Invasive fungal infections during the neonatal period: diagnosis, treatment and prophylaxis

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**Introduction:** The incidence of preterm births seems to be increased in many countries around the world, in parallel to the advances in neonatal medicine. However, this has resulted in longer hospital stays and more exposure to invasive interventions, both of which can lead to an increase in late-onset nosocomial infections in the newborn period. In addition to bacteria, fungi are thought to be an important cause of hospital infections.

**Areas covered:** The present article reviews the diagnosis, treatment and prophylaxis of invasive fungal infections (IFIs) during the neonatal period. IFIs are associated with high morbidity and mortality in preterm neonates. The main risk factors are multiple antibiotics, central venous catheters, parenteral nutrition, immunodepression, very low birth weight, and fungal colonization. Successful management of IFIs relies on early recognition and rapid initiation of effective treatment.

**Expert opinion:** Invasive-fungal-infection-related morbidity and mortality is a major concern for most neonatal intensive care units worldwide. Incidence rates are increasing for preterm neonates. Preterm infants display clinical characteristics that make them prone to fungal infections, and there is a high frequency of neurodevelopmental sequelae in those who survive after neonatal fungal infections. Specific prevention – rather than treatment – should be the optimal strategy. Both uconazole and nystatin prophylaxis reduce the incidence of IFI and fungal colonization in very preterm infants.

**Keywords:** diagnosis, invasive fungal infection, neonates, prevention, treatment
Invasive fungal infections in neonates

**Article highlights.**

- The leading fungal pathogens in the neonatal period are of the Candida species.
- Although there have been advances in the diagnosis of fungal infections, most of the methods are of limited use in the newborn.
- Clinical signs of invasive candidiasis can be nonspecific and diagnosis is still based mostly on blood cultures.
- Newborns of very low birth weight with high risk for developing invasive fungal infections should be identified and given fluconazole prophylaxis.
- Although treatments with new antifungals used in the neonatal period are encouraging, larger prospective and comparative studies are needed to determine neonatal invasive fungal infection and the optimal dose for use in this population.

This box summarizes key points contained in the article.

IFIs [11]. Among infants admitted to the neonatal intensive care unit (NICU), colonization with Candida spp. seems to be associated with the development of invasive infections [12,13]. Also, the increased risk in low-birth-weight infants can be attributed to an immature immune system and longer stays in the NICU. Risk factors associated with late-onset infections are shown in Box 1.

2. Candida species

Candida spp. can cause invasive, potentially fatal, systemic infections of any organ as well as more benign, local mucocutaneous infections. Candida infections make up 70 – 90% of all IFIs. Candida albicans is the most commonly isolated species in neonatal candidiasis, followed by the non-Candida spp. in neonates [14-17].

Newborns were reported to be under high risk for candida infections as well as immunocompromised children, recipients of bone marrow or solid organ transplants and children in intensive care units [10,18,19]. Additionally, a study on neonatal candidiasis concluded that the risk factors associated with the development of candidiasis on the third day of life were birth weight, cephalosporin administration, gender and lack of enteral feeding [20].

Colonization of the host with Candida spp. is a prerequisite for invasive infection and there is a correlation between the risk for invasive disease and the number of colonized sites [12,13,21,22]. Colonization of the gastrointestinal tract and skin act as important reservoirs for the development of Candida infection [23]. Surveillance of fungal colonization of multiple sites revealed that 62% of extremely-low-birth-weight (ELBW: < 1000 g) infants were colonized during the first 6 weeks of life, and colonization was inversely related to gestational age [18]. Two-thirds of all invasive candidal infections have been reported in small infants and infants with congenital anomalies [24,25]. The incidence of invasive candidiasis is 1 – 5% in VLWB infants and 10% in ELBW infants [26]. Notably, the incidence of invasive candidiasis in VLWB infants has been reported to be 25 – 35 times higher than the incidence in full-term infants who require neonatal intensive care [27].

Over the past decade, Candida parapsilosis has emerged as an important nosocomial pathogen in VLWB infants. In animal models, C. parapsilosis seems to be a less virulent pathogen than C. albicans, as it is less able to adhere to and penetrate the endothelium. However, a recent systematic review of 34 observational studies was unable to determine whether the clinical course of C. parapsilosis infection is less severe (less end-organ involvement and mortality) than C. albicans infection in VLWB infants. There is a need for a large, prospective, population-based study to assess the relative severity of infection from these organisms in order to inform infection control and treatment strategies. The emergence of C. parapsilosis as an important pathogen in VLWB infants may be related to changes in neonatal intensive care practices. More extremely preterm infants now survive beyond the first few days after birth and are exposed to invasive procedures that predispose to nosocomial infection. In vitro, C. parapsilosis proliferates in high concentrations of glucose and can form biofilms on synthetic materials. The use of central venous catheters to deliver parenteral nutrition is a risk factor for IFI; some authors did not find a significant difference in the frequency of central venous catheter use between infants infected with C. parapsilosis and those infected with C. albicans [24-27]. We can say that infants with C. parapsilosis infection were less likely to have evidence of central vascular line colonization than infants with C. albicans infection.

