteral nutrition — day				0.26	0.35
Mean	10.9±13.0	11.3±11.0	12.8±12.0		
95% CI	12.0–18.0	13.0–18.0	12.0–23.0		
Vancomycin — %	14.0	20.0	22.0	0.16	0.87
Early-onset neutropenia — %	13.0	10.1	11.8	0.84	0.54
Antibiotic therapy — day				0.73	0.90
Mean	14.8±17.0	13.9±11.0	14.1±13.0		
95% CI	15.0–27.0	16.0–21.0	12.0–18.0		
Systemic corticosteroids — day				<0.001	0.02
Mean	6.6±3.0	6.3±5.0	5.0±3.0		
95% CI	4.0–9.0	2.0–10.0	3.0–13.0		
Umbilical catheter — day				1.0	1.0
Mean	6.0±3.0	6.0±3.0	6.0±3.0		
95% CI	5.0–6.0	5.0–7.0	4.0–7.0		
Intubation — day				0.83	0.28
Mean	11.9±10.0	10.7±9.0	12.2±11.0		
95% CI	9.0–16.0	9.0–18.0	5.0–21.0		
Oxygen therapy — day				0.16	0.05
Mean	16.0±11.0	17.0±12.0	14.0±10.0		
95% CI	12.0–24.0	9.0–30.0	10.0–26.0		
Histamine-receptor antagonist — day				0.83	0.63
Mean	14.2±12.8	14.7±12.7	13.8±14.6		
95% CI	13.0–21.0	14.0–22.0	8.0–28.0		
Theophylline — day				0.51	0.37
Mean	19.4±13.1	19.0±12.3	20.6±13.6		
95% CI	20.0–26.0	19.0–26.0	17.0–32.0		
Secondary end point					
Sepsis — %					
Gram-negative bacteria	17	17	22	0.40	0.49
Gram-positive bacteria	12	18	20	0.13	0.86
Necrotizing enterocolitis (requiring surgery or defined by pneumatosis on radiography) — %	5.3	4.8	4.9	1.0	1.0
Threshold retinopathy of prematurity (requiring surgery) — %	14	15	18	0.58	0.71

Table 2. (Continued.)

Variable	Fluconazole		Placebo (N=106)	P Value†	
	6 mg (N=112)	3 mg (N=104)		6 mg of Fluconazole vs. Placebo	3 mg of Fluconazole vs. Placebo
Severe (grade 3 or 4) intraventricular hemorrhage — %	9	9	11	0.66	0.65
Bronchopulmonary dysplasia — %	18	16	24	0.32	0.23
Major surgery (including ligation of PDA)	16	19	13	0.57	0.27
Death from any cause before hospital discharge — no. (%)	9 (8.0)	9 (8.7)	10 (9.4)	0.81	1.0
Mean time in NICU — day				0.36	0.13
Mean	54±28	56±28	51±19		
95% CI	48–60	50–62	45–58		
		Odds Ratio‡ (95% CI)		P Value	
Multivariate logistic analysis adjusted for the most important risk factors					
Fluconazole		0.23 (0.08–0.63)		0.001	
Corticosteroids — per day		0.88 (0.87–1.04)		0.18	
Oxygen therapy — per day		0.93 (0.77–1.08)		0.28	
Third-generation cephalosporins — per day		1.21 (0.96–1.06)		0.55	
Central venous catheter — per day		1.15 (0.92–1.04)		0.61	
Total parenteral nutrition		1.11 (0.93–1.04)		0.68	

* Plus–minus values are means ±SD. CI denotes confidence interval, PDA patent ductus arteriosus, and NICU neonatal intensive care unit.
† P values were calculated with the use of Fisher's exact two-tailed test for comparing proportions and the t-test for comparing continuous variables (e.g., birth weight).
‡ Odds ratios are for the fluconazole groups versus the placebo group.

nonfatal invasive fungal infections, one each in the 6-mg group and the placebo group.

