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Prophylactic Fluconazole Is Effective in Preventing Fungal Colonization and Fungal Systemic Infections in Preterm Neonates: A Single-Center, 6-Year, Retrospective Cohort Study

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ABSTRACT

OBJECTIVE. Despite the promising preliminary results observed in extremely low birth weight (ELBW) populations, the use of fluconazole to prevent fungal colonization and infection in preterm neonates in the NICU is still an open question and not yet recommended as a standard of care. We have reviewed our 6-year series to assess the effectiveness and safety of this form of prophylaxis.

METHODS. This retrospective study consisted of 465 neonates who weighed <1500 g at birth and were admitted to our NICU in the period 1998–2003. Those who were born between 1998 and 2000 and did not receive fluconazole prophylaxis (group A, $n = 240$) were compared with those who were born between 2001 and 2003 and treated with fluconazole until the 30th day of life (45th for neonates <1000 g at birth; group B, $n = 225$). Weekly surveillance cultures were obtained from all patients. Incidence of fungal colonization, incidence of systemic fungal infection (SFI), rate of progression from colonization to infection, and mortality rates attributable to fungi were calculated for both groups and separately for neonates who were <1000 g (ELBW) and were 1001 to 1500 g (NE-VLBW) at birth.

RESULTS. Overall fungal colonization was significantly lower in group B (24.0%) than in group A (43.8%; relative risk [RR]: 0.406; 95% confidence interval [CI]: 0.273–0.605). The same was true of neonates with colonization in multiple sites (2.6% vs 5.8%) and of those with colonization from high-risk sites (5.8% vs 19.2%). SFI incidence was significantly lower in group B (10 of 225 cases; 4.4%) than in group A (40 of 240 cases; 16.7%; RR: 0.233; 95% CI: 0.113–0.447). Reduction of both colonization and SFI in group B was greater in the ELBW neonates and also significant in the NE-VLBW neonates. Rate of progression from colonization to infection was significantly lower in group B (0.17 vs 0.38; RR: 0.369; 95% CI: 0.159–0.815). Crude mortality rate attributable to *Candida* species

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Key Words
fluconazole, very low birth weight infant, *Candida*, infection, colonization, prophylaxis

Abbreviations
SFI—systemic fungal infection
LOS—late-onset sepsis
VLBW—very low birth weight
ELBW—extremely low birth weight
NE-VLBW—very low birth weight neonates, excluding ELBW neonates
LAmB—liposomal amphotericin B
dol, day of life
RR—relative risk
CI—confidence interval
MIC—minimal inhibitory concentration

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was 1.7% (4 of 240) in group A vs 0% (0 of 225) in group B. Overall mortality rate (any cause before hospital discharge) was similar in the two groups (11.2% vs 10.6%), but in colonized infants (n = 159), it was significantly lower in group B (3.7% vs 18.1%; RR: 0.174; 95% CI: 0.039–0.778). The incidence of natively fluconazole-resistant fungal species did not increase over the years, and patterns of sensitivity to fluconazole remained the same. No adverse reaction related to fluconazole occurred.

CONCLUSIONS. Prophylactic fluconazole significantly reduces the incidence of colonization and systemic infection by *Candida* species in both ELBW and NE-VLBW neonates and decreases the rates of progression from initial colonization to massive colonization and to systemic infection. All VLBW neonates may benefit from fluconazole prophylaxis.

PRETERM NEONATES IN NICUs are highly prone to systemic fungal infection (SFI) as a result of the mostly unavoidable presence of several risk factors[1–5] and the long, aggressive, intensive, and invasive care needed by not yet immunocompetent patients. Colonization by *Candida* species, multiple courses of antibiotics, parenteral nutrition, the presence of a central venous catheter, and the use of histamine receptor subtype 2 antagonists are most frequently associated with an increased risk for SFI.[4–10]

Candida species is the third most frequent causal agent of late-onset sepsis (LOS) in preterm neonates,[1–3] with an estimated incidence of 1.6% to 9% in very low birth weight (VLBW)[2,4] and of 10% to 16% in extremely low birth weight (ELBW) neonates in the NICU[5,11] and a crude mortality of 30% to 75%.[1,5] These estimates may be too low, because diagnosis is hampered by the nonspecificity of clinical symptoms, the frequent unavailability of *Candida* isolates from blood cultures (their sensitivity for fungi may be as low as 50%[12,13]), and poor sensitivity of the serum inflammatory markers.[14]

Extreme suspicion and early diagnosis are imperative, because a late commencement of systemic antifungal treatment is a well-documented cause of increased mortality.[12,15] Management of SFI involves the use of both efficient means of securing an early diagnosis[16–18] and effective prevention. However, prevention in NICUs must go beyond reduction of the often unavoidable risk factors.

Prophylactic use of fluconazole in both adult[19–22] and pediatric patients with hematologic malignancies and immunodeficiencies is well established,[20,23] whereas controversial results have been observed in the few small-scale studies devoted to prophylaxis with antifungal agents in neonates. Two studies showed that oral or intravenous fluconazole[24] and oral nystatin[25,26] can prevent rectal colonization by *Candida*, whereas topical miconazole oral gel failed to prevent colonization and systemic infection.[27] The efficacy of fluconazole in reducing both colonization and infection in ELBW neonates has been demonstrated in 1 study.[11,28,29]

As in most NICUs, in the late 1990s, our tertiary intensive and subintensive 40-bed unit faced a dramatic increase in frequency and severity of SFI, related to expansion of instrumental activities and procedures and to an increase in the number of preterm births and survival rates of the most immature infants. In light of the protocols that are used for infants with hematologic malignancies and on the strength of our personal observations, in January 2001, we started to give oral or intravenous prophylactic fluconazole to all VLBW neonates, both those with birth weight <1000 g (ELBW) and those with birth weight 1001 to 1500 g (NE-VLBW). The results that were achieved until the end of 2003 are reviewed in this article.