3. Aspergillus species

Aspergillus fumigatus is the most common cause of invasive aspergillosis, followed by Aspergillus flavus. Aspergillosis is far less common than invasive candidiasis in newborns and is usually a primary cutaneous disease [28,29].

The species of Aspergillus causing disease in the neonatal population are similar to those in the pediatric or adult populations. One of the main risk factors for development of invasive aspergillosis in the neonatal period is prematurity. In a review of previous published data by Groll et al. [30], 43.2% of 44 neonates with invasive aspergillosis were premature. A birth weight of < 1500 g is also a clear risk factor, given that it often requires both nutrition and mechanical ventilation in the presence of immature neonatal skin.

4. Zygomycosis

Neonates have been described in individual case reports as a population at risk for zygomycosis [31]. Zygomycosis starts as a skin infection and later progresses to a necrotizing soft-tissue infection and is associated with high mortality of up to 61% [32]. It was reported in a review that most of the neonates infected with zygomycosis (77%) were born prematurely [33].
In this study of 59 neonates with zygomycosis, the most common forms of zygomycosis in neonates were reported to be gastrointestinal (54%) and cutaneous (36%). Pulmonary and rhinocerebral infections, as well as infections of other sites, were found in a total of six (10%) cases. Among these 59 patients with zygomycosis, there were 38 (64%) deaths reported and 85% of reported cases of disseminated disease died. The mortality was reported to be especially high (78%) in neonates who developed gastrointestinal disease [33].

5. An important risk factor for invasive fungal infections: intravascular catheters

Neonates with ELBW often require central vascular catheterization (CVC) for parenteral nutrition and administration of antibiotics during their long hospitalization periods. The main types of CVCs and means of access to neonates are umbilical catheters, central catheters inserted through a peripheral vein (PICC), central venous nontunneled catheters through direct puncture of the femoral, jugular or subclavian veins, and tunneled central venous catheters surgically implanted by means of venous dissection [34-37]. The most common life-threatening complication of CVCs is catheter-related bloodstream infections (CR-BSI). CR-BSIs are also associated with high costs [38-40].

CVCs are generally presumed to be associated with higher risk of sepsis compared with peripheral venous catheters (PVCs); however, stricter infection control measures taken during the insertion of CVCs may actually be lowering the risk. Geffers et al. [41] reviewed surveillance data from the German national nosocomial surveillance system for VLBW infants, which consisted of 2126 infants from 22 neonatal centers, to describe the relationship between the use of CVCs/PVCs and the risk of nosocomial, primary, laboratory-confirmed BSI. In this study, 261 (12.3%) of all infants had developed a BSI; had lower birth weight, vaginal delivery, CVC use or PVC use within 2 days before developing BSI, and the individual neonatal centers were found to be significant independent risk factors for BSI. In the same study, with regard to the adjusted hazard ratios in the multivariate analysis, there was only a minor difference between the types of catheter (PVC or CVC), which is also supported by some other studies [42,43].

The indwelling duration of PVCs or CVCs is a major risk factor for development of BSIs. Usage of PICCs for > 16.4 days and umbilical catheters for > 4 days were found to increase the risk of sepsis dramatically [44]. In a study by Sengupta et al. [45], the incidence rate of catheter-related BSI was reported to increase during the first 18 days after PICC insertion, decreased between 19–35 days, and then increased again between 36 and 60 days by 33%/day, even more highly compared with the first 18 days. These findings show that catheter duration is a significant risk factor for development of CR-BSI in the NICU, and it may be advisable to replace or displace the catheter after 35 days given the substantial increase in the risk of associated BSI.

6. Diagnosis

Successful management of IFIs relies on early recognition and rapid initiation of effective treatment. A high index of suspicion and the use of additional laboratory and clinical tests, including retinal examination, echocardiography and renal ultrasonography, may be needed to confirm the suspected diagnosis. Although there have been advances in the diagnosis of fungal infections, most of the methods are of limited use in the newborn period. The best diagnostic test in the management of a suspected fungal infection is to isolate the causative agent from a relevant clinical specimen. Modern imaging techniques and detection of fungal cell wall components and DNA in blood and other body fluids are also helpful in the diagnosis of IFIs [46].