MINIMAL INHIBITORY CONCENTRATION

Sensitivity to fluconazole as measured by the minimal inhibitory concentration (MIC) required to inhibit the growth of 90% of the isolates (MIC_{90}) did not vary during the study period, and all isolates remained sensitive to fluconazole (*C. albicans*, 0.125 to 2.0; and *C. parapsilosis*, 0.25 to 2.0). MICs of fluconazole in the isolated strains did not increase in any of the neonates at any center during the study period. Satisfactory sensitivity (MIC_{90}: 1 [colonizing isolates], 2.0 and 8 [infecting isolates]) continued for *C. glabrata*. All infecting and colonizing isolates were sensitive to amphotericin B and flucytosine (MIC_{90}, 0.125 to 1.0 and 0.125 to 0.25, respectively).

SECONDARY OUTCOMES

There were no significant differences in secondary outcomes (Table 5). No serious adverse events or fluconazole-related toxic effects were recorded. Drug administration was not discontinued because of presumed adverse events, intolerance, or potentially dangerous interactions with other drugs. At 4 weeks of age, infants who received fluconazole had increased levels of aspartate aminotransferase and alanine aminotransferase. For aspartate aminotransferase, the mean (±SD) levels were 16.8±11.0 U per liter in the fluconazole groups combined and 13.1±10.0 U per liter in the placebo group (P=0.004); for alanine aminotransferase, the mean levels were 22.8±16.0 U per liter in the fluconazole groups combined and 19.5±11.0 U per liter in the placebo group (P=0.06). These modifications were not observed at 6 weeks or at hospital discharge. Elevations in levels of more than two times the range of normal in serum aspartate aminotransferase and alanine aminotransferase were recorded in 4 neonates who received fluconazole and in no neonates in the placebo group (P=0.31 for both comparisons); such increases in γ-glutamyltransferase levels occurred in 13 neonates who received fluconazole and in 6 who received placebo (P=1.0). At 6 weeks of age, three infants in the fluconazole groups (none with previous alterations in levels) main-

Table 3. Rates of Invasive Fungal Infection, Progression from Colonization to Systemic Infection, Previous Colonization, and Mortality.*

Variable	Treatment Group			
	6 mg and 3 mg of Fluconazole (N=216)	6 mg of Fluconazole (N=112)	3 mg of Fluconazole (N=104)	Placebo (N=106)
	no./total no. (%)			
Invasive fungal infection				
All patients	7/216 (3.2)	3/112 (2.7)	4/104 (3.8)	14/106 (13.2)
Birth weight				
<1000 g	3/98 (3.1)			6/43 (14.0)
1000–1500 g	4/118 (3.4)			8/63 (12.7)
<750 g	1/44 (2.3)			3/18 (16.7)
750–1500 g	6/172 (3.5)			11/88 (12.5)
Gestational age <27 wk	2/35 (5.7)			3/12 (25.0)
Infections caused by natively fluconazole-resistant candida species	1/7 (14.3)	1/3 (33.3)	0	1/14 (7.1)
Progression from colonization to invasive fungal infection				
All patients	7/19 (36.8)	3/11 (27.3)	4/8 (50.0)	14/31 (45.2)
Colonization				
Overall colonization (at least 1 site)	19/216 (8.8)	11/112 (9.8)	8/104 (7.7)	31/106 (29.2)
Natively fluconazole-resistant candida species	3/216 (1.4)	2/112 (1.8)	1/104 (1.0)	2/106 (1.9)
At baseline	9/216 (4.2)	5/112 (4.5)	4/104 (3.8)	5/106 (4.7)
Birth weight				
<1000 g	9/98 (9.2)			14/43 (32.6)
1000–1500 g	10/118 (8.5)			17/63 (27.0)
750–1500 g	14/172 (8.1)			26/88 (29.5)
Death				
From any cause before hospital discharge	18/216 (8.3)	9/112 (8.0)	9/104 (8.7)	10/106 (9.4)
Attributable to fungal infection	0	0	0	2/106 (1.9)

tained serum levels that were slightly above normal (one for aspartate aminotransferase and two for γ-glutamyltransferase), as compared with two infants (both for γ-glutamyltransferase) in the placebo group. These abnormalities were transient and absent at discharge. No infants reached levels of more than three times the normal range of values or had clinical signs of hepatotoxicity or cholestasis, and none required treatment for cholestasis. None of the neonates required phototherapy to treat hyperbilirubinemia.