METHODS

Study Design

A retrospective, nonrandomized intervention study with historical control subjects was undertaken by reviewing the clinical and microbiologic records of all VLBW neonates who were admitted to our NICU in the period 1998–2003. Those who were born in the period 1998–2000 and did not receive fluconazole prophylaxis were compared with those who were born in the period 2001–2003, all of whom received fluconazole.

Population

The study was conducted at the Neonatology and Hospital NICU of the Sant'Anna Hospital (Turin, Italy). This is a level 3 unit located in the greater Turin area (1 500 000 inhabitants and 15 000 births per year) with a mean delivery rate of 4000 per year and 400 admissions to its neonatal subintensive and intensive care unit. Neonates who were born from January 1, 1998, when all medical records of NICU patients were stored in computerized database, to December 31, 2003, and survived >3 days were included in the study.

All cases were extracted from our database and reviewed by examining their demographic, gestational, and perinatal data as well as antenatal risk factors (specifically those associated with maternal and fetal diabetes), septic episodes, type and duration of nutrition, clinical and microbiologic-culture results, laboratory data, treatments, and outcome. A check was also made to ensure that all neonates with a diagnosis of SFI displayed the microbiologic laboratory and clinical criteria required. Two groups were formed: (1) group A, neonates who were born in the period 1998–2000 when fluconazole prophylaxis was not used, and (2) group B, neonates who were born in the period 2001–2003, all of

whom received prophylactic fluconazole. Neonate characteristics are illustrated in Table 1.

Other causes that might have affected the frequency or relative weight of factors that increased or decreased the risk for SFI were ruled out. In detail, infection control policies were not significantly different in our unit or in the hospital as a whole during the period and followed the criteria expressed in protocols produced and regularly checked by a dedicated Control of Nosocomial Infections Committee. Furthermore, the quarterly surveillance reports that were issued by this committee never disclosed either an increase of fungal isolates that might have been related to problems in infection control or any episodic increase in *Candida parapsilosis* isolates (often related to the spread of hospital-acquired fungal infections). Finally, there were no changes in the policies and protocols regarding the use of antenatal and neonatal antibiotics or steroids or neonatal H2-antagonists or in nutritional protocols and policy. Informed written parental consent was obtained before any investigation or treatment.

Definition of SFI

Microbiologically documented fungal infection (proven fungal infection) was defined as a positive culture (1) from blood (drawn from peripheral sites) or (2) from urine (collected by suprapubic sterile puncture or sterile bladder catheterization, with growth of >10 000 fungal organisms/mL) or (3) from cerebrospinal fluid or (4) from intravascular catheter tip (only considered proof of microbiologically documented fungal infection in patients with previous peripheral colonization by the same species; otherwise, positivity was considered as high-risk site colonization).

Clinically documented fungal infection (presumed fungal infection) was defined as clinical worsening with septic features confirmed by laboratory data (increase in serum C-reactive protein levels [>15 mg/dL], with leukocytosis [>30 000/mm³] and/or leukopenia [<6000/mm³] and/or neutrophilia and/or neutropenia [Mouzinho reference values[30]] and/or thrombocytopenia [<50 000/mm³]) despite aggressive broad-spectrum antibiotic therapy for at least 2 days, with no isolation of

TABLE 1 **Neonate Characteristics**

	Group A	Group B	*P*[a]
No. of patients (*N* = 465)	240	225	
Gender (male/female)	125/115	107/118	.42
White race, %	89	85	.42
Birth weight, mean (±SD; median), g	1212 (±270; 1285)	1108 (±268; 1170)	.001
Gestational age, mean (±SD; median), wk	29.7 (±3; 29)	28.2 (±3; 28)	.06
Neonates <1000 g (ELBW), *n* (%) (*N* = 129)	57 (24)	72 (32)	.03
<750 g, *n*	23	26	
>750<1000 g, *n*	34	46	
Neonates >1001 to <1500 g (NE-VLBW), *n* (%) (*N* = 336)	183 (76)	153 (68)	
Vaginal delivery, %	38	43	.21
Apgar score at 1 min	4 ± 3	3 ± 4	.40
Apgar score at 5 min	6 ± 2	6 ± 2	.47
Mothers with preeclampsia, %	16	25	.02
Antenatal steroids, %	68	79	.08
Antenatal antibiotics, %	79	91	.11
Surfactant received (at least once), %	78	89	.21
Use of steroids, %	25	24	.39
Use of TPN, %	86	94	.10
Antibiotic therapy, mean ± SD, d	13 ± 11	14 ± 13	.26
Use of third-generation cephalosporins, %	26	37	.18
Use of vancomycin, %	15	23	.11
Incidence of early-onset neutropenia, %	11	15.3	.09
Central venous line(s) positioned, %	60	76	.11
Umbilical line at birth until dol 3, %	89	94	.30
Intubation (at least 1 d), %	60	77	.08
Oxygen therapy (at least 2 d), %	96	99	.42
Major surgery, %	12	18	.08
AST mean serum values at the fourth week of life, mg/dL; mean (±SD; median)	21.7 (±23; 24)	28.5 (±31; 30)	.19
ALT mean serum values at the fourth week of life, mean (±SD; median), mg/dL	30.1 (±20; 31)	35.2 (±26; 36)	.12
Use of H2 blockers	33%	28%	.16
Systemic fungal infection onset, mean ± SD, dol	15.0 ± 8.4	18.1 ± 8.2	.11
Mean duration of stay in NICU, d	32 ± 28	37 ± 31	.12
Proportion of sepsis by nonfungal agents, %	41	45	.22
Proportion of NEC (surgical), %	3	4.2	.18
Proportions of death (before hospital discharge, not attributable to fungi)	27/240 (11.2%)	24/225 (10.6%)	.44

