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The Epidemiology and Diagnosis of Invasive Candidiasis Among Premature Infants

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Abstract

Invasive candidiasis is a leading infectious cause of morbidity and mortality in premature infants. Improved recognition of modifiable risk factors and antifungal prophylaxis have contributed to the recent decline in the incidence of this infection among infants. Invasive candidiasis typically occurs in the first six weeks of life and presents with non-specific signs of sepsis. Definitive diagnosis relies on growth of *Candida* in blood culture or cultures from other normally sterile sites, but this may identify fewer than half of cases. Improved diagnostics are needed to guide initiation of antifungal therapy in premature infants.

Keywords

neonatal candidiasis; *Candida*; premature infants; risk factors

Background

Invasive candidiasis is a leading infectious cause of morbidity and mortality in extremely premature infants. It affects 4–8% of extremely low birth weight (ELBW; birth weight <1000 g) infants, and is associated with 30% mortality.[1–8] Infants with invasive candidiasis who survive frequently have long-term neurological impairment including cerebral palsy, blindness, hearing impairment, cognitive deficits, and periventricular leukomalacia.[2, 5, 9–11]

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The incidence of neonatal candidiasis rose rapidly in the 1980's and 1990's with the improved survival of ELBW infants and the increased use of central venous catheters.[12] However, this trend has reversed, with the incidence of invasive candidiasis among premature infants declining substantially over the past 15 years.[13–15] In one study that included data from 322 neonatal intensive care units (NICUs), the incidence of invasive candidiasis decreased from 3.6 episodes per 1000 infants in 1997 to 1.4 episodes per 1000 infants in 2010.[15] Fluconazole prophylaxis, reduced use of broad-spectrum antibacterial antibiotics, empirical antifungal therapy, and improved care of central venous catheters have contributed to the declining incidence of invasive candidiasis.[13, 15]

Pathogenesis

Candida species are yeast that frequently colonize skin, the gastrointestinal (GI) tract, and the female genitourinary tract.[16] Infants admitted to the NICU are colonized by *Candida* rapidly after birth, with the GI and respiratory tracts being the most frequent sites during the first two weeks of life.[17–21] Colonization during this age period may be related to the birthing process; infants delivered vaginally have higher rates of colonization than infants born by Caesarean section and the colonizing *Candida* species are identical to those isolated from the maternal genitourinary tract in the majority of cases.[17, 20–22] Colonization of infants >2 weeks of age frequently occurs on the skin and may be related to contact with maternal skin or the hands of health care providers.[20] In particular, health care workers may be the primary source of *Candida parapsilosis* colonization in the NICU environment.[22, 23]

Colonization of infants by *Candida* species is not sufficient for the development of invasive candidiasis (Figure 1), although up to 5–10% of very low birth weight (VLBW; birth weight <1500 g) infants colonized by *Candida* develop invasive disease.[18, 20, 24, 25] Premature infants are predisposed to invasive candidiasis for several reasons. First, the typical barriers to invasion by *Candida* species are not fully developed in premature infants. The epidermis of the infant born at <30 weeks gestational age is thin and poorly formed compared with the skin of term infants.[26] Moreover, immaturity of the barrier and immune functions of the GI tract predispose to translocation by *Candida*.[27] Cellular immunity is also impaired; premature infants have fewer neutrophils and T lymphocytes than term infants, and both groups have altered neutrophil chemotaxis and phagocytosis compared with older children and adults.[28–30] Finally, virulence factors of the colonizing yeast isolate also appear to be important in determining risk of progression to invasive disease. Bliss et al. observed enhanced virulence characteristics among more than half of *Candida* isolates from infants with invasive candidiasis.[31]

Once *Candida* species have invaded across mucosal surfaces or entered the bloodstream, they have a predilection for tissue invasion in the central nervous system, kidneys, liver, spleen, heart, and retina. Within the central nervous system, *Candida* can cause a meningoencephalitits, cerebral abscesses, and ventriculitis with obstructive hydrocephalus.[32, 33] *Candida* can also infiltrate with or without abscess formation in the liver, spleen, and (most commonly) the kidneys.[32, 34] Finally, endocarditis and endogenous endophthalmitis may result from seeding of the heart valves or eyes during fungemia.