6.1 Blood culture

Clinical signs of invasive candidiasis can be nonspecific, and diagnosis is still based mostly on blood cultures; however, blood cultures are thought to be positive for Candida in only 40–60% of cases [47]. Despite a low yield of positivity, blood culture is the main diagnostic method in neonatal period. In neonates, only 0.5–1 ml of blood is routinely available for culture, probably decreasing the sensitivity of the test substantially [48]. In addition to this, it should be kept in mind that Candida spp. require as much as 36 h to grow and deep organ involvement may be associated with negative blood cultures [49]. Rapid differentiation of C. albicans from non-albicans species by a germ tube test can be done directly from BactAlert blood culture bottles [50]. Finally, blood cultures are almost always negative in disseminated aspergillosis [51].

6.2 Hematologic markers

Hematologic markers are mostly used to predict the presence of fungal infections, but their sensitivity and specificity is low. They are not helpful in differentiating bacterial and fungal etiologies. Most patients with fungal infections have normal white
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blood cell counts. Thrombocytopenia is another frequently used marker, but it is neither specific nor sensitive for fungal infections [48].

6.3 Fungal antigens

6.3.1 Galactomannan (invasive aspergillosis)

Culture-based methods of detection lack sensitivity and often need invasive procedures for an exact diagnosis of invasive aspergillosis. Although histopathology has high specificity, it has limited sensitivity, requires the removal of tissue and may not be helpful in the early stages of infection. For this reason, clinicians preferred performing rapid diagnostic tests. One example is the galactomannan antigen ELISA, which detects the galactomannan antigen in patient’s serum [19].

Galactomannan is a polysaccharide present in the cell wall of *Aspergillus* spp. False positivity of the galactomannan antigen ELISA is frequent in adult and pediatric patients, but it is especially higher in the neonatal period. This may occur because of cross-reactivity with the *Bifidobacterium* species, which comprises up to 91% and 75% of the total fecal microflora in breast-fed and formula-fed infants, respectively [52-54]. False-positive results may also occur in patients receiving the antibiotics piperacillin, amoxicillin or ticarcillin, alone or combined with a beta-lactamase inhibitor [55].

6.3.2 Beta-D glucan and mannan

The two fungal antigens used most frequently for diagnostic purposes are (1,3)-β-D glucan and mannan. Mannan is a high-molecular-weight polysaccharide present in the cell wall of *Candida*. (1,3)-β-D glucan is an important structural component of the fungal cell wall [48], a fungal-cell-wall polysaccharide that is released into the bloodstream of patients with invasive candidiasis, invasive aspergillosis and some other species simultaneously, low specificity, high false-positive results due to sample contamination and difficulty in sample preparation. Additionally, these tests are understudied in the neonatal population [48].

The detection of fungemia using in-house PCR methods has been evaluated in the neonatal and pediatric intensive care unit (PICU) setting [61]. Small blood volumes such as 0.2 ml for detecting fungal DNA were reported to be enough in patients with blood culture-proven candidemia. These data indicate that molecular diagnostics may be a useful adjunctive method to culture.

Polymerase chain reaction testing shows promise as a tool for the diagnosis of invasive candidiasis. The limitations of this test include absence of probes that can detect multiple *Candida* species simultaneously, low specificity, high false-positive results due to sample contamination and difficulty in sample preparation. Additionally, these tests are understudied in the neonatal population [48].

Once a diagnosis of systemic candidiasis is suspected or confirmed, patients should be investigated for sites of dissemination. These investigations should include a direct ophthalmologic examination, abdominal ultrasound and echocardiography. Also, neuroimaging is strongly encouraged in the setting of suspected or proved meningitis. Serial imaging studies can be considered in the setting of persistently positive cultures [48].

7. Treatment

There are no dedicated guidelines outlining the choices for optimal therapy in the treatment of neonatal or pediatric fungal disease. There are guidelines for some IFIs in adults which comment on the treatment of these infections in children. On the basis of these adult guidelines, the epidemiology of IFIs in children and available pediatric pharmacokinetic data, recommendations can be made for optimal therapy in treating neonatal and pediatric candidiasis, aspergillosis, zygomycosis and other fungi.

Amphotericin B (AmB) deoxycholate is a polyene antifungal drug with a very broad spectrum of activity. For decades, AmB has been considered to be the best antifungal agent available.
against most systemic fungal infections \(^{(62,63)}\). Concerns about its toxicity, however, have led to the development of lipid-based formulations that have been shown to be less nephrotoxic while maintaining a broad antifungal spectrum \(^{(64)}\). In addition, several new broad-spectrum antifungal agents have recently been developed, including the echinocandins (e.g., caspofungin, anidulafungin, micafungin) and new azoles (voriconazole, posaconazole, ravuconazole) \(^{(65,66)}\). But only micafungin is licensed in the UK for use in neonates. Table 1 lists several antifungals and associated dosing information. Another important issue that needs to be considered in the treatment of IFI is the resistance against antifungals (see Table 2).