TPN indicates total parenteral nutrition; AST, aspartate aminotransferase; ALT, alanine aminotransferase; NEC, necrotizing enterocolitis.

[a] χ^2 for relative frequencies, and *t* test for continuous variables (eg, birth weight)

other microorganisms in any cultures in the last 2 days, in patients who were already heavily colonized by *Candida* species (≥3 colonization sites at the onset of sepsis or previous positive culture from intravascular catheter tip).

These diagnostic criteria implemented guidelines from international Consensus documents,[16,17] as well as the Italian Neonatology Society's Fungal Infections Task Force recommendations.[18]

Treatment of SFIs

All SFI episodes were treated with intravenous liposomal amphotericin B (LAmB) given at 2.5 (start) to 5.0 (steady state) mg/kg per day for 12- to 28-day courses, as decided by the physician in charge.[15,31] When diagnosing an episode, removal of central vascular catheters was the standard policy for the management of central intravascular lines.[32] When SFI was presumed, fluconazole was suspended and LAmB was given empirically until the culture results were known.[13] 5-Fluorocytosine was added in 5 cases with end-organ damage and 1 neonate received LAmB plus itraconazole. No agent-related adverse effects or reactions were recorded, and there were no discontinuances in fluconazole prophylaxis.

Fungal Isolation and Identification From Cultures

From 5 to 16 of the following cultures per neonate were obtained:

- Cultures of surveillance (1 at day of life [dol] 1, 7, 14, 21, and 28 and also at dol 35 and 42 in ELBW infants): ear canal swab at birth, and then weekly stool, gastric aspirate, and nasopharynx secretions (endotracheal if intubated) cultures
- Cultures from surgical and mechanical devices when removed: endotracheal tubes and intravascular catheter, as well as from drains and similar devices
- Cultures from any site indicated by the physician and justified clinically (eg, skin, respiratory secretions).

Any surveillance cultures collected after dol 30 (dol 45 in ELBW infants) were not taken into consideration to exclude cases with unusually complicated courses. Baseline fungal colonization was defined as (1) ear canal swab at birth positive for fungi or (2) isolation of fungi from any site during dol 1 to 2. Low- and high-grade colonizations were defined as 1 to 2 and 3 to 4 sites colonized, respectively. Each site was considered once only, even when repeatedly positive. High-risk site colonization was defined as the isolation of fungi from urine (if <10 000 organisms/mL), catheter tip (without contemporary peripheral colonization), and endotracheal tube; low-risk colonization was defined as the isolation from skin, stool, ear swab, gastric aspirate, and nasopharynx secretions.

Stool, gastric aspirates, surgical and intravascular devices were collected in sterile containers; respiratory secretions were obtained with an infant mucus sterile extractor kit supplied with two 3.3-mm suction catheters (Vygon, Ecouen, France); skin, ear, and nasopharynx specimens were obtained on swabs (Labobasi, Novazzano, Switzerland); blood draws for culture were submitted in dedicated specimens (BacT/Alert PF; BioMerieux Inc, Durham, NC). Urine samples were obtained by sterile urethral catheterization or suprapubic aspiration of the bladder; samples that were collected from indwelling catheters or from urine bags were not considered.

For the identification of fungi, all specimens were inoculated onto chromogen culture plates (Albicans ID; BioMerieux Inc), which allow for rapid *Candida albicans* identification through the blue staining of the colonies after 48 hours of incubation at 37°C. Differently stained colonies were speciated through a miniaturized system of biochemical tests (Vitec Yeast; BioMerieux Inc). The surveillance and culture collection and analysis procedures and methods were not changed at any time during the period studied.

Prophylaxis With Fluconazole in Group B Neonates

The group B regimen was (and still is) 6 mg/kg fluconazole (Diflucan; Pfizer Italia s.r.l., Latina/Roma, Italy) every 72 hours in the first week of life, then every 48 hours from the second week until dol 30 for NE-VLBW and dol 45 for ELBW neonates or until earlier discharge or until the need for systemic antifungal therapy (mostly with LAmB) as a result of the onset of proven or presumed SFI. Fluconazole was administered starting from dol 1 as a single dose intravenously or orally, depending on the availability of a venous line and/or on the tolerance of oral feeding. This schedule was based on experiences and published pharmacokinetic data from preterm neonates.[24,33–36]

Routine surveillance (serum alanine aminotransferase and aspartate aminotransferase concentrations checked twice) was performed to record the presence of drug-related adverse effects or toxicity. Neonates were also screened for interactions between fluconazole and other concomitantly prescribed drugs and for any clinical manifestation that might be related to the use of fluconazole.