Risk Factors

Neonatal candidiasis generally occurs after the first two weeks of life in the setting of extreme prematurity or among infants of any gestational age with GI pathology.[35] Over the past decade, investigators have identified risk factors for invasive candidiasis in several large cohorts of infants.

Prematurity & Birth Weight

Extreme prematurity is the strongest risk factor for the development of invasive candidiasis.[2, 4, 15] The incidence of invasive candidiasis is low (0.06%) among infants admitted to the NICU with birth weight >1500 g.[36] In comparison, invasive candidiasis develops in 2–5% of VLBW infants, while 4–16% of ELBW infants have historically been affected.[2, 6, 15, 37–39] The incidence of invasive candidiasis is inversely related to birth weight even among ELBW infants, with infants born at <750 g being at least twice as likely to develop invasive candidiasis as infants with birth weights between 751 and 1000 g.[2, 15] Mortality from invasive candidiasis is also inversely related to birth weight, approaching 50% for infants <750 g.[10]

NICU site

NICU site is also strongly related to risk of invasive candidiasis.[8] In a cohort of ELBW infants admitted to 12 NICUs, the incidence of invasive candidiasis ranged from 2.4% to 20.4%.[2] Empirical use of third-generation cephalosporins correlated with the center-specific incidence observed in this study.[2] Use of antifungal prophylaxis might also contribute to the differing incidence by center. However, substantial variation in the incidence of invasive candidiasis was still observed among infants receiving placebo in several recent trials of antifungal prophylaxis (Table 1).[24, 25, 40–42]

Broad-spectrum antibiotics

The strongest modifiable risk factor is antibiotic exposure and, more importantly, the choice of antibiotics for routine empiric therapy. Antibacterial therapy increases the density of *Candida* colonization by reducing the competitive pressure exerted by commensal bacteria, and receipt of broad-spectrum antibacterial antibiotics (e.g., third-generation cephalosporins) is among the most consistently identified risk factors for neonatal candidiasis.[2, 4, 8, 17, 23, 37, 43] Studies suggest that exposure to third-generation cephalosporins is associated with an approximate doubling of the risk of invasive candidiasis among ELBW infants.[2, 38] Carbapenems are likely to be increasingly used in NICUs with the emergence of multi-drug resistant Gram-negative bacteria.[44] In one study of VLBW infants, receipt of a carbapenem or third-generation cephalosporin in the prior seven days was associated with invasive candidiasis, although no studies have assessed risk specifically associated with carbapenem use.[4]

Central venous catheters

Central venous catheters are indispensable in the treatment of critically ill premature infants, minimizing the need for venipuncture and facilitating the administration of parenteral nutrition, blood products, and inotropic therapy. However, these devices also play a critical

role in the pathogenesis of invasive candidiasis, providing a portal of entry for *Candida* as well as a foreign surface for adhesion and biofilm formation.[1, 3, 8, 43] The portion of central venous catheters that is within the vessel lumen frequently becomes covered by a fibrin sheath.[45] *Candida* species can grow within this fibrin matrix while remaining protected from host immune defenses and antifungal therapy.[46] As a result, central venous catheter removal is often necessary to clear candidemia, while delayed catheter removal (>1 day after initiation of antifungal therapy) is associated with an increased risk of death or neurodevelopmental impairment from invasive candidiasis.[38]