### 7.1 Amphotericin B

#### 7.1.1 Amphotericin B deoxycholate

Amphotericin B deoxycholate, the traditional mainstay of antifungal therapy, is still in use in pediatric patients despite its dose-limiting nephrotoxicity. In contrast to adults, AmB is generally well tolerated in full- and preterm infants \(^{(65)}\), but close monitoring of patients is still an important component of care as side effects are not negligible \(^{(67)}\). Side effects observed in neonates include electrolyte abnormalities and nephrotoxicity \(^{(68)}\). There have been no reported studies comparing different doses of the drug for treating documented fungal infections. Therefore, the therapeutic dose of the drug able to produce nephrotoxicity is uncertain. Glomerular toxicity occurs less frequently in children than in adults because of their greater nephronal reserve; nevertheless, hypokalemia due to tubular toxicity can be particularly severe in children and neonates. In a cohort of 34 neonates receiving AmB deoxycholate for candidiasis, 16 (47%) required supplementation of potassium due to drug treatment \(^{(69)}\). AmB is highly protein bound, and studies in adult populations have suggested that penetration into extracellular fluid spaces, including cerebrospinal fluid (CSF), is poor. However, there is evidence that CSF concentrations that are 40 – 90% of plasma concentrations can be achieved with standard dose regimens in VLBW infants \(^{(68)}\). Pharmacokinetic data in the neonatal population indicate a dosage regimen of AmB deoxycholate in neonates that is similar to that used in adults \(^{(64)}\).

#### 7.1.2 Lipid complex formulations of amphotericin B

Lipid complex formulations of AmB are less cytotoxic and can be given at higher total doses because the active drug is delivered directly to the site of action on the fungal cell membrane. At present, there are three lipid formulations of AmB that were developed with the aim of reducing the nephrotoxicity associated with the deoxycholate formulation; liposomal AmB (L-AmB); AmB lipid complex (ABLC); and AmB colloidal dispersion (ABCD). There has been little investigation into the pharmacokinetic properties of these formulations in neonates \(^{(64)}\). The literature on the use of liposomal preparations in neonates is limited primarily to case reports and case series \(^{(70,71)}\). There is some concern about the ability of the lipid preparations to clear renal infections because the drugs concentrate in lesser amounts in the kidney compared with AmB deoxycholate \(^{(72)}\). A study of 56 infants with candidiasis (including 52 preterm infants), showed no differences in mortality or time to resolution of candidemia between neonates receiving conventional AmB, L-AmB or ABCD \(^{(69)}\). The decision to prescribe a lipid formulation of AmB should be based on the potential of reducing nephrotoxicity or infusion-related toxicity rather than anticipated therapeutic benefit.

### 7.2 Flucytosine

Flucytosine (5-FC) is a fluorine analog of cytosine that works by disrupting DNA synthesis \(^{(64)}\). Flucytosine demonstrates broad antifungal activity against most Candida spp., with the exception of C. krusei \(^{(73)}\). The compound is available only as an oral formulation. Flucytosine is rarely used for treating infants because of potential toxicity, lack of a parenteral formulation and concerns that resistance may develop rapidly if used as a monotherapy. Because flucytosine achieves good CSF penetration, its main use has been in combination with AmB for treating fungal meningitis. It must be used cautiously in premature infants. Dosage is 50 – 150 mg/kg/day divided every 6 h \(^{(74)}\).

### 7.3 Azoles

The azoles are a class of antifungal agents that inhibit the production of ergosterol, the major sterol component of the fungal cell membrane. It is important to note that the azoles are fungistatic, but not fungicidal. 14-α-sterol demethylase is a cytochrome P-450-dependent enzyme, therefore the azoles may interfere with the pharmacokinetics of other medications and may be associated with hepatotoxicity \(^{(75)}\). Fluconazole, itraconazole, voriconazole and posaconazole demonstrate similar activity against most Candida spp. Each of the azoles has less activity against Candida glabrata and C. krusei \(^{(75)}\). All azoles have the potential for hepatotoxicity, and therapeutic drug monitoring is important.