DISCUSSION

This prospective, randomized, multicenter study showed that prophylactic fluconazole prevents colonization and infection by candida species in very-low-birth-weight infants. Our findings are similar to those from single-NICU studies[21-26] and to those observed in immunocompromised adults and children.[15-19] Colonization is considered a major risk factor for invasive fungal infection in the preterm neonate.[10,11,14,37] In our study, fluconazole was effective in preventing rather than treating colonization.

Exposure of newborns to fungi is a complex problem and results from both horizontal and vertical transmission. Treatment of maternal vaginal candidiasis and good hand hygiene of health care workers should reduce the risk of "new entry" candida in the NICU. Breaking the horizontal NICU transmission cycle of hand coloni-

6 mg and 3 mg of Fluconazole vs. Placebo		6 mg of Fluconazole vs. Placebo		3 mg of Fluconazole vs. Placebo	
Risk Ratio (95% CI)	P Value	Risk Ratio (95% CI)	P Value	Risk Ratio (95% CI)	P Value
0.25 (0.10–0.59)	0.001	0.20 (0.06–0.69)	0.005	0.29 (0.10–0.86)	0.02
0.22 (0.06–0.84)	0.02				
0.27 (0.08–0.85)	0.03				
0.14 (0.02–1.23)	0.07				
0.24 (0.09–0.70)	0.007				
0.23 (0.04–1.21)	0.10				
2.00 (0.15–27.4)	1.0	4.67 (0.39–55.5)	0.33	0	1.0
0.82 (0.40–1.65)	0.77	0.60 (0.21–1.71)	0.47	1.11 (0.50–2.45)	1.0
0.30 (0.18–0.51)	<0.001	0.34 (0.18–0.63)	<0.001	0.26 (0.13–0.55)	<0.001
0.74 (0.12–4.34)	0.67	0.95 (0.14–6.60)	1.0	0.51 (0.05–5.53)	1.0
0.88 (0.30–2.57)	0.78	0.95 (0.28–3.18)	1.0	0.82 (0.23–2.95)	1.0
0.28 (0.13–0.60)	0.001				
0.31 (0.15–0.64)	0.001				
0.27 (0.10–0.43)	<0.001				
0.88 (0.42–1.85)	0.83	0.85 (0.36–2.01)	0.81	0.92 (0.39–2.17)	1.0
0	0.10	0	0.23	0	0.50

* The exclusion of four infants who were originally assigned to the 3-mg group but actually received the 6-mg dose of fluconazole yields the following risk ratios (as compared with the placebo group): for colonization, risk ratio, 0.34; 95% confidence interval (CI), 0.18 to 0.67; P<0.001; for invasive fungal infection, risk ratio, 0.22; 95% CI, 0.07 to 0.74; P=0.007. However, the error in assignment does not change the P value for progression from colonization to invasive fungal infection (P=0.47) and does not change the P value for mortality, since none of the four infants died.

zation from caregivers to neonates and vice versa by fluconazole prophylaxis should reduce the risk that preterm infants pose to one another.

Rates of candidemia in the NICU vary greatly among institutions. NICU-related factors (e.g., the average use of broad-spectrum antibiotic per infant[5]) may be implicated, in addition to the well-known preterm-related factors. Decreasing the incidence of infection would benefit a NICU's present and future preterm infants, a factor that may lead to the adoption of intermittent prophylactic regimens with the targeting of prophylaxis to ever-more-selected subgroups of high-risk neonates.[9,37] Prophylaxis should be adapted to the infection rate in each NICU to optimize the number needed to treat, in line with guidelines recommending antifungal prophylaxis in "carefully selected patients in units with high rates of invasive candidiasis."[38] In our study, the number needed to treat was eight (five among extremely-low-birth-weight infants) to prevent invasive fungal infection.