Other risk factors

Translocation across the GI tract is generally thought to be the most frequent source of invasive candidiasis.[47] Necrotizing enterocolitis, congenital GI anomalies (e.g. gastroschisis),[3, 32] spontaneous intestinal perforation,[48] and prior abdominal surgery[43, 49] are all associated with an increased risk of invasive candidiasis among premature infants. Histamine-2-receptor antagonists (H2 antagonists) encourage overgrowth of *Candida* in the GI tract through suppression of gastric acid production, and may facilitate invasion by *Candida* species through inhibition of the neutrophil oxidative burst.[50, 51] In a study conducted among infants in six NICUs, H2 antagonists more than doubled the risk of invasive candidiasis.[1] Data also suggest that corticosteroid treatment increases the risk of invasive candidiasis among premature infants.[52, 53] Corticosteroids alter number and function of T lymphocytes and result in hyperglycemia, which facilitates growth and inhibits phagocytosis by *Candida* species *in vitro*.[54, 55] In a placebo-controlled trial, dexamethasone increased the risk of sepsis and meningitis among VLBW infants, with *Candida* species accounting for roughly one-quarter of these infections.[53] Finally, in a prospective cohort study of more than 1500 infants, presence of an endotracheal tube increased the risk of invasive candidiasis by more than 50%.[8] Although the mechanism for this association has not been defined, endotracheal intubation can result in abrasion of the respiratory mucosa, which may enable invasion by *Candida* species.[56]

Microbiology

Although there are more than 150 species of *Candida*, the majority of cases of invasive candidiasis among infants are caused by a relatively small number of species. *Candida albicans* is generally the most commonly isolated species, accounting for 45–55% of episodes of invasive candidiasis among infants.[2, 3, 8, 13, 57] In the majority of cohorts, *C. parapsilosis* is the most frequent non-albicans *Candida* species (20–35%), followed by *Candida tropicalis* (1–6%).[3, 13, 39, 58] Non-albicans species may be responsible for a growing proportion of neonatal candidiasis.[13, 14, 57] *Candida krusei* and *Candida glabrata* warrant special consideration given their inherent or potential resistance to fluconazole.[57] However, these species still account for a relatively small proportion (<5%) of neonatal candidiasis, and no increase in disease caused by these species was observed in recent cohorts.[8, 13, 14, 39]

C. albicans is also the most pathogenic species of *Candida*. In a number of studies, mortality associated with invasive candidiasis caused by *C. albicans* was higher than for disease caused by *C. parapsilosis*.[2, 12, 22, 59, 60] Moreover, the mortality differences in several of

these studies were substantial, as in a cohort where the case fatality rates for invasive candidiasis caused by *C. albicans* and *C. parapsilosis* were 24% and 3%, respectively.[59] However, this mortality difference was not observed in some studies, and a recent meta-analysis concluded that invasive candidiasis caused by *C. parapsilosis* is associated with a mortality rate of approximately 10% among premature infants.[37, 58]

Diagnosis

Delayed initiation of appropriate antifungal therapy is associated with increased mortality from invasive candidiasis.[61, 62] However, identification of infants with candidiasis is challenging as infants typically have non-specific symptoms and diagnostic capabilities are currently limited.

Clinical Findings

Infants with invasive candidiasis frequently present with features suggestive of sepsis, including lethargy or apnea, feeding intolerance, cardiorespiratory instability, and hyperbilirubinemia.[46] Hyperthermia, a generally unreliable marker for infection in infants, is present in only half of infants with invasive candidiasis.[35, 39] Thrombocytopenia lacks specificity for a diagnosis of invasive candidiasis, and studies reporting the sensitivity of this finding yielded conflicting results.[4, 63, 64] Glucose intolerance and leukopenia or leukocytosis are also common findings, although white blood cell count is normal in 40% of infants with fungal sepsis.[39] Finally, C-reactive protein and procalcitonin are often elevated in infants with fungal sepsis, but the specificity of these results is poor.[65, 66]

Clinical judgment in determining risk of invasive candidiasis among ELBW infants was evaluated in one study.[8] At the time that blood cultures were obtained for sepsis, the bedside clinician was asked to estimate the probability of invasive candidiasis. Of the sepsis episodes resulting in a diagnosis of invasive candidiasis, only 25% were deemed to have been “probably” or “highly likely” to be caused by *Candida* by the treating clinician.[8] Moreover, the accuracy of clinical judgment was similar across levels of medical training (resident, fellow, and attending).[8]