Of the azoles, fluconazole is the only agent with significant data on use in neonatal populations and has the greatest penetration into the CSF and vitreous body, achieving concentrations of at least 50% of those in serum \(^{(76)}\); for this reason, it is used in the treatment of central nervous system (CNS) and intraocular Candida infections. Fluconazole has potent activity against Cryptococcus neoformans and the majority of Candida spp. The volume of distribution of fluconazole is greater and more variable in neonates than in infants and children. There is also a slow elimination of fluconazole; however, with a mean half-life of 88.6 h at birth, decreasing to ~ 55 h by 2 weeks of age. Neonates should be treated with a higher dose of fluconazole to compensate for their increased volume of distribution, but the frequency of dosing needs to be decreased because of their slow elimination \(^{(75)}\). The dosing interval of fluconazole should be every 72 h during the first 2 weeks of life, and then every 48 h during the following 2 weeks \(^{(77)}\).
Clinical experience of treating infants with the newer triazoles is limited. Voriconazole is a second-generation triazole, a derivative of fluconazole, and is available in intravenous and oral forms. Voriconazole is licensed in children from 2 years of age for the treatment of invasive aspergillosis, candidiasis, scedosporiosis and fusariosis. The drug has a broader spectrum of antifungal activity against *Candida* spp. than fluconazole. Voriconazole has been found to be active in vitro against *C. glabrata* and *C. krusei*. Voriconazole has not been studied in infants, and because of reports of adverse visual events in adults and pediatric patients, there is concern over unknown interactions with the developing retina. For this reason, the neonatal literature is limited to some cases of preterm infants treated with voriconazole.

Children require higher doses of voriconazole than adults to attain similar serum concentrations over time because the drug exhibits nonlinear pharmacokinetics in adults, but exhibits linearity in children. Recently, Shima et al. examined 16 children aged < 18 years (6 of whom were < 3 years of age) with malignancies who received voriconazole as primary antifungal prophylaxis. Voriconazole was administered orally every 12 h. As a result, the optimal oral dosages for children < 3 and ≥ 3 years of age were 17 and 8 mg/kg, respectively. It was concluded that children aged < 3 years required higher optimal daily doses, but had greater variations compared with those for older children; this results in complicated optimal dose adjustments. Therefore, plasma concentration monitoring of voriconazole is recommended for optimal and less toxic voriconazole treatments, especially for children < 3 years of age. In another report, voriconazole (4 mg/kg/dose twice a day) was used along with other cardiac drugs in two critically ill newborns with cardiac disease, and there were no significant drug interactions or side effects. Additional studies are required to determine the definitive optimal dosages for infants.

### 7.4 Echinocandins

The echinocandins are the newest class of antifungal agents and target the cell wall by specific inhibition of the (1,3)-β-D-glucan synthase enzyme complex that forms glucan

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<th>Table 1. Antifungals used in the neonatal period.</th>
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<td><strong>Families</strong></td>
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<td>Polyene antibiotics</td>
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<td>Pyrimidines</td>
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<td>Azoles</td>
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<td>Echinocandins</td>
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<td>NA: Not available.</td>
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<th>Table 2. Antifungals used in the neonatal period and susceptibility of fungus.</th>
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<td><strong>Species</strong></td>
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<td>Candida albicans</td>
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<td>Candida tropicalis</td>
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<td>Candida parapsilosis</td>
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<td>Candida glabrata</td>
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<td>Candida krusei</td>
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<td>Candida lusitaniae</td>
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<tr>
<td>Cryptococcus neoformans</td>
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<td>Aspergillus fumigatus</td>
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<td>Mucor sp.</td>
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<td>Zygomycetes</td>
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<td>Fusarium sp.</td>
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<td>Scedosporium apiospermum</td>
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<td>Scedosporium prolificans</td>
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Resistance data are summarized based on information from [62] and [73].

I: Intermediately susceptible; R: Resistant; S: Susceptible; S-DD: Susceptible dose-dependent.
polymers, the major components of the fungal cell wall. Side effects of caspofungin seem to be minimal because the target enzyme, 1,3-β-D-glucan synthase, is not present in humans. At present, three drugs in this class have been licensed for use (caspofungin, micafungin, and anidulafungin) and are available only as parenteral preparations [65]. These drugs have been well studied in the adult population, but pharmacokinetic and treatment studies are still in progress and/or in development in the neonatal population. Although there are pharmacokinetic studies assessing the use of micafungin and anidulafungin in pediatric patients, caspofungin is approved for pediatric patients aged 3 months to 17 years by the FDA. As mentioned before, micafungin is also licensed in the UK for use in neonates.

Caspofungin is the first echinocandin to be approved for the treatment of fungal infections in pediatric patients. In vitro, caspofungin is fungicidal against Candida spp. and fungistatic against Aspergillus spp., in that it inhibits growth and replication of fungal hyphae [80]. However, it has limited utility for the treatment of non-Aspergillus molds and Cryptococcus neoformans (Table 2). Although caspofungin is active against most Candida spp., a tendency for higher minimum inhibitory concentrations has been reported for Candida guilliermondii, Candida lusitaniae and C. parapsilosis [80].