Patterns of Sensitivity of *Candida* Species to Fluconazole During the Observation Period

Fungal isolates were tested for susceptibility to fluconazole at the hospital's microbiology service at the time of isolation. The standardized microbroth dilution assay was used according to the National Committee for Clinical Laboratory Standards standard recommendations.[37] The interpretative breakpoints of fluconazole resistance were defined as ≥64 μg/mL, as recommended by the National Committee for Clinical Laboratory Standards.[37]

Statistical Analysis

The following variables were analyzed in both groups and for ELBW and NE-VLBW separately:

- Incidence of colonization during the first month of stay in NICU;
- Incidence of baseline colonization;
- Incidence of low- and high-grade colonization;
- Incidence of low- and high-risk site colonization;
- Incidence of SFI (total, proven, and presumed);
- Overall mortality rate expressed as proportions (mortality for all causes before hospital discharge);
- Mortality rate (expressed as proportions) attributable to fungal infections: defined as death within 3 days from the last positive culture from a high-risk site in the absence of any other cause of death or as isolation of *Candida* from autopsy specimens;
- Rate of progression from colonization to systemic infection (expressed as proportions);
- Incidence of isolation of natively fluconazole-resistant fungal species;
- Patterns of sensitivity to fluconazole of fungal isolates; and
- Incidence of secondary outcomes in group B, particularly those potentially related to the use of fluconazole.

Yates' corrected χ^2 to compare proportions and relative risk (RR; risk ratio) estimates, differences in risk to compare incidence rates among groups, and t test for continuous variables were calculated with SPSS 8.0 for Windows statistical software (SPSS Inc, Chicago, IL). Power calculations were performed according to S Plus 2000 (MathSoft, Cambridge, MA).

RESULTS

Population

Neonate characteristics are illustrated in Table 1. The records showed that 490 VLBW neonates (all inborn) had been admitted to our NICU and had survived >3 days. Twenty-five (12 in group A, 13 in group B) were excluded because of 1 of the following: incomplete or unavailable data, incorrect prophylaxis, ineligibility (1 group B neonate with abnormal serum liver enzyme levels on enrollment), or absence of parental consent. None of the 3 neonates who were born to HIV-positive mothers was HIV-positive at 1 year of age. One had received prophylactic fluconazole during the period 1998–2000 and therefore was excluded. The other 2 were treated in the period 2001–2003 and were free from colonization and infection by fungi. The remaining 465 neonates were considered eligible for the study and divided into 2 groups: (1) group A, neonates who were born in the period 1998–2000, when fluconazole prophylaxis was not used (n = 240: 57 ELBW, 183 NE-VLBW), and (2) group B, neonates who were born in the period 2001–2003, all of whom received prophylactic fluconazole (n = 225: 72 ELBW, 153 NE-VLBW).

There were no significant differences at the baseline between groups A and B in terms of presence of major (including antenatal) risk factors for fungal colonization and systemic infection, as defined by most reports.[1–10] Mean birth weight was significantly lower in group B (1108 vs 1212 g; P = .001), in keeping with the tendency to admit more complicated pregnancies and deliveries to our department. For confirming the importance of the results, the a posteriori power for subgroup analysis was .97 in the ELBW group and .11 for the NE/VLBW group.

Mortality

See Tables 1 and 2. Overall mortality was similar in both groups (11.2% in group A vs 10.6% in group B; P = .44). In colonized infants (n = 159), it was significantly lower in group B (3.7% vs 18.1%; 95% confidence interval [CI]: 0.039–0.778; P = .007). The decrease was significant in ELBW (10.5% vs 39.0%; 95% CI: 0.037–0.905; P = .01) but not in NE-VLBW colonized neonates (3.0% vs 4.7%; P = .26). Mortality attributable to *Candida* was 0% (0 of 225) in group B (instead of the 1.9% expected) vs 1.7% (4 of 240) in group A (RR: 0.146; P = .07)

Incidence of SFI

The incidence of SFI was significantly lower in group B than in group A (P < .0001), both in ELBW and in NE-VLBW neonates (P < .0001 and P = .02, respectively). Significance was also maintained (P = .001) when the proven episodes that were diagnosed from catheter tips (5 of 27 in group A, 1 of 5 in group B) were excluded. The difference in presumed SFI was not significant between groups (P = .06).

We also analyzed the ELBW group further, examining the infants who weighed <750 g (n = 49) and the infants who weighed 750 to 999 g (n = 80), for the presence of SFI. Data show that reduction in SFI obtained with fluconazole was significant in both subgroups (P < .0001 in infants <750 g, P = .02 in infants 750–999 g, respectively).

There were 4 cases of fatal SFI (10% of SFI episodes), all in group A (3 in ELBW neonates): 2 were proven episodes, 1 (in an ELBW patient) was presumed, and the fourth was presumed and became proven after autopsy. *C albicans* was detected in all 4 fatal cases. There were no fatal cases in group B (P = .07), instead of the 1.9 expected. No clusters of SFI occurred. In 11 cases of SFI, neutropenia prompted the use of short courses of filgrastim (granulocyte colony-stimulating factor; always before detection of SFI).