Culture-Based Methods

Blood culture remains the gold standard for diagnosis of neonatal candidiasis. However, autopsy studies suggest that the sensitivity of blood culture for invasive candidiasis is <50% even when an optimal volume of blood (7.5–10 mL) is obtained for culture.[67, 68] Blood culture yield varies based on the number of organs that are involved, ranging from 28% when one vital organ is involved to 78% when at least four vital organs are involved.[67] Blood culture sensitivity may be even lower in premature infants because of the small volumes that are typically used to inoculate blood culture bottles in this patient population. Substantial improvements in blood culture technology were made since these original autopsy studies. The precise impact of these advances on the yield of blood cultures for invasive candidiasis is unknown, but blood culture likely remains an insensitive test for invasive candidiasis.[69]

Once growth of *Candida* in blood culture occurs, a lengthy process to identify the species generally ensues. Over the past decade, a number of techniques became available that can reduce the time needed for identification of yeast species from positive blood cultures. Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) emerged as a powerful technique for the rapid identification of bacteria and fungi from growth on solid media. MALDI-TOF MS uses mass spectrometry to identify bacterial and fungal species based on the ribosomal protein patterns, often providing results in less than one hour.[70] Several studies confirm that the MALDI-TOF identifies *Candida* species from solid growth with 90–95% accuracy, effectively reducing the time needed for species identification following blood culture positivity.[70–72]

The peptide nucleic acid fluorescent *in situ* hybridization (PNA-FISH) Yeast Traffic Light Assay (AdvanDx, Inc., Woburn, MA) enables rapid detection of *Candida* directly from liquid media, including positive blood cultures.[73, 74] This assay uses species-specific fluorescent probes and is capable of identifying the five most commonly isolated species of *Candida* within 90 minutes.[73] When viewed under a fluorescent microscope, green fluorescence is seen in the presence of *C. albicans* or *C. parapsilosis*, yellow fluorescence with *C. tropicalis*, and red fluorescence in the presence of *C. glabrata* or *C. krusei*.[73, 74] For each of the probes, the sensitivity and specificity are above 90%, and this assay generally identifies blood culture isolates more quickly than the MALDI-TOF as it does not require growth on solid media.[73, 74]

Polymerase chain reaction (PCR) holds great promise for earlier identification of *Candida* species from positive blood cultures. Several PCR-based assays are commercially available that can identify yeast species from positive blood cultures with sensitivity and specificity >98%.[75–77] Moreover, like the PNA-FISH Yeast Traffic Light Assay, PCR does not require growth on solid media, and the total time needed for this technique is generally <4 hours.[75] Given its ability to detect small quantities of fungal DNA, PCR is also being evaluated for the direct detection of *Candida* species from whole blood.[78–80] Among the most studied assays for this purpose is the LightCycler SeptiFast (Roche Diagnostics), which can detect *Candida* species from whole blood in approximately 60% of patients with culture-confirmed candidemia.[78]

Fungal Antigens

There are a number of fungal antigens that may be detectable in the blood of patients with invasive candidiasis. These include mannan, a component of the outer cell wall of *Candida* species, and 1-3-β-D-glucan, found in the middle layers of the cell wall.[81] The Platelia *Candida* Antigen Plus assay (Bio-Rad, Marnes-la-Coquette, France) is most frequently used for detection of mannan antigen in blood. The available data indicate that the specificity of this assay for invasive candidiasis in adults is excellent (>90%), but sensitivity is poor (30–60%).[81–84] Few studies have been conducted in infants, although mannan antigen was positive in 11 of 12 infants with proven invasive candidiasis in one small study.[85] Mannan is poorly expressed by *C. parapsilosis* and the sensitivity of mannan antigen for invasive candidiasis caused by this species is likely to be lower.[82, 83]