Two randomized, placebo-controlled studies

Table 4. Distribution of Candida Fungal Species and Sites of Colonization and Infection.

Variable	All Infants	6 mg and 3 mg of Fluconazole	Placebo
		number	
Fungal isolates from cultures	133	36	97
Infants with positive cultures	50	19	31
Colonization			
Fungal species isolates			
Candida albicans	98	24	74
C. parapsilosis	25	7	18
C. glabrata	3	1	2
C. tropicalis	5	2	3
C. guilliermondii	1	1	0
C. krusei	1	1	0
Site			
Stool	55	16	39
Gastric aspirate	22	6	16
Nasopharynx	9	3	6
Skin	6	0	6
Mechanical devices	19	5	14
Other	22	6	16
Invasive fungal infection			
Fungal species isolates*			
C. albicans	16	4	12
C. parapsilosis	2	1	1
C. glabrata	2	1	1
C. tropicalis	1	0	1
C. guilliermondii	1	1	0
Site			
Blood	13	4	9
Urine	7	3	4
Cerebrospinal fluid	1	0	1

* One infant was infected with both *C. parapsilosis* and *C. glabrata*.

of prophylactic fluconazole have been conducted among preterm infants, one in which 3 mg per kilogram of the drug was administered and the other in which 6 mg per kilogram was administered.[21,22] Our study was underpowered to detect differences in the outcome of invasive fungal infection between the two groups, owing to the low incidence of such infection. Power analysis indicated that approximately 1100 patients would be required for this evaluation.

A post hoc analysis was performed to evaluate the effect of at least 3 mg per kilogram of fluconazole by comparing both the 6-mg group and the 3-mg group with the placebo group. This analysis requires cautious interpretation. The higher dose could be more effective against strains that are dose-susceptible to fluconazole but could also be more toxic because of increased drug exposure. In this study, at 4 weeks treated infants showed higher levels of alanine aminotransferase and significantly higher levels of aspartate aminotransferase, although the levels remained within the normal reference range. However, at 6 weeks and at discharge, these modifications were not observed, and all treated neonates were discharged without abnormalities possibly caused by fluconazole. Mild and transient increases of liver enzymes, without clinical implications, are described in infants receiving fluconazole.[21,22,39]

Another concern regarding fluconazole prophylaxis is the emergence of fungal resistance.[18] In this small, brief study, sensitivity of susceptible strains remained unchanged. (MIC_{90} remained below the cutoff of NCCLS guidelines.) Another concern is the selection of natively resistant candida species (e.g., *C. glabrata* and *C. krusei*), which are occasionally associated with fluconazole exposure.[40,41] Consistent with other, short-term studies,[19,42] there was no significant change in candida ecology among the three groups, and MICs for *C. glabrata* remained in the susceptible range during the study period. Nevertheless, our study was underpowered to detect a change in fungal ecology because of its short duration. Thus, we were unable to detect shifts in candida species or the establishment of acquired resistance mutations. Long-term surveillance of fungal ecology will be important for NICUs that adopt a prophylaxis strategy.

In this multicenter study, fluconazole prophylaxis reduced fungal colonization and infection in preterm neonates. Future studies should further refine the identification of neonates who are at highest risk for infection for whom prophylaxis would be most optimally suited. Such trials should also help to define the lowest effective dose for such prophylaxis and the possible long-term emergence of fungal resistance.