The 50 episodes of SFI were caused by *C albicans* (42

TABLE 2 **Invasive SFI, Before Colonization, Progression From Colonization to SFI, and Mortality in the 2 Groups**

	Overall	Group A	Group B	RR	95% CI	*P*
SFI						
Total SFI, *n* (%)	50 (10.8)	40 (16.7)	10 (4.4)	0.233	0.113–0.477	<.0001
Proven SFI, *n* (%)	32 (7.1)	27 (11.5)	5 (2.2)	0.179	0.068–0.474	<.0001
Presumed SFI, *n* (%)	18 (3.7)	13 (5.2)	5 (2.2)	0.397		.06
Total SFI in ELBW, *n* (%)	26 (20.2)	22 (38.6)	4 (5.6)	0.094	0.030–0.293	<.0001
Proven SFI, *n* (%)	18 (13.9)	16 (28.0)	2 (2.8)	0.098	0.068–0.355	<.0001
Presumed SFI, *n* (%)	8 (6.3)	6 (10.6)	2 (2.8)			.08
Total SFI in ELBW with birth weight 750–999 g, *n* (%) (*N* = 80)	16 (20.0)	14 (41.1)	2 (4.4)	0.065	0.013–0.313	<.0001
Proven SFI in ELBW 750–999 g, *n* (%)	12 (15)	11 (32.3)	1 (2.2)	0.046	0.006–0.382	<.0001
Presumed SFI in ELBW 750–999 g, *n* (%)	4 (5)	3 (8.8)	1 (2.2)			.12
Total SFI in ELBW with birth weight <750 g, *n* (%) (*N* = 49)	10 (20.4)	8 (34.7)	2 (7.6)	0.156	0.028–0.837	.02
Proven SFI in ELBW with birth weight <750 g, *n* (%)	6 (12.2)	5 (21.7)	1 (3.8)	0.144	0.015–1.340	.07
Presumed SFI in ELBW with birth weight <750 g, *n* (%)	4 (8.2)	3 (13)	1 (3.8)			.11
Total SFI in NE-VLBW, *n* (%)	24 (7.1)	18 (9.8)	6 (3.8)	0.374	0.145–0.948	.02
Proven SFI, *n* (%)	14 (4.2)	11 (6.0)	3 (1.9)	0.228	0.156–0.788	.009
Presumed SFI, *n* (%)	10 (2.9)	7 (3.8)	3 (1.9)			.12
Previous colonization						
No. (%) of infants with SFI among patients with low-grade colonization	38/139 (27.3)	31/91 (34.0)	7/48 (14.6)	0.351	0.244–0.858	.002
No. (%) of infants with SFI among patients with high-grade colonization	12/20 (60.0)	9/14 (64.2)	3/6 (50.0)			.08
No. (%) of infants with SFI among patients with low-risk colonization	18/100 (18.0)	14/59 (23.7)	4/41 (9.7)	0.389	0.195–0.782	.002
No. (%) of infants with SFI among patients with high-risk colonization	32/59 (54.2)	26/46 (56.5)	6/13 (46.7)			.15
Progression from colonization to SFI, *n* (%)						
All neonates	50/159 (0.31)	40/105 (0.38)	10/54 (0.17)	0.369	0.159–0.815	.009
ELBW neonates	26/60 (0.43)	22/41 (0.54)	4/19 (0.21)	0.230	0.165–0.814	.009
NE-VLBW neonates	24/99 (0.25)	18/64 (0.28)	6/35 (0.16)	0.459	0.198–0.988	.05
SFIs caused by fluconazole natively resistant fungal species (and % on total neonates)	7/465 (1.5)	4/240 (1.7)	3/225 (1.3)			.58
Mortality, *n* (%)						
Overall mortality (before hospital discharge, not attributable to fungi)	51/465 (11.0)	27/240 (11.2)	24/225 (10.6)			.44
Overall mortality in colonized neonates	21/159 (13.2)	19/105 (18.1)	2/54 (3.7)	0.174	0.039–0.778	.007
Overall mortality in ELBW colonized neonates	18/60 (30)	16/41 (39.0)	2/19 (10.5)	0.184	0.037–0.905	.01
Overall mortality in NE-VLBW colonized neonates	3/99 (3.0)	3/64 (4.7)	0/35 (0)			.26

cases), *C parapsilosis*,[6] *Candida glabrata*,[4] *Candida krusei*,[2] *Aspergillus fumigatus*,[1] *Candida tropicalis*,[1] and *Candida guilliermondii*.[1] Seven neonates were infected by 2 species, but neither was a natively fluconazole-resistant fungal species.

Incidence of Colonization

Baseline colonization rate was similar: 3.7% in group A vs 4.9% in group B ($P = .15$). Prophylactic fluconazole decreased all colonization rates (overall, low-grade, high-grade, low-risk, and high-risk colonization rate) in group B neonates. Clustering the analysis for weight range, prophylactic fluconazole caused an equally significant decrease of colonization in ELBW (from 71.9% to 26.4%; $P < .0001$) and NE-VLBW neonates (from 35.0% to 22.2%; $P = .01$; Table 3).