There are a number of commercially available kits for the detection of 1-3-β-D-glucan from clinical specimens, including the Fungitell assay (Associates of Cape Cod, Inc., Falmouth, MA) and the Fungitec G-test (Seikagaku Corporation, Tokyo, Japan). Several reports suggest that 1-3-β-D-glucan may be a useful screening test for invasive fungal infection in certain populations.[86–88] A recent meta-analysis including 19 studies concluded that 1-3-β-D-glucan assays have a sensitivity of 81% and a specificity of 81% for diagnosis of invasive candidiasis, although most of the included data were from adults.[89] Goudjil et al. retrospectively examined serum 1-3-β-D-glucan levels in 61 infants with clinical suspicion of fungal infection. Among 18 infants who were diagnosed with invasive candidiasis, the mean 1-3-β-D-glucan level was 364 pg/mL (range 131–976) compared with 89 pg/mL (range 30–127) among non-infected control infants.[87] However, healthy children and infants have higher 1-3-β-D-glucan levels than adults, suggesting that age-specific cutoffs may be necessary, and larger prospective studies are needed before use of 1-3-β-D-glucan assays can be recommended for the diagnosis of neonatal candidiasis.[90, 91]

Treatment

Indications and neonatal dosing for specific antifungal agents are discussed in detail elsewhere in this issue. However, for the bedside clinician, two aspects of invasive candidiasis warrant special consideration: 1) involvement of the kidneys should guide choice of antifungal therapy, and 2) central nervous system involvement should be presumed in the infant with invasive candidiasis. More specifically, liposomal formulations of amphotericin B should not be used for infants with renal candidiasis given the sub-optimal penetration of these agents into the renal parenchyma.[92] Central nervous system involvement should be presumed because the incidence of meningoencephalitis exceeds 15% in neonates with invasive candidiasis,[33] cerebrospinal fluid parameters (white blood cells, glucose, protein) and culture unreliably detect disease, and imaging is not sufficiently sensitive to exclude central nervous system involvement.

The optimal duration of antifungal therapy for neonatal candidiasis has not been defined, but guidelines are available from the Infectious Diseases Society of America.[92] Candidemia without evidence of end-organ dissemination should be treated with 3 weeks of antifungal therapy from clearance of blood cultures and resolution of signs of infection. Infants with *Candida* meningoencephalitis should receive antifungal therapy until these conditions are met and cerebrospinal fluid abnormalities have completely resolved. Native valve endocarditis should be treated with ≥6 weeks of antifungal therapy and may require valve replacement. Central venous catheters should be promptly removed or replaced in the setting of bloodstream infection as this reduces duration of candidemia, the rate of end-organ dissemination, and mortality.[32, 38, 59]

Conclusions

Although improved recognition of risk factors led to a substantial reduction in neonatal candidiasis over the past decade, this infection remains a barrier to achieving further reductions in the morbidity and mortality associated with extreme prematurity. The diagnosis of invasive candidiasis continues to rely on clinical suspicion and the detection of

candidemia. Several methods were recently developed that can shorten the duration of time needed for identification of yeast from positive blood cultures. However, improved diagnostics that can rapidly identify infants with invasive candidiasis and permit initiation of prompt antifungal therapy are still needed.

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Key Points

- Invasive candidiasis occurs primarily in extremely premature infants and is associated with substantial morbidity and mortality.
- The incidence of invasive candidiasis is strongly related to gestational age and birth weight, but most cases are preventable.
- The diagnosis of invasive candidiasis relies on clinical suspicion and detection of *Candida* in blood culture or cultures from other normally sterile sites.
- Several methods were recently developed that can shorten the time needed for identification of yeast from a positive culture, but improved diagnostics are still needed.

Best Practices Box

What is the current practice?

- Blood cultures are sent routinely from premature infants with signs of sepsis.
- The decision of whether to start empirical antifungal therapy is determined by the clinician's suspicion for invasive candidiasis based on local epidemiology and infant- specific risk factors.
- Widespread variation exists in the practices of antifungal prophylaxis and empirical antifungal therapy for premature infants with sepsis.

What changes in current practice are likely to improve outcomes?

- Further research to determine what impacts the variation in the incidence of invasive candidiasis across NICUs.
- Improved molecular or fungal antigen-based diagnostics that can rapidly identify *Candida* species from blood or other normally sterile sites.
- Determining optimal duration of antifungal therapy for invasive candidiasis among premature infants.

Is there a clinical algorithm?