Caspofungin use is yet not approved by the FDA for use in infants under 3 months of age. Caspofungin treatment of persistent fungemia in 13 critically ill neonates was reviewed by Natarajan et al. [81]. Eleven of these patients with deep-seated infection were treated with caspofungin in combination with at least one other antifungal. Blood sterilization occurred in 11 patients after 3 days of caspofungin (range 1 – 21 days). Adverse events included thrombophlebitis, hypokalemia and elevation of liver enzymes. Three infants had a second episode of candidemia and seven patients died. Results from a pharmacokinetic study indicated that the caspofungin half-life is shorter in children (2 – 11 years of age) than in adults [82]. Other studies have shown that patients aged < 3 months with documented or highly suspected candidiasis seemed to require a lower dosage of caspofungin than older patients to achieve comparable plasma concentrations [83]. Sáez-Llorens et al. [84] evaluated the plasma concentrations, safety and tolerability of single and multiple once-daily doses of caspofungin in neonates aged < 3 months. The results indicated that in these children doses of 25 mg/m²/day resulted in similar plasma concentrations to those seen in adults receiving 50 mg/m²/day. However, it was not possible to determine the caspofungin CSF concentrations achievable in this age group with a dose of 25 mg/m²/day. While these case series are encouraging, larger prospective and comparative studies are needed to determine caspofungin’s role in neonatal candidiasis and the optimal dose to use in this population.

Micafungin has very good antifungal activity against a wide range of Candida spp. in vitro. It has a favorable pharmacokinetic profile allowing for once-daily administration, has few drug–drug interactions and reports of resistance are rare. Micafungin is metabolized mainly in the liver and has low kidney toxicity. It is reported to have no effect on pharmacokinetics in renally impaired patients [85]. Although the pharmacokinetics of micafungin is similar for adults and adolescents, a shorter half-life and faster clearance is observed in premature infants and young children. Heresi et al. [86] evaluated 18 premature infants with a mean gestational age of 26.4 weeks who received single doses of 0.75 – 3 mg/kg micafungin. In examining the safety and pharmacokinetics in this age group, the authors reported larger volumes of distribution (0.435 liters/kg) and higher clearance rates (38.9 ml/h/kg) in the premature infants compared with older children and adults. Therefore, they suggested doses of 5 – 7 mg/kg/day for premature infants. It is also important to consider the need to define a dose level of micafungin sufficient to achieve adequate CNS exposure in young infants, given the propensity for Candida to invade the CNS in neonatal candidiasis. Children should be treated with 2 – 4 mg/kg/day, but neonates may require as much as 10 – 12 mg/kg/day to achieve therapeutic concentrations [87]. Recently, micafungin (0.5 mg/kg – 1.0 mg/kg) was given to four premature infants in whom other antifungals were ineffective, or in whom the side effects made it impossible to continue treatment. Micafungin was well tolerated, effective and without side effects [88]. Benjamin et al. [89] evaluated the safety and pharmacokinetics of micafungin in 13 infants aged 48 h to 120 days old with suspected or proved invasive candidemia. They treated infants weighing ≥ 1000 g and < 1000 g with doses of 7 and 10 mg/kg/day, respectively, for a minimum of 4 – 5 days. As a function of bodyweight, micafungin clearance was higher in infants weighing < 1000 g as compared with infants weighing ≥ 1000 g. The doses used were well tolerated and no deaths or discontinuations from treatment were reported in this study. However, more studies are required to ascertain the efficacy and optimal duration of treatment with micafungin in neonates.

8. Target therapy

Treatment algorithms for infants suggest giving empirical antifungal therapy as soon as IFI is suspected in order to minimize sepsis progression and deep-organ involvement.

8.1 Invasive candidiasis

Antifungal therapy initiated 3 days earlier relative to the first positive culture in infected ELBW infants has been associated with improved mortality and morbidity [90]. The lack of available dosing and safety data limits the therapeutic options in this age group primarily to AmB deoxycholate, AmB lipid preparations and fluconazole. However, the cure rate is about 60 – 90% for each drug alone [72,91]. Comparison of the efficacy between AmB deoxycholate and fluconazole has demonstrated no significant difference in treatment success or mortality rate in preterm neonates [92].