Relationship Colonization/SFI

Statistical analysis showed an association between the occurrence of SFI and the presence of colonization. The infection/colonization ratio was lower in group B (0.17) than in group A (0.38), showing that fluconazole reduced or prevented the progression to infection in more than half of the colonized neonates in group B (RR: 0.369; 95% CI: 0.159–0.815). This trend was unchanged when proven and presumed SFI were analyzed separately. Fluconazole was most effective in reducing or preventing progression from colonization to infection in neonates with low-grade colonization and in those with low-risk colonization ($P = .002$ in both cases), regardless of their birth weight, and in ELBW infants as a whole ($P = .009$), whereas it was much less effective in neonates who were already heavily colonized ($P = .08$) or colonized in high-risk sites ($P = .15$; Table 4).

TABLE 3 **Colonization by *Candida* Species in the 2 Groups**

Colonization	Overall	Group A	Group B	RR	95% CI	*P*
Total no. of cultures obtained	3685	1901	1784			.20
Total no. (%) of fungal isolates obtained from cultures	351 (9.5)	275 (14.4)	76 (4.2)	0.342	0.253–0.705	<.0001
Mean no. of isolates from each colonized patient	2.2	2.6	1.2	0.344	0.205–0.770	<.0001
Overall colonization (at least 1 site), *n* (%)	159/465 (34.2)	105/240 (43.8)	54/225 (24.0)	0.406	0.273–0.605	<.0001
Incidence of "baseline" colonization, *n* (%)	20/465 (4.3)	9/240 (3.7)	11/225 (4.9)			.15
Colonization in ELBW neonates, *n* (%)	60/129 (46.5)	41/57 (71.9)	19/72 (26.4)	0.140	0.064–0.305	<.0001
Colonization in NE-VLBW neonates, *n* (%)	99/336 (29.5)	64/183 (35.0)	35/153 (22.2)	0.550	0.340–0.895	.01
Colonization low grade (1–2 sites), *n* (%)	139/465 (29.8)	91/240 (38.0)	48/225 (21.3)	0.580	0.405–0.844	.008
Colonization high grade (3–4 sites), *n* (%)	20/465 (4.3)	14/240 (5.8)	6/225 (2.6)	0.402	0.191–0.895	.01
High-grade/low-grade colonization ratio, *n* (%)	0.14	0.16	0.12	0.596	0.201–0.824	.01
Colonization in low-risk sites, *n* (%)	100/465 (27.5)	59/240 (24.5)	41/225 (18.2)	0.571	0.452–1.086	.09
Colonization in high-risk sites, *n* (%)	59/465 (10.5)	46/240 (19.2)	13/225 (5.8)	0.305	0.226–0.602	<.0001
High-risk/low-risk colonization ratio, *n* (%)	0.59	0.77	0.32	0.355	0.245–0.602	.004

TABLE 4 **Relationships Between Fluconazole Prophylaxis and Isolation of Fluconazole Natively Resistant Fungal Species**

	Overall	Group A	Group B	RR	95% CI	*P*
No. of neonates with cultures positive for fluconazole[a] natively resistant fungal species (and % on total neonates)	18/465 (3.8)	9/240 (3.8)	9/225 (3.9)			.51
No. of SFIs caused by fluconazole[a] natively resistant fungal species (and % on total neonates)	7/465 (1.5)	4/240 (1.7)	3/225 (1.3)			.58
No.of isolates of fluconazole[a] natively resistant fungal species (% on total isolates)	29/351 (8.2)	15/275 (0.5)	14/76 (18)	0.388	0.155–1.052	.04
No. of deaths (not attributable to fungi) in neonates from whom fluconazole[a] natively resistant fungal species had been isolated	2/18	2/9	0/9			.20

[a] *C glabrata* (ie, *Torulopsis glabrata*) and *C krusei*.

Incidence of Fluconazole-Resistant Fungal Species Isolates

There were no significant between-group differences in the incidence of potential, demonstrated, or natively fluconazole-resistant species such as *C krusei* or *C glabrata* ($P = .51$). The number of isolates from these species was very low in group B (4 *C krusei* and 5 *C glabrata*). More important, the overall crude number of isolates from natively fluconazole-resistant species was 14 in group A vs 15 in group B, indicating the absence of a shift toward resistant species (the place left vacant by the nonresistant species was still vacant after 3 years). In addition, the number of episodes that were caused by *C krusei* and *C glabrata* was 4 in group A and 3 in group B (RR: 0.895; 95% CI: 0.456–1.320), and none was fatal. Mortality rate for nonfungal causes in this subgroup was lower in group B (0 of 9 vs 2 of 9; $P = .20$).

Patterns of Sensitivity of *Candida* Species to Fluconazole During the Observation Period

Sensitivity to fluconazole of fungal isolates (as expressed by minimal inhibitory concentration for 90% of colonies [MIC90] values) did not change significantly, and all isolates remained sensitive to fluconazole during the first 3 years and at the fourth and sixth years (MIC [μg/ml] of *C albicans*: 0.125–2.0; of *C parapsilosis*: 1.0–4.0; and of *C glabrata*: 2–16; in this last case, the value 16 is indicative of dose-dependent susceptibility rather than true sensitivity). The highest recorded values of MIC90 of fluconazole (expressed as μg/ml) for the fungal subspecies for whom data allowed analysis, during the 6-year survey, were 2.0 in group A and 1.0 and 2.0 in group B (at the first and third years of prophylaxis, respectively) for *C albicans* and 4.0 in group A and 4.0 and 2.0 in group B (at the first and third years of prophylaxis, respectively) for *C parapsilosis*.

