Combination antifungal therapy with voriconazole for persistent candidemia in very low birth weight neonates

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SUMMARY: Turan Ö, Ergenekon E, Hirfanoğlu İM, Önal EE, Baş VN, Türkyılmaz C, Koç E, Atalay Y. Combination antifungal therapy with voriconazole for persistent candidemia in very low birth weight neonates. Turk J Pediatr 2011; 53: 19-26.

The purpose of this article is to report our experience with intravenous voriconazole therapy in the treatment of persistent *Candida* septicemia in very low birth weight (VLBW) neonates. Candidiasis was defined if an infant had a positive blood culture. Ten VLBW newborns developed *Candida* sepsis, and candidemia persisted in 6 of them despite 3 to 21 days of antifungal therapy with amphotericin B, either conventional or liposomal, and fluconazole. After the addition of voriconazole, clearance of *Candida* was achieved within 3-7 days of treatment. Antifungal therapy combination with liposomal amphotericin B and voriconazole was continued for at least two weeks after two negative cultures 48 hours apart. We conclude that considering the hazardous effects of *Candida* infections in preterm newborns, voriconazole can be added to the treatment of fungal sepsis in newborns who still have persistent candidemia despite conventional antifungal management. More clinical information is needed before voriconazole can be used as a first-line drug in antifungal therapy in newborns.

Key words: voriconazole, newborn.

Candida species are becoming increasingly important pathogens in neonates. Signs and symptoms of *Candida* sepsis may be nonspecific and difficult to differentiate from those of bacterial sepsis.

Candida infections are responsible for 2.4% of early-onset neonatal infections and 10% to 12% of all late-onset or nosocomially acquired infections in neonates[1,2]. Any candidal infection in the neonate can be life-threatening, and delay in diagnosis often results in significant morbidity or mortality. Many risk factors have been implicated in the pathogenesis of invasive candidiasis in the Neonatal Intensive Care Unit (NICU): high burden of colonization with *Candida* species, prematurity (especially gestational age <28 weeks), very low birth weight (VLBW, <1500 g), prolonged broad spectrum antimicrobial therapy, delayed enteral feedings, total parenteral nutrition for longer than five days, use of lipids for longer than seven days, use of H2 receptor antagonists, presence of catheters and tubes, surgery, postnatal steroids, and prolonged hospitalization[3-5].

Currently, amphotericin B is the standard therapy for *Candida* infections. However, new antifungal agents like echinocandins or new-generation azole derivatives have been developed in the past decade, and some clinical experience has been reported about the treatment of VLBW infants with *Candida* infections. There is very little data on voriconazole therapy in neonates. The appropriate dose and interval, and its safety, efficacy and adverse effects are unknown.

Voriconazole is the preferred agent for treatment of invasive aspergillosis and is also effective in the treatment of *Candida* infections[6,7]. In this report, our experience with intravenous (IV) voriconazole in VLBW newborns with persistent *Candida* septicemia is presented.