The expert panel of Infectious Diseases Society of America (IDSA) guidelines is similar between adults and pediatric
patients [73]. However, there is a special guidance recommendation for neonatal candidiasis. According to these guidelines, in case of disseminated candidiasis in the neonatal period, AmB deoxycholate (1 mg/kg daily) is recommended. If urinary tract involvement is excluded, L-AmB (3 – 5 mg/kg daily) can be used [73]. In a prospective study done during 1995 – 2001 in 37 neonates (36 of the 37 were premature infants with very low birth weights) with systemic candidiasis, high-dose liposomal amphotericin B (5 – 7 mg/kg/day) was effective and safe [93]. Also, fluconazole (12 mg/kg daily) is a reasonable alternative [73,94,95]. In NICUs that prefer fluconazole for prophylaxis, a different antifungal should be selected for treatment. Echinocandins should be used attentively and in situations in which resistance or toxicity precludes the use of fluconazole or AmB-deoxycholate [73].

The duration of therapy varies widely according to the extent of infection, clinical response and drug toxicity. The recommended duration of therapy for uncomplicated candidemia is 14 days after the clearance from the bloodstream and resolution of all symptoms [1,96]. However, treatment for deep candidal infection frequently requires prolonged therapy.

As in older patients, removal of a CVC within 24 hof a positive blood culture is an important component of treatment [97]. Delayed removal of CVCs in cindemic neonates has been associated with increased mortality and morbidity, including worse neurodevelopmental outcomes. A dilated retinal examination (preferably by an ophthalmologist) and a lumbar puncture are recommended in neonates with sterile body fluid and/or urine cultures positive for *Candida* spp. Imaging of the genitourinary tract, liver and spleen should be done if the results of sterile body fluid cultures are persistently positive.

### 8.2 Treatment of aspergillosis and zygomycosis

When neonates are afflicted with *Aspergillus* or a zygomycete, it is often a cutaneous infection at the site of skin trauma from an intravenous catheter or adhesive tape [28,33]. These cutaneous infections do carry a risk of dissemination; thus, the initiation of prompt therapy is mandatory. Although there have been recent case reports on successful treatment of neonatal aspergillosis with newer azoles and echinocandins, AmB is the agent of choice for these neonatal mold infections [98]. Similarly, among newer antifungal agents with activity against filamentous fungi (such as voriconazole, posaconazole and echinocandins), posaconazole seems to have greater activity against zygomycetes [99]; however, the pharmacokinetics and clinical efficacy of posaconazole are not known in neonates. Therefore, the decision to use these agents is based on the concern that the infant’s worsening medical condition might represent dissemination of the extensive cutaneous aspergillosis that is not responding clinically to treatment with only AmB. Similar to adults, surgical therapy may be an important adjunct for the treatment of localized *Aspergillus* infection [100]. However, infants, particularly preterm infants, may not tolerate surgical resection, especially if cutaneous lesions are extensive.

### 9. Prevention

Systemic fungal infection can develop in neonates who have mucocutaneous candidiasis and gastrointestinal colonization for *Candida* spp. It occurs mainly in the second week of life and in VLBW infants, and it has been associated with increased mortality [101]. Studies have shown that preterm infants weighing < 1000 g at birth are at a higher risk for candidemia and fungal-associated mortality than those infants with birth weights of 1000 – 1500 g [3,13,102]. During the past decade, strategies to prevent IFI have focused on adherence to good infection control policy, early introduction of enteral feeds, avoidance of broad-spectrum antimicrobial agent use and prompt removal of infected devices. More recently, there have been numerous reports detailing the success of fluconazole prophylaxis in reducing fungal colonization and invasive infection in VLBW infants [102-106].

Fluconazole has been a preferred drug for prophylaxis of IFI because it was reported to reduce colonization at sites such as the skin, gastrointestinal tract and respiratory tract [107]. Nevertheless, routine use of fluconazole is not recommended in all newborns [108]. In 2009, the IDSA and the American Academy of Pediatrics (AAP) Committee on Infectious Diseases made statements supporting the use of fluconazole prophylaxis in high-risk preterm infants with birth weights of < 1000 g [73,109]. Recently, Reed et al. [110] reviewed the literature for fluconazole prophylaxis and stated that the existing literature does not support the use of fluconazole prophylaxis based on birth weight or gestational age alone, but it may be reasonable to recommend prophylaxis in at-risk populations in which this practice seems to have some benefit. In addition, the authors indicated that neonates with additional risk factors predisposing them to invasive fungal disease, such as central venous access or exposure to broad-spectrum agents for extended periods of time, seemed to receive the most benefit from prophylaxis.

