Review

Recent Challenges in Diagnosis and Treatment of Invasive Candidiasis in Neonates

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Recent Challenges in Diagnosis and Treatment of Invasive Candidiasis in Neonates

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Abstract: Invasive *Candida* infections represent a significant cause of morbidity and mortality in the neonatal intensive care unit (NICU), particularly among preterm and low birth weight neonates. The nonspecific clinical presentation of invasive candidiasis, resembling that of bacterial sepsis with multiorgan involvement, makes the diagnosis challenging. Given the atypical clinical presentation and the potential detrimental effects of delayed treatment, empirical treatment is often initiated in cases with high clinical suspicion. This underscores the need to develop alternative laboratory methods other than cultures, which are known to have low sensitivity and a prolonged detection time, to optimize therapeutic strategies. Serum biomarkers, including mannan antigen/anti-mannan antibody and 1,3-β-D-glucan (BDG), both components of the yeast cell wall, a nano-diagnostic method utilizing T2 magnetic resonance and Candida DNA detection by PCR-based techniques have been investigated as adjuncts to body fluid cultures and have shown promising results in improving diagnostic efficacy and shortening detection time in neonatal populations. This review aims to provide an overview of the diagnostic tools and the current management strategies for invasive candidiasis in neonates. Timely and accurate diagnosis followed by targeted antifungal treatment can significantly improve the survival and outcome of neonates affected by Candida species.

Keywords: invasive candida infections; neonatal candidiasis; candida diagnosis; antifungal treatment

1. Introduction

1.1. Epidemiology

Invasive candidiasis (IC) represents one of the leading causes of morbidity and mortality in neonatal intensive care units (NICUs), and is reported to be the third most common cause of late-onset neonatal sepsis [1,2]. The incidence of IC shows considerable variation across geographic areas and even between different centers in the same region [2–8]. Preterm and/or low birth weight neonates represent the most vulnerable population, and the prevalence of IC is inversely correlated with gestational age and birth weight [9]. The reported incidence among NICU admissions is estimated to be between 0.5 and 2%; however, among the most susceptible population, the extremely low birth weight (