Material and Methods

All VLBW neonates admitted to our NICU between January 1, 2006 and January 31,

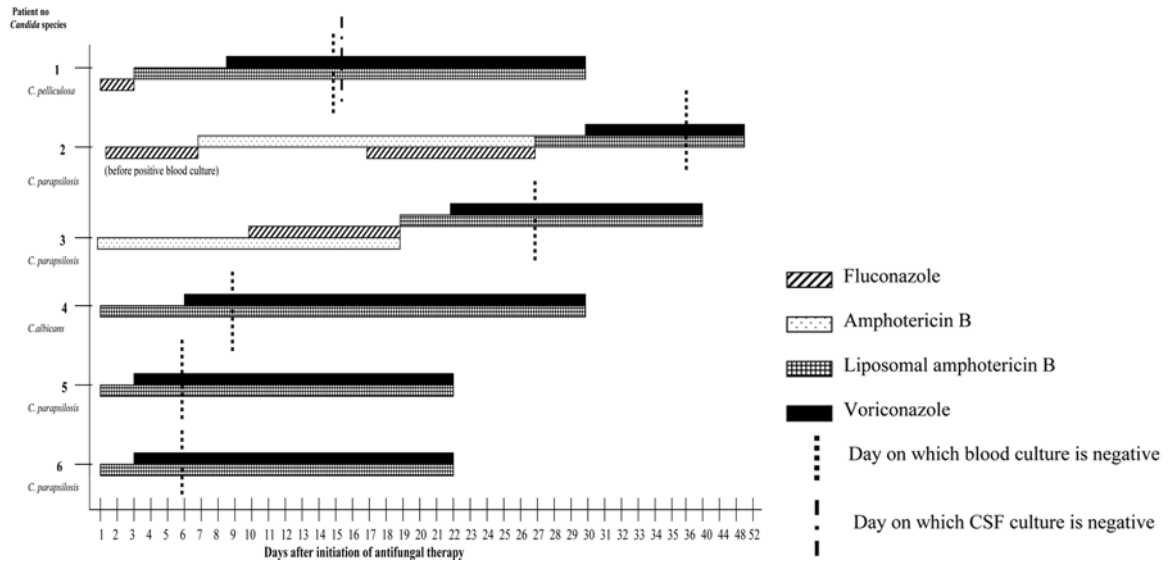

Fig. 1. Antifungal treatment combination and duration of therapy

2009 were evaluated retrospectively with regards to *Candida* infection. The following data were recorded: birth weight, gestational age, gender, history of multiple pregnancy, blood, urine, cerebrospinal fluid (CSF) and central catheter cultures, postnatal age at the time of infection, antibiotic use, parenteral nutrition and lipid solution infusion, days with central catheters (percutaneously placed central venous catheters [PICC] and umbilical venous catheters [UVC]).

Blood cultures were obtained by sterile technique from infants with the signs and symptoms of sepsis. These cultures were processed by the microbiology laboratory with BACTEC systems. One ml of blood was inoculated per blood culture; 2 ml of blood was obtained from those who were already on antibiotics. CSF and urine cultures were also obtained. Candidiasis was defined if an infant had a positive blood culture drawn from a peripheral site. Follow-up blood cultures were obtained every 1-2 days until the cultures yielded negative results. Antifungal therapy was chosen according to the attending physician's decision. Fluconazole was not given prophylactically.

In vitro susceptibility tests were performed for fluconazole, amphotericin B, voriconazole, and caspofungin, through the Clinical and Laboratory Standards Institute (CLSI). The minimum inhibitory concentrations (MICs) of antifungal drugs were evaluated by microdilution methods.

Blood count, blood urea nitrogen, creatinine, electrolytes, hepatic transaminases, and urine output levels were determined, and these tests were repeated during antifungal treatment. Liver function test abnormalities were defined as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels >3 times the upper limit of normal (n: 0-40 U/L). Direct hyperbilirubinemia was defined as conjugated bilirubin >2.0 mg/dl. Hypokalemia was defined as serum potassium <3 mmol/L and hypophosphatemia was defined as serum phosphorus <4.5 mg/dl. Thrombocytopenia was defined as a platelet count <150,000/μL. All patients received a maintenance dose of electrolytes.

Once *Candida* infection was diagnosed, ophthalmologic examination, echocardiogram, transfontanel and abdominal ultrasound were done and repeated weekly. Microbiologic response to therapy was accepted as two negative blood cultures with a 48-hour interval. Voriconazole levels were not analyzed for this study.