Table 5. Adverse Events.*

Event or Value	Fluconazole						Placebo (N=106)
	6 mg (N=112)	P Value	3 mg (N=104)	P Value	6 mg and 3 mg (N=216)	P Value	
AST (week 4) — U/liter							
All infants	15.3±10.0	0.11	18.5±12.0	<0.001	16.8±11.0	0.004	13.1±10.0
<1000 g	23.2±13.0	0.96	26.5±15.0	0.10	24.8±14.0	0.32	23.1±15.0
1000–1500 g	12.1±9.0	0.31	14.8±11.0	0.006	13.4±10.0	0.03	10.8±10.0
ALT (week 4) — U/liter							
All infants	20.5±12.0	0.52	25.2±19.0	0.008	22.8±16.0	0.06	19.5±11.0
<1000 g	29.5±18.0	0.02	33.5±18.0	0.46	31.4±18.0	0.07	35.4±19.0
1000–1500 g	17.1±11.0	<0.001	20.0±13.0	<0.001	18.5±12.0	<0.001	11.3±10.0
γ-Glutamyltransferase (week 4) — U/liter							
All infants	66.9±68.0	0.97	53.9±42.0	0.12	60.6±57.0	0.38	67.3±77.0
<1000 g	86.5±88.0	0.80	75.1±72.0	0.20	81.0±81.0	0.39	89.5±88.0
1000–1500 g	55.4±48.0	0.91	38.9±34.0	0.004	47.5±42.0	0.11	56.2±51.0
Direct bilirubin (week 4) — mg/dl							
All infants	2.1±1.8	0.67	2.5±2.1	0.06	2.3±2.0	0.19	2.0±1.7
<1000 g	2.8±2.2	0.18	2.9±2.4	0.12	2.8±2.3	0.14	2.4±2.2
1000–1500 g	1.9±1.8	0.38	2.2±1.8	0.03	2.0±1.8	0.14	1.7±1.5
Elevation >2×ULN — no. (%)							
Week 4							
AST	2 (1.8)	0.5	2 (1.9)	0.24	4 (1.9)	0.31	0
ALT	2 (1.8)	0.5	2 (1.9)	0.24	4 (1.9)	0.31	0
γ-Glutamyltransferase	8 (7.1)	0.79	5 (4.8)	1.0	13 (6.0)	1.0	6 (5.7)
Week 6							
AST or ALT	0	1.0	1 (1.0)†	0.49	1 (0.5)	1.0	0
γ-Glutamyltransferase	1 (0.9)†	0.61	1 (1.0)†	1.0	2 (0.9)	1.0	2 (1.9)†
Elevation of direct bilirubin — no. (%)							
Week 4							
>2 mg/dl	16 (14.3)	0.55	17 (16.3)	0.32	33 (15.3)	0.40	12 (11.3)
>5 mg/dl	1 (0.9)	1.0	2 (1.9)	0.62	3 (1.4)	1.0	1 (0.9)
Week 6							
>2 mg/dl	6 (5.4)	0.75	7 (6.7)	0.37	13 (6.0)	0.60	4 (3.8)
>5 mg/dl	0	1.0	1 (1.0)†	0.50	1 (0.5)	1.0	0

* Plus–minus values are means ±SD. All P values, which were calculated with the use of Fisher's exact two-tailed test for comparing proportions and the t-test for comparing continuous variables, are for comparisons with the placebo group. Infants with a birth weight of less than 750 g had no notable differences in the incidence of adverse effects, as compared with infants who weighed more than 750 g. AST denotes aspartate aminotransferase, ALT alanine aminotransferase, and ULN upper limit of normal.

† Infants were not among those with previously noted abnormalities.

Supported by Pfizer Italia.

Some data from this study were presented in preliminary form at the 46th annual Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, September 27–30, 2006.

No potential conflict of interest relevant to this article was reported.