Six-Year Time Trend

The 6-year time trends of incidence of colonization, SFI, and progression rate (Table 5) show that an abrupt drop in all 3 parameters appeared as early as the first year of prophylactic fluconazole.

TABLE 5 **Time Trend of Colonization and SFI During the Years of Survey**

Year of Survey	VLBW Enrolled Patients, *n*	Standard Prophylactic Regimen	Colonized Infants, *n*	SFI, *n*	Colonization/ Infection Progression Rate
1998	71	None	34	12	0.35
1999	80	None	37	14	0.37
2000	89	None	34	14	0.41
2001	74	Fluconazole	18	3	0.16
2002	79	Fluconazole	23	4	0.17
2003	72	Fluconazole	13	3	0.23

Secondary Outcomes

There were no significant differences in secondary outcomes between the 2 groups. The incidence of gastroesophageal reflux, bronchopulmonary dysplasia, intraventricular hemorrhage, major surgery (eg, ligation of patent ductus arteriosus) was not significantly different. A point of great importance is that the frequency of bacterial infections (particularly those by *Staphylococcus epidermidis*) and the incidence of necrotizing enterocolitis (both surgical and nonsurgical) did not change with the use of fluconazole. Only the frequency of grades 3 and 4 retinopathy of prematurity was significantly lower in group B.

There were no adverse effects or toxicity related to fluconazole. There was never an unacceptably high increase in serum hepatic enzymes or liver function impairment or clinical signs of hepatotoxicity. Mean serum values of aspartate aminotransferase and alanine aminotransferase were not different between the 2 groups (see Table 1). Incidence of hyperbilirubinemia was the same in both groups. Fluconazole administration was never discontinued on account of adverse effects or intolerance.

DISCUSSION

Despite convincing evidence of the efficacy of prophylactic fluconazole in immunocompromised adult[19–22] or infant patients,[20,23] only 2 studies involving preterm high-risk neonates are available.[11,24] They demonstrate that fluconazole prevents rectal colonization in VLBW[24] and colonization and infection in ELBW neonates.[11] Both studies were designed for partial objectives and leave some questions unanswered.[28,29]

Our study focuses on the effects of prophylactic fluconazole on a widespread NICU population over a period of 3 years. This policy proved very effective in all VLBW neonates, not only in ELBW neonates as previously reported.

Fluconazole decreased the incidence of all grades of *Candida* species colonization in all sites in VLBW neonates. It is important to remember that colonization by fungi is undoubtedly the most significant risk factor for the development of SFI in any patient, including preterm neonates,[6,7,10] and always precedes an SFI episode.[31,38–40] Baley et al[6] in 1986 calculated that among 100 VLBW neonates in the NICU, 33 developed fungal colonization and 7 progressed to SFI. A relationship or an association between colonization and a subsequent SFI has been demonstrated for all peripheral sites open to investigation with serial cultures.[6–9,39,40] Overall incidence of colonization is ~5% at birth in VLBW neonates and increases to 64% in the first month of life in the NICU.[6–9,24] The incidence of colonization in our population before the use of fluconazole prophylaxis is clearly comparable with other findings.[4–7,11,24] The reduction in incidence after prophylaxis may not seem as dramatic as the reduction in incidence of SFI obtained in the same period. We are aware that a 24% incidence after prophylaxis is high. However, nearly 90% of colonized infants in the fluconazole group were low-grade or low-risk colonized, because fluconazole produced a massive reduction in the number of high-grade and high-risk colonized infants ($P = .01$ and $P < .0001$, respectively): in group B, only 10% of the neonates were high-grade or high-risk colonized vs 25% in group A. Furthermore, the high-grade/low-grade and the high-risk/low-risk colonization ratios decreased from 0.16 to 0.12 ($P = .01$) and from 0.77 to 0.32 ($P = .004$), respectively. These data show that prophylactic fluconazole also limits the severity and the intensity of fungal colonization.

The percentage of colonized preterm neonates who progressed to infection ranged from 7% to 28% in previous studies.[6–8] Colonized neonates are obviously the first to benefit from prevention as well as those for whom effective prevention is most urgent.[28] In our study, prophylactic fluconazole significantly reduced the rate of progression from colonization to infection (0.17 in group B vs 0.38 in group A; $P = .009$). Once again, fluconazole was particularly effective in the low-grade ($P = .002$) and low-risk ($P = .002$) colonized neonates and saved from progression nearly 8 of 9 neonates with low-grade or low-risk colonization (2 of 3 in group A), as well as in ELBW colonized neonates, who progressed in only 1 of 5 cases vs 1 of 2 of those who did not receive prophylaxis ($P = .009$). We calculated that in these neonates, fluconazole saved 6 episodes of SFI. Efficacy of fluconazole in stopping progression was still significant in NE-VLBW colonized neonates ($P < .05$) but was lower and not significant in heavily or severely colonized neonates whatever their birth weight; we may presume, in agreement with other studies, that the antifungal drug limitation of progression by fluconazole wanes beyond a certain intensity of candidal colonization.[6,9,22,28,38,39] This finding supports starting prophylactic fluconazole on dol 1, as in the study, and not only after colonization is detected; in this way, not only is progression to infection prevented in many cases, but also colonization is limited to low risk or low grade so that infants will be at lower risk for infection.