Although research in this area has grown over the last decade, concerns remain regarding the risk of fluconazole resistance development among clinical isolates, as well as the risk of selecting for fluconazole resistant strains of *Candida* such as *C. glabrata* and *C. krusei*. However, many studies reveal no evidence of the development of fluconazole-resistant *Candida* spp. [102,104,111,112]. Manzoni et al. have published a retrospective study reported no significant changes in the emergence of resistant strains such as *C. krusei* and *C. glabrata* [111]. Other concerns include adverse effects of fluconazole therapy and the lack of data demonstrating its impact on long-term morbidity and mortality [100], but fluconazole seems to be well tolerated in prophylactic doses in most neonates [102,103,106,111,112].

The most effective dosing schedule for fluconazole prophylaxis is an early initiation to prevent colonization, ideally on the first postnatal day, and then a continuation of dosing throughout the period of highest infection risk. Within these limitations, most infants would receive fluconazole prophylaxis
for 3 – 6 weeks [105]. In randomized, placebo-controlled clinical trials, both 3 and 6 mg/kg twice-weekly dosing beginning near birth have decreased the incidence of invasive candidiasis in centers with a high burden of Candida infections, without the emergence of resistance [103,111,113]. Therefore, 3 mg/kg given twice a week is the optimal dosing schedule maximizing efficacy, safety and cost effectiveness [110].

There is evidence that prophylactic topical or oral nonabsorbed antifungal agents (e.g., nystatin and miconazole) in VLBW infants decrease fungal colonization on the skin and mucosal surfaces, including the gastrointestinal tract [96]. Nystatin is a polyene antifungal that is not absorbed from the gastrointestinal tract, so oral nystatin acts as a topical agent to reduce colonization. Oral nystatin is inexpensive, less toxic than fluconazole and was shown to be highly effective in clinical trials for reducing the incidence of invasive candidiasis and candidal rectal colonization in neonates and for preventing Candida infection [114-117]. However, several observational studies did not find nystatin effective once the colonization had occurred [118,119]. Ozturk et al. [114] demonstrated that invasive candidiasis was insignificantly lower in the neonates who were identified as yeast carriers (oral moniliasis) and treated with oral nystatin than in the infants who did not receive oral nystatin (5.6 and 14.2%, respectively).

10. Expert opinion

Candida and Aspergillus species are the causative agents in most of the IFIs in the newborn period as well as childhood. The clinical findings of fungal infections in the neonatal period are nonspecific, which makes early diagnosis a challenge. Newer methods have been developed for diagnosis of IFIs in children and adults, but their use in the newborn period is limited. Therefore, blood cultures are still the gold standard method. Many newer azoles and echinocandins have been approved by the FDA for use in children and adults; but none in the neonatal period. However, there are case reports of neonates who were refractory to conventional treatments and successfully treated with echinocandins (caspofungin and micafungin) and newer azoles (voriconazole). Because diagnosis and treatment are often delayed, recent research has focused on empirical and prophylactic treatment strategies. Selective antifungal prophylaxis has reduced invasive fungal sepsis in NICUs without evidence of emerging fluconazole resistance. Therefore, VLBW newborns with high risk of developing IFIs should be identified and given fluconazole prophylaxis. In addition, the main preventive measures are still the following: i) avoiding modifiable risk factors, in particular restricted use of broad-spectrum antibiotics; ii) minimizing the duration of use of parenteral nutrition and endotracheal intubation; and iii) avoiding gastric acid suppressants. Finally, to reduce associated morbidity and mortality, more studies are needed on the diagnosis, treatment and prevention of IFIs of the newborn period.

Invasive fungal infections are major causes of morbidity and mortality in both preterm and term neonates. Successful management of IFIs relies on early recognition and rapid initiation of effective treatment. The ultimate goal in this field is to reduce fungal colonization and invasive infections.

The efficacy and safety of fluconazole prophylaxis in preterm infants has been reported in four sufficient randomized, controlled trials and a systematic review, with no significant adverse effects reported. Very preterm newborns with high risk of developing IFIs should be identified and given prophylaxis with fluconazole or nystatin. Prophylactic nystatin and fluconazole reduce the incidence of colonization and IFI in very preterm newborns. We believe that nystatin is an alternative to fluconazole because nystatin is safe, inexpensive, well tolerated and effective.

Fungal infections constitute an important problem in the neonatal intensive care unit and a better understanding of the incidence, diagnosis, clinical management, treatment and prophylaxis is important to reduce morbidity and mortality. The identification of high-risk preterm infants and the implementation of prophylactic measures and early treatment may improve the outcome of these patients.

Declaration of interest

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89. Prophylaxis is important and emphasized in this study.


Data indicate importance of antifungal prophylaxis in preterms weighing < 1000 g.


Important data focus on prophylaxis for preterm neonates.


Very important data about nystatin is as effective as fluconazole.


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