REFERENCES

1. Stoll BJ, Hansen N, Fanaroff AA, et al. Late-onset sepsis in very low birth weight neonates: the experience of the NICHD Neonatal Research Network. Pediatrics 2002;110:285-91.
2. Makhoul IR, Sujov P, Smolkin T, Lusky A, Reichman B. Epidemiological, clinical, and microbiological characteristics of late-onset sepsis among very low birth weight infants in Israel: a national survey. Pediatrics 2002;109:34-9.
3. Kaufman D, Fairchild KD. Clinical microbiology of bacterial and fungal sepsis in very-low-birth-weight infants. Clin Microbiol Rev 2004;17:638-80.
4. Kaufman D. Fungal infection in the very low birthweight infant. Curr Opin Infect Dis 2004;17:253-9.
5. Cotten CM, McDonald S, Stoll B, Goldberg RN, Poole K, Benjamin DK Jr. The association of third-generation cephalosporin use and invasive candidiasis in extremely low birth-weight infants. Pediatrics 2006;118:717-22.
6. Smith PB, Morgan J, Benjamin DK, et al. Increased costs associated with neonatal candidemia. Presented at the 2006 annual meeting of the Pediatric Academic Societies, San Francisco, April 29–May 2, 2006.
7. Stoll BJ, Hansen NI, Adams-Chapman I, et al. Neurodevelopmental and growth impairment among extremely low-birth-weight infants with neonatal infection. JAMA 2004;292:2357-65.
8. Benjamin DK Jr, Stoll BJ, Fanaroff AA, et al. Neonatal candidiasis among extremely low birth weight infants: risk factors, mortality rates, and neurodevelopmental outcomes at 18 to 22 months. Pediatrics 2006;117:84-92.
9. Feja KN, Wu F, Roberts K, et al. Risk factors for candidemia in critically ill infants: a matched case-control study. J Pediatr 2005;147:156-61.
10. Saiman L, Ludington E, Pfaller M, et al. Risk factors for candidemia in neonatal intensive care unit patients. Pediatr Infect Dis J 2000;19:319-24.
11. Huang YC, Li CC, Lin TY. Association of fungal colonization and invasive disease in very low birth weight infants. Pediatr Infect Dis J 1998;17:819-22.
12. Saiman L, Ludington E, Dawson JD, et al. Risk factors for Candida species colonization of neonatal intensive care unit patients. Pediatr Infect Dis J 2001; 20:1119-24.
13. Johnsson H, Ewald U. The rate of candidaemia in preterm infants born at a gestational age of 23-28 weeks is inversely correlated to gestational age. Acta Paediatr 2004;93:954-8.
14. El-Nasry FA, Neal TJ, Subhedar NV. Risk factors for invasive fungal infection in neonates. Acta Paediatr 2002;91:198-202.
15. Gotzsche PC, Johansen HK. Routine versus selective antifungal administration for control of fungal infections in patients with cancer. Cochrane Database Syst Rev 2002;2:CD000026.
16. Laverdiere M, Rotstein C, Bow EJ, et al. Impact of fluconazole prophylaxis on fungal colonization and infection rates in neutropenic patients: the Canadian Fluconazole Study. J Antimicrob Chemother 2000;46:1001-8.
17. Ho KM, Lipman J, Dobb GJ, Webb SA. The use of prophylactic fluconazole in immunocompetent high-risk surgical patients: a meta-analysis. Crit Care 2005; 9:R710-R717.
18. Castagnola E, Machetti M, Bucci B, Viscoli C. Antifungal prophylaxis with azole derivatives. Clin Microbiol Infect 2004;10:Suppl 1:86-95.
19. Playford EG, Webster AC, Sorrell TC, Craig JC. Antifungal agents for preventing fungal infections in non-neutropenic critically ill and surgical patients: systematic review and meta-analysis of randomized clinical trials. J Antimicrob Chemother 2006;57:628-38.
20. Kaufman D. Strategies for prevention of neonatal invasive candidiasis. Semin Perinatol 2003;27:414-24.
21. Kicklighter SD, Springer SC, Cox T, Hulsey TC, Turner RB. Fluconazole for prophylaxis against candidal rectal colonization in the very low birth weight infant. Pediatrics 2001;107:293-8.