Fluconazole significantly decreased the incidence of both proven and presumed SFI episodes in ELBW and NE-VLBW neonates. In the latter, it saved 10 and in the former 5 SFI episodes over the 3-year period. We calculate that 1.9 expected deaths were avoided. The reduction of morbidity and mortality by *Candida* species confirms data from other categories of patients, both adults and infants, in which this strategy has already been implemented as a routine preventive practice.[20–22]

The increase of the presumed/proven SFI ratio in the fluconazole group (1.0 vs 0.48 in group A) could mean an increased frequency of less paradigmatic (and less severe) fungal infectious features in the neonates who

undergo prophylaxis. The use of prophylactic fluconazole may have enhanced the host response to fungal agents once the infection has occurred[20]; however, blood cultures, whose sensitivity is basically poor,[12] may have given more often negative results depending on the continuous administration of low-dosage fluconazole.

In brief, fluconazole provided our preterm neonates with a pattern of protective ability against fungi at the different levels in which this is needed: avoiding colonization, limiting progression to infection, decreasing the number of infectious events, and avoiding fatal cases. The overall mortality rate was also significantly lower in colonized neonates who received prophylaxis, particularly in the ELBW neonates.

The benefits of topical antifungal treatment in preventing colonization at specific sites have been illustrated in preterm neonates.[25–27] An initial fungal colonization at a peripheral site in the first days of life may well be better managed by topical rather than systemic antifungal agents, as it mostly depends on the inefficiency of the host barrier defense. However, this treatment cannot be proposed for all of the potential sites in every preterm neonate, because it is unknown which sites will become colonized, and some sites cannot be treated topically. Thus, topical antifungal treatment cannot be an alternative to fluconazole as a prophylactic strategy in the NICU.

There are some differences between our schedule (dose, duration, and administration route) and that of Kaufman et al.[11] We used a higher dose (6 mg/kg instead of 3 mg/kg) to achieve efficacy against strains that may be dose-sensitive to fluconazole. Furthermore, we set out to minimize the invasiveness of the prophylaxis while nonetheless allowing (by the oral route) its completion in neonates who no longer required an intravenous access, thus avoiding discontinuances before 30 and 45 days in NE-VLBW and ELBW neonates, respectively. Several clinical[24] and pharmacokinetic studies have demonstrated the equal bioavailability of intravenous and oral fluconazole,[34–36,40] and in such neonates, continuing the prophylaxis is advisable because the risk factors for SFI do not disappear when the intravenous access is removed.

No adverse effect related to fluconazole was detected in group B neonates, particularly no liver function impairment putatively related to fluconazole. None of the 225 treated infants discontinued the prophylaxis. We thus agree with those who have used fluconazole to treat neonates with invasive fungal infection and found only mild and transient elevation of plasma levels of hepatic enzymes as a relatively common side effect.[33]

A major concern over the widespread use of prophylactic fluconazole is the eventual emergence of fungal resistance in the NICU, as well as a change in the relative weight among the isolates from *Candida* subspecies to the advantage of those that are natively resistant to fluconazole.[41] A shift toward nonalbicans species, in fact, has been noted in other high-risk patients.[25]

Our data nevertheless show that the patterns of sensitivity of *Candida* to fluconazole remained the same during the 3-year period (as reported by Kaufman et al[42,43]), and there was no significant increase in rate of colonization and infection by the natively resistant *Candida* species (*C krusei* and *C glabrata*),[22] as expressed by the crude number of their isolates in group B. "It would be surprising if antifungal prophylaxis had no effect on the pattern of pathogens causing infection in patients receiving prophylaxis,"[20] but changes in this pattern do not seem related to an increased risk for colonization and infection. It thus may be supposed that our use of prophylactic fluconazole was not associated with any significant increase in the isolation of potentially more dangerous nonalbicans species. In any event, SFI by both natively fluconazole-resistant and nonresistant species can be treated successfully with LAmB. These data provide additional support for the view that fluconazole is a reliable preventive drug in the management of the specific fungal infection risk for preterm neonates in the NICU.

A question arising from previous studies concerned the preterm neonate subpopulation that would benefit most from fluconazole prophylaxis. On the basis of our data, we have demonstrated that every VLBW neonate in the NICU can receive substantial advantages from prophylaxis. We calculate that we could prevent 1 SFI episode and save 1 life out of every 14 and 100 protected patients, respectively, with no harm as a result of adverse effects and no significantly negative outcome in terms of selection of fluconazole-resistant species. These data support its use in prophylaxis in a less limited way than at present (1 life saved should have no cost).

We are aware that our study provides retrospective historical data from only 1 NICU and that a multicenter, randomized trial would be needed to assess definitively the impact of this preventive strategy in NICUs. Nevertheless, the silence of the literature after the only single-center, randomized trial[11] prompted us to describe our experience to provide the neonatology community with some additional data.

That the most protection was obtained in ELBW neonates, that the overall mortality rate in ELBW colonized neonates under prophylaxis is lower, and that fluconazole stopped progression both from low- to high-grade colonization and from colonization itself to infection suggest that early prophylaxis is advisable for all ELBW neonates and, of the NE-VLBW neonates, at least for those who become colonized during their stay in a NICU. The endpoints of prophylactic administration of an antifungal drug in a high-risk neonatal population must be a decrease in both fungus-induced morbidity and mortality and the risk that fungal colonization will result in SFI. Subject to these intrinsic limits of this study, our

data show that these endpoints can be attained by fluconazole prophylaxis.

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