Results

One hundred thirty-six VLBW newborns were admitted to the NICU during the study period, and 10 VLBW newborns developed *Candida* sepsis, six of whom had persistent candidemia despite antifungal treatment and were given IV voriconazole therapy. They were all <32 weeks gestation and birth weight ranged from 790 to 1477 g. Five newborns were the product of

Table 1. Demographic Characteristics of the Six Neonates

Patient No	GA (weeks)	Gender (F/M)	BW (g)	Postnatal age at the time of Candida infection (days)	Blood, urine, CSF and catheter cultures for Candida	Candida species	UVC duration before positive blood culture (days)	UVC duration after positive blood culture (days)	PICC duration before positive blood culture (days)	PICC duration after positive blood culture (days)	Antibiotic therapy prior to Candida sepsis (days)	TPN/ lipid	Mechan- ical ventilation prior to Candida sepsis (days)	End organ dissemination
1	29 1	F	790 (singleton)	7	Blood CSF PICC	C. pelliculosa	8	3	4	8	Ampicillin (6) Amikacin (6)	+/+	1	Endocarditis Central nervous system candidiasis
2	27 6	M	1120	19	Blood	C. parapsilosis	12	-	7	12	Ampicillin (10) Amikacin (10)	+/+	9	-
3	27 6	F	1100	20	Blood	C. parapsilosis	9	-	6	2	Ampicillin (10) Amikacin (10)	+/+	12	-
4	31 5	M	1000	50	Blood Urine	C. albicans	-	-	-	-	Ceftazidime (10) Netilmicin (10)	+/+	7	Renal candidiasis
5	29 6	M	1477	10	Blood PICC	C. parapsilosis	10	1	3	2	Ampicillin (7) Amikacin (7)	+/+	6	-
6	29 6	M	1111	9	Blood UVC	C. parapsilosis	11	2	4	1	Ampicillin (5) Amikacin (5)	+/+	5	-

GA: Gestational age. BW: Birth weight. CSF: Cerebrospinal fluid. UVC: Umbilical venous catheters. PICC: Peripherally inserted central catheter. TPN: Total parenteral nutrition.

multiple pregnancy. Demographic data of the six newborns are presented in Table I.

All infants had all predisposing factors for *Candida* infection, such as prior antibiotic therapy, mechanical ventilation, total parenteral nutrition given by central or peripheric venous catheter, use of lipids, prematurity, VLBW, and prolonged hospitalization.

Candida parapsilosis were the most common species isolated from 4 blood and 2 catheter-related candidal infections. *C. albicans* was isolated from 1 blood culture. *C. pelliculosa* was positive in 2 blood cultures and 1 CSF culture. All *Candida* species were susceptible to fluconazole, amphotericin B, caspofungin, and voriconazole. Amphotericin B MICs ranged from <0.03 to 1 μg/ml and fluconazole MIC was 16 μg/ml. Caspofungin MICs ranged from <0.03 to 4 μg /ml and voriconazole MICs from <0.25 to 2 μg/ml.

The time course of antifungal therapy of the six patients is shown in Figure 1. After diagnosis of *Candida* infection, patients were started on conventional amphotericin B, conventional amphotericin B plus fluconazole or liposomal amphotericin B (L-AmB), according to the attending physician's choice. Patient 2 received fluconazole for 7 days, and 4 days after discontinuation of the drug, *C. parapsilosis* was isolated in the blood culture. Fluconazole (Triflucan®) was administered as 12 mg/kg loading dose, then 6 mg/kg per dose IV infusion by syringe pump over 30 minutes. The dosing interval was based on postmenstrual age and postnatal age. Amphotericin B (Fungisome®) was administered 1 mg/kg every 24 hours as IV infusion over 2 hours. Amphotericin B liposome (AmBisome®) was administered as 5 mg/kg every 24 hours as IV infusion by syringe pump within 2 hours. We used the antifungal drug dose and dosing interval chart in Neofax® 2006.

Voriconazole was added to the antifungal therapy due to the persistence of *Candida* septicemia despite treatment with L-AmB or conventional amphotericin B plus fluconazole. IV voriconazole (Vfend®) was administered as 6 mg/kg per dose every 8 hours by syringe pump in 2 hours. Dose was determined according to the literature[8]. Blood cultures became negative within 3-7 days of voriconazole treatment. CSF culture was sterile after the 7th day of voriconazole therapy. The duration of voriconazole treatment was 18-24 days. Antifungal therapy was discontinued 2 weeks after the last 2 negative blood cultures 48 hours apart.