22. Kaufman D, Boyle R, Hazen KC, Patrie JT, Robinson M, Donowitz LG. Fluconazole prophylaxis against fungal colonization and infection in preterm infants. N Engl J Med 2001;345:1660-6.
23. Bertini G, Perugi S, Dani C, Filippi L, Pratesi S, Rubaltelli FF. Fluconazole prophylaxis prevents invasive fungal infection in high-risk, very low birth weight infants. J Pediatr 2005;147:162-5.
24. Healy CM, Baker CJ, Zaccaria E, Campbell JR. Impact of fluconazole prophylaxis on incidence and outcome of invasive candidiasis in a neonatal intensive care unit. J Pediatr 2005;147:166-71.
25. Manzoni P, Arisio R, Mostert M, et al. Prophylactic fluconazole is effective in preventing fungal colonization and fungal systemic infections in preterm neonates: a single-center, 6-year retrospective cohort study. Pediatrics 2006;117:e22-e32.
26. Uko S, Soghier LM, Vega M, et al. Targeted short-term fluconazole prophylaxis among very low birth weight and extremely low birth weight infants. Pediatrics 2006; 117:1243-52.
27. Neely MN, Schreiber JR. Fluconazole prophylaxis in the very low birth weight infant: not ready for prime time. Pediatrics 2001;107:404-5.
28. Long SS, Stevenson DK. Reducing Candida infections during neonatal intensive care: management choices, infection control, and fluconazole prophylaxis. J Pediatr 2005;147:135-41.
29. Fanaroff AA. Fluconazole for the prevention of fungal infections: get ready, get set, caution. Pediatrics 2006;117:214-5.
30. Kaufman D, Smith PB, Benjamin DK Jr. Fluconazole prophylaxis in the nursery. Pediatr Infect Forum 2005;VII:2-16.
31. McGuire W, Clerihew L, Austin N. Prophylactic intravenous antifungal agents to prevent mortality and morbidity in very low birth weight infants. Cochrane Database Syst Rev 2004;1:CD003850.
32. Austin NC, Darlow B. Prophylactic oral antifungal agents to prevent systemic candida infection in preterm infants. Cochrane Database Syst Rev 2004;1:CD003478.
33. Munoz P, Burillo A, Bouza E. Criteria used when initiating antifungal therapy against Candida spp. in the intensive care unit. Int J Antimicrob Agents 2000;15:83-90.
34. Ascioglu S, Rex JH, de Pauw B, et al. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. Clin Infect Dis 2002;34:7-14.
35. Manzoni P, Pedicino R, Stolfi I, et al. Criteria for the diagnosis of systemic fungal infections in newborns: a report from the Task Force on Neonatal Fungal Infections of the GSIN. Pediatr Med Chir 2004; 26:89-95. (In Italian.)
36. National Committee for Clinical Laboratory Standards (NCCLS). Reference method for broth dilution antifungal susceptibility testing of yeasts: approved standard M27-A. Wayne, PA: NCCLS, 1997.
37. Fridkin SK, Kaufman D, Edwards JR, Shetty S, Horan T Changing incidence of Candida bloodstream infections among NICU patients in the United States: 1995–2004. Pediatrics 2006;117:1680-7.
38. Pappas PG, Rex JH, Sobel JD, et al.

Guidelines for treatment of candidiasis. Clin Infect Dis 2004;38:161-89.
39. Kaufman D, Boyle R, Hazen KC, Patrie JT, Robinson M, Grossman LB. Twice weekly fluconazole prophylaxis for prevention of invasive Candida infection in high-risk infants of <1000 grams birth weight. J Pediatr 2005;147:172-9.
40. Hope W, Morton A, Eisen DP. Increase in prevalence of nosocomial non-*Candida albicans* candidemia and the association of *Candida krusei* with fluconazole use. J Hosp Infect 2002;50:56-65.
41. Marr KA, Seidel K, White TC, Bowden RA. Candidemia in allogeneic blood and marrow transplant recipients: evolution of risk factors after the adoption of prophylactic fluconazole. J Infect Dis 2000;181:309-16.
42. Blot S, Janssens R, Claeys G, et al. Effect of fluconazole consumption on long-term trends in candidal ecology. J Antimicrob Chemother 2006;58:474-7.