Major Recommendations

- Minimize exposure to modifiable risk factors for invasive candidiasis (broad-spectrum antibacterials, central venous catheters) in caring for premature infants.
- Consider empirical antifungal therapy for premature infants with signs of sepsis, particularly in the setting of established infant risk factors.
- Infants with invasive candidiasis should be treated presumptively for central nervous system disease.

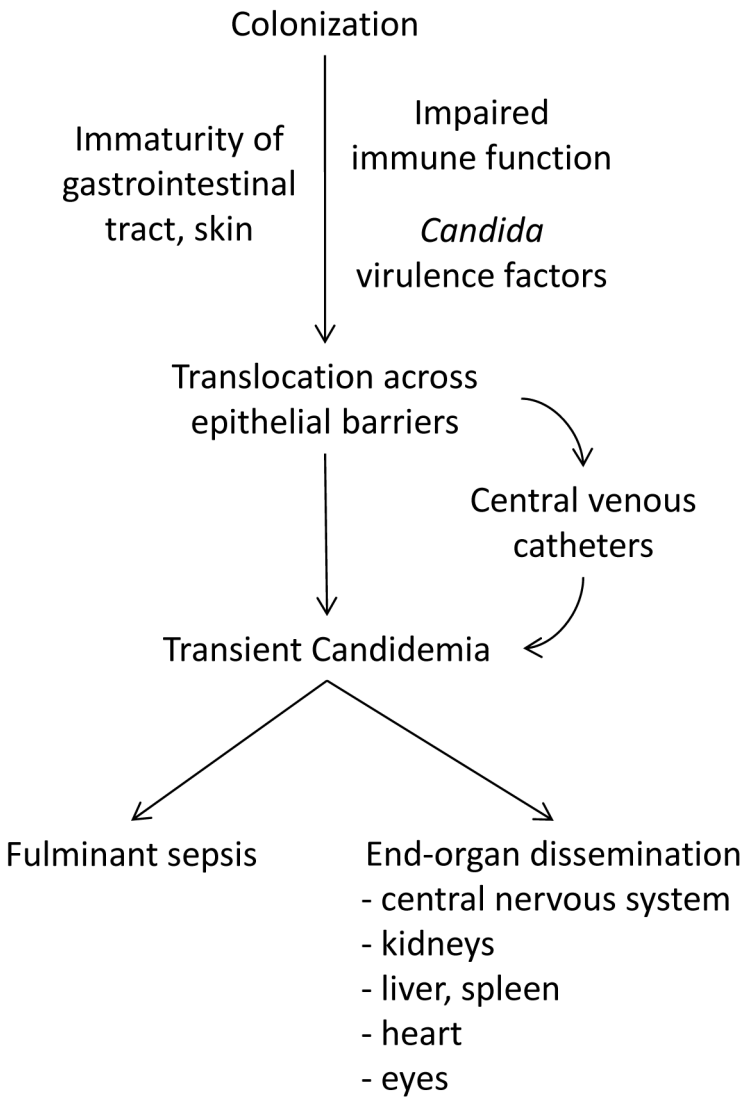

Figure 1.
Pathophysiology of invasive candidiasis in premature neonates.

Table 1

Variation in the cumulative incidence of definite invasive candidiasis among placebo recipients in recent clinical trials of antifungal prophylaxis among premature infants.

Study	Year	Patient Population (Birth Weight, Age)	Patients (N)	Cumulative Incidence of Invasive Candidiasis	Patients, Birth Weight <750 g (*n*)	Cumulative Incidence of Invasive Candidiasis, Birth Weight <750 g
Kicklighter et al.[24]	2001	<1500 g, 0–28 days	N=50	0%	*n*=10	0%
Kaufman et al.[25]	2001	<1000 g, 0–42 days	N=50	20%	*n*=24	25%
Manzoni et al.[45]	2007	1000–1500 g, 0–30 days <1000 g, 0–45 days	N=106	13%	*n*=18	17%
Parikh et al.[46]	2007	<1500 g, 0–28 days	N=60	25%	*n*=0	-
Benjamin et al.[44]	2014	<750 g, 0–42 days	N=173	7%	*n*=173	7%