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Recognition and diagnosis of invasive fungal infections in neonates

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Summary Fungal infections largely caused by *Candida* species are responsible for a significant disease burden in neonates and invasive candidiasis in hospitalised neonates has high associated morbidity and mortality. Early initiation of antifungal treatment improves outcome but the recognition and diagnosis of systemic fungal infection in this population is difficult due to the non-specific clinical presentation and the high false negative rate of cultures. There is a need for a practical, sensitive and rapid diagnostic test to enhance identification and early treatment. Serum detection of (1,3)-β-d-glucan and *Candida* PCR are promising candidates but at present limited data exists for their use in the neonatal intensive care setting. Until such investigations are validated, early initiation of antifungal treatment on the basis of risk factor profile and clinical features remains the safest practical approach.

Background, epidemiology and risk factors

Neonatal invasive fungal disease is largely synonymous with invasive *Candida* infection, although rarer nosocomial infections with the yeast *Malassezia*[1,2] and mucocutaenous infections with moulds including *Aspergillus* species[3–5] and Zygomycetes have been reported in preterm infants.[6] Due to the different nature of these two groups of fungal infections and the much greater clinical importance of *Candida* disease, the discussion of clinical recognition and diagnostics will focus on invasive candidiasis (IC) only.

Neonates are particularly susceptible to IC, with a 3- to 5-fold higher incidence compared with children or adults.[7,8] A combination of host and external factors contribute to creating particularly favourable conditions for the development of invasive *Candida* infections (Table 1).

Birth weight and gestational age are the most important risk factors both for developing IC and for IC associated mortality.[14] Neonates born below 28 weeks of gestation and/or <1000g birth weight (ELBW; extreme low birth weight) have the highest risk of developing IC, while the risk is generally low in neonates born after 32 weeks of gestation

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Table 1 Neonatal patient factors which are associated with an increased susceptibility to invasive *Candida* infection (derived from [9–13]).

Host	External
Extreme prematurity (<28 weeks gestational age)	Indwelling prosthetic devices
Very low birth weight (<1500 g)	Mechanical ventilation
Immature immune system	Broad spectrum antibiotics (particularly third-generation cephalosporins) or polymicrobial use
Impaired barrier function of skin & mucosa	Use of steroids
Candida colonization	Use of H_2-blockers
Hyperglycaemia	Gastrointestinal pathology ± abdominal surgery
	Parenteral nutrition or lipid emulsions

and/or >1500g birth weight.[15] Neonates with abdominal pathology requiring surgery also have significantly increased risk of IC, related to the GI tract being the major site of *Candida* colonisation.[16,17]

Despite increasing numbers of the highest risk infants for IC due to the improved survival of extremely preterm and low birth weight infants, epidemiological data demonstrates that the overall incidence of neonatal IC has been declining since the late 1990s, likely attributable to a combination of reduced use of broad spectrum antibiotics, improved infection control measures and the introduction of antifungal prophylaxis.[12] Recent reported incidences of neonatal candidaemia are around 0.15% of all neonatal admissions[8,12] and between 2–9% of ELBW infants,[18,19] although results can vary up to 10-fold amongst centres.[10,19]

The commonest species accounting for neonatal candidaemia are *C. albicans* and *C. parapsilosis*,[20–22] with smaller numbers due to *C. glabrata*, *C. tropicalis*, *C. krusei* and *C. lusitaniae.* The occurrence of neonatal candidaemia peaks in the 2nd week of life with a median age at diagnosis of 11 days for *C. albicans* and 18 days for *C. parapsilosis*.[21] *Candida* colonisation is a risk factor for subsequent infection, with *C. albicans* transmission more likely to be acquired by vertical transmission whereas *C. parapsilosis* is most likely to be transmitted by healthcare workers or the environment.[23–26]

The diagnosis of IC in a neonate is associated with a poor prognosis both in terms of survival and neuro-developmental outcomes; 26% of ELBW infants with IC die with an attributable mortality of 11.9%[8] and up to 60% of survivors have neurodevelopmental impairment.[14,27,28] Early initiation of treatment improves outcome;[28–30] hence, prompt clinical recognition and the availability of robust diagnostic tests are greatly important.

Clinical recognition

The commonest presentation of invasive fungal disease in neonates is a generalised sepsis which is indistinguishable from the clinical picture seen with late-onset bacterial sepsis. There are however blood markers and risk factors which increase the likelihood of a diagnosis of invasive candidiasis. Neonates with IC develop thrombocytopenia more frequently compared to those with bacteraemia, with both a lower thrombocyte nadir as well as a longer duration.[31–33] In combination with existing risk factors as listed in Table 1, candidemia should be suspected in a neonate with sepsis and new thrombocytopenia. Hyperglycemia is also a common feature of neonatal fungal sepsis, and the findings of the Neonatal Research Network (NRN) *Candida* study confirmed the significance of this amongst a large cohort of ELBW neonates as the only other blood parameter – along with thrombocytopenia – that was a clinical predictor for IC, with odds of IC increasing as blood glucose level rose.[19]

A very small minority of neonatal *Candida* infections are acquired prenatally from ascending maternal infection and present at or around the time of birth. These cases of congenital candidiasis are rare but worthy of note, as clinically the infection behaves differently, usually manifesting as a widespread vesiculopustular or erythematous skin eruption, which in a term infant largely follows a benign course. Congenital candidiasis can, however, evolve into severe sepsis and death, with preterm infants being the most vulnerable to complications. Therefore any preterm infant with suspected congenital candidiasis or term infant with suspicious skin eruption and signs of respiratory distress or sepsis should receive antifungal therapy.[34]

Fungal diagnostics in neonates

Culture

Culture of blood or other normally sterile body fluid – the current gold standard for diagnosing IC – is particularly challenging in neonates due to the practical difficulty of obtaining an appropriate volume for analysis.[35]

Even with optimal volumes sensitivity of sterile site culture is poor; blood cultures are negative in approximately 50% of cases of autopsy proven disseminated candidiasis,[36] and guidelines from the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) on diagnostic procedures advise that daily blood culture from three separate sites with a total culture volume of 2–4 ml for infants <2 kg is required to optimise detection in suspected candidaemia; even in this context sensitivity of culture only reaches 50–75%.[37] In everyday clinical practice the number of false negatives obtained from neonatal blood cultures will therefore be high.[38,39]

Urine cultures positive for *Candida* spp. are highly significant, and must be treated as invasive systemic candida disease in neonates. ELBW infants with candiduria have almost equivalent rates of neurodevelopmental impairment and death as those with positive blood cultures suggesting that candiduria is a sign of IC.[40] Nevertheless, microbiological proof of IC is hard to obtain, further reflecting the poor