Once *Candida* infection was diagnosed, ophthalmologic examination, echocardiogram, transfontanel and abdominal ultrasound were done and repeated weekly. Papilla, macula and retinal vessels were normal. One patient had right atrial and ventricular fungal masses requiring tissue plasminogen activator (t-PA) treatment, and 1 patient had renal candidiasis. All transfontanel ultrasound results were normal, but *Candida* meningitis was present in 1 patient (Table I).

Central catheters were removed within 1-12 days. In 2 of the patients, central lines were removed on the 8th and 12th day of infection due to IV access problems (Patients 1, 2). In the other 4 patients, they were removed as soon as the *Candida* sepsis was diagnosed.

During antifungal therapy, side effects like elevation of liver function tests (n:4) (highest value AST: 363 U/L, ALT: 239 U/L, direct bilirubin: 5.4 mg/dl), hypokalemia (n:2) (lowest value K: 2.1 mmol/L), hypophosphatemia (n:6) (lowest value P: 1.1 mg/dl), and thrombocytopenia (n:2) (lowest value: 33 x10.e3/μL) were observed. Laboratory data before beginning the antifungal therapy, and at initiation and completion of voriconazole therapy of the 6 patients are presented in Table II. The patients who developed elevated liver enzymes already had cholestasis before antifungal treatment, and liver functions returned to normal between 11-95 days after the end of treatment. Hypokalemia and hypophosphatemia were treated with supplements and returned to normal within 2-5 days after the cessation of antifungal therapy. Thrombocytopenia did not require treatment.

Seizures were observed in Patients 4 and 5 and phenytoin was used for treatment. Voriconazole dose was increased to 7 mg/kg in Patient 4 because of increased metabolism of the drug due to phenytoin.

All newborns with *Candida* sepsis were discharged from the NICU.

Discussion

Effective antifungal drugs, appropriate supportive care and interventions to prevent infection are important treatment and management measures of neonatal candidiasis.

Table 2. Laboratory Data of the Six Newborns Before Antifungal Therapy, and at Initiation and Completion of Voriconazole Therapy

Patient no		Na (mmol/L)	K (mmol/L)	BUN (mg/dl)	Cre (mg/dl)	T. bil (mg/dl)	D. bil (mg/dl)	AST (U/L)	ALT (U/L)	P (mg/dl)	Hct (%)	Plt (μL)
	Before antifungal therapy	138	3.0	8	0.6	3.5	0.4	49	8	3.7	48.8	95,000
1	Initiation voriconazole	142	4.0	4	0.5	1.7	0.5	30	13	2.9	30.9	45,900
	Completion voriconazole	140	4.1	6	0.3	5.1	3.9	212	88	4.5	38.6	159,000
	Before antifungal therapy	141	3.8	18	0.6	8.0	0.4	20	6	3.2	48.5	183,000
2	Initiation voriconazole	137	4.1	13	0.4	1.2	0.4	35	15	3.3	36.7	79,900
	Completion voriconazole	137	4.0	5	0.6	3.3	2.1	132	38	4.9	23.2	333,000
	Before antifungal therapy	135	4.6	10	0.6	1.4	0.4	26	5	1.3	35.8	358,000
3	Initiation voriconazole	136	4.4	9	0.5	10.0	0.4	24	15	1.5	36.4	168,000
	Completion voriconazole	143	4.5	4	0.3	3.1	2.7	57	16	4.2	28.2	273,000
	Before antifungal therapy	134	4.8	14	0.1	16.4	5.1	233	207	6.3	37.4	157,000
4	Initiation voriconazole	140	3.8	8	0.2	6.1	4.4	363	101	1.3	34.6	203,000
	Completion voriconazole	137	6.0	9	0.1	13.9	5.4	317	239	4.7	32.9	90,500
	Before antifungal therapy	136	4.1	13	0.6	5.1	0.3	23	5	3.7	29.0	206,000
5	Initiation voriconazole	141	4.4	6	0.7	1.1	0.5	19	6	2.9	32.2	89,200
	Completion voriconazole	139	2.1	10	0.6	0.6	0.4	14	3	5.6	37.2	308,000
	Before antifungal therapy	144	4.3	19	0.8	4.1	0.4	27	5	2.9	47.6	163,000
6	Initiation voriconazole	141	3.8	10	0.6	0.6	0.3	20	3	3.6	36.7	122,000
	Completion voriconazole	138	4.9	2	0.4	1.6	0.3	60	10	5.4	31.6	171,000

Na: Sodium. K: Potassium. BUN: Blood urea nitrogen. Cre: Creatinine. T. bil: Total bilirubin. D. bil: Direct bilirubin. ALT: Alanine aminotransferase. AST: Aspartate aminotransferase. P: Phosphorus. Hct: Hematocrit. Plt: Platelet.

Three classes of antifungals are commonly used in the treatment of systemic fungal infections in neonates: the polyene macrolides (e.g. amphotericin B [deoxycholate and lipid preparations]); the azoles (e.g. fluconazole); and the fluorinated pyrimidines (e.g. flucytosine). There are more data for the liposomal and lipid complex preparations of amphotericin B and for fluconazole compared to echinocandins and new-generation azole derivatives. The echinocandins (e.g. caspofungin and micafungin) are a newer class of antifungals currently being studied in neonates. However, little information is available regarding the use of the new azole derivative voriconazole in VLBW infants with *Candida* infections.

Amphotericin B is the primary antifungal agent for the treatment of candidemia, and disseminated and invasive candidiasis in NICUs. Most *Candida* species are susceptible to amphotericin B. However, some trials have reported that *C. lusitaniae* has resistance and *C. glabrata* and *C. krusei* have reduced susceptibility to amphotericin B[9,10]. Neonates tolerate this drug and its minimal side effects include renal insufficiency, hypokalemia, hypomagnesemia, anemia, thrombocytopenia, and increase in hepatic enzymes.

Liposomal amphotericin B (L-AmB), amphotericin B lipid complex (ABLC) and amphotericin B cholesterol sulfate complex (ABCD) are lipid-associated formulations and alternatives to standard amphotericin B. Fungal susceptibility is the same, but they are more expensive than conventional amphotericin B deoxycholate. The main advantage of these drugs is their lower levels of toxicity[11,12].

Fluconazole is the most commonly used azole but it is not fungicidal. Several studies of successful use of fluconazole in neonates have been published in the treatment of invasive candidiasis[13,14]. Transient thrombocytopenia and increase in creatinine, bilirubin and liver transaminases have been reported in neonates. In our group, three newborns were started on fluconazole empirically after sepsis work-up. All of them required addition of amphotericin B due to persistent candidemia.

Voriconazole, a second-generation triazole and synthetic derivative of fluconazole, is more potent and has a broader spectrum of activity than fluconazole[11]. It is available both in IV and oral formulations. Voriconazole inhibits the synthesis of ergosterol in the fungal plasma membrane. It is fungicidal against *Aspergillus* and fungistatic against *Candida* species[15].

Voriconazole metabolism is nonlinear in adults. In contrast, elimination of voriconazole seems to be linear in children after doses of 3 mg/kg and 4 mg/kg every 12 hours[16]. Children require higher doses of voriconazole than adults to achieve similar serum concentrations over time. Therefore, using voriconazole at recommended doses for adults may lead to clinical failures in children. Pediatric patients have a higher capacity for elimination of voriconazole than adults[16]. However, neonatal capacity for elimination is not known.

Voriconazole is distributed into the brain, lung, liver, kidney, and spleen. Clearance of this drug is mostly hepatic, metabolized by the human cytochrome P-450 isoenzymes CYP2C19, CYP2C9 and CYP3A4[17]. The activity of the isoenzyme CYP2C19 is highly dependent on genetic characteristics with at least two allelic polymorphisms that affect individual metabolism[18]. As a result, due to a point mutation of the gene encoding CYP2C19, some people are poor metabolizers, while others are rapid metabolizers of the drug[19]. Furthermore, drug interactions may occur with other medications[17,18]. Phenytoin increases voriconazole clearance whereas voriconazole decreases phenytoin's clearance, both requiring dose modifications if the drugs are to be used together. Thus, phenytoin levels must be monitored carefully. On the other hand, use of carbamazepine and barbiturates together with voriconazole is not recommended since they decrease effective plasma concentrations of voriconazole.

There is limited experience with voriconazole in neonates. In the literature, a preterm infant with disseminated fluconazole-resistant *C. albicans* infection was treated with IV voriconazole and

L-AmB. IV voriconazole 6 mg/kg every 8 hours was administered safely. In our group, the dose was determined according to this literature[8].

In very few studies, plasma levels of voriconazole have been reported in children. Pasqualotto et al.[20] reported that plasma voriconazole concentrations were unpredictable, and subtherapeutic levels were frequently observed, despite progressive increments in dosage. The dose administered was generally not correlated with blood drug levels. Voriconazole plasma levels were determined either by bioassay or liquid chromatography-tandem mass spectrometry. Voriconazole levels were

not analyzed in this study, but monitoring of plasma levels may be helpful to determine the exact dose for an individual patient.

In our group, voriconazole was started in four infants receiving L-AmB who still had positive blood cultures despite 3-6 days of treatment. Two infants were given voriconazole on the 21st day of antifungal therapy (19 days conventional amphotericin B + fluconazole, 2 days L-AmB) for persistent candidemia (Patients 2, 3).

Efficacy of combinations with older or newer antifungal agents is largely unknown. Voriconazole is given in combination with L-AmB and caspofungin. In our study, antifungal therapy combination with L-AmB and voriconazole was continued for at least two weeks after the two negative cultures 48 hours apart.

Voriconazole's side effects include reversible dose-dependent visual disturbances, such as increased brightness, blurred vision, photophobia, elevated hepatic transaminases, and skin reactions[16,18,21]. The visual abnormalities are transient, and the liver enzyme abnormalities often resolve when the drug is discontinued or the dose is decreased. Treatment-related toxicity rarely develops into serious adverse effects if the patient is closely monitored. Due to the reports of visual adverse events in children and adults, there is concern over the unknown interactions with the developing retina. In our group, all infants had periodical retinal exams and no retinopathy was observed.

No renal failure was observed during voriconazole treatment. Hypokalemia returned to normal within two days after the treatment was completed. Hypophosphatemia and elevated liver function tests continued longer, most likely due to prematurity, sepsis and total parenteral nutrition- associated cholestasis.

Since *Candida* infections seem to be increasing in NICUs, antifungal therapy is being used with increased frequency. Persistence of candidemia should be searched with daily blood cultures once the diagnosis is established since it will determine the duration of treatment. Depending on the antifungal drug, side effects require monitoring. Efficacy and side effects of amphotericin B and fluconazole are relatively well studied in newborns compared to the new-generation antifungals like voriconazole. In our group of six patients, no significant side effects of voriconazole requiring discontinuation of the drug were observed, and clearance of *Candida* was achieved within 3-7 days of treatment. With prompt catheter removal at the time of diagnosis and appropriate dosing of antifungals, it would be expected to clear candidemia within 3-7 days.

Therefore, we conclude that considering the hazardous effects of *Candida* infections in preterm newborns, voriconazole can be added to the treatment of fungal sepsis in newborns who still have persistent candidemia despite conventional antifungal management. However, the drug needs to be studied in VLBW infants with regards to safety issues, since more clinical information is needed before it can be used as a first-line drug in antifungal therapy in this vulnerable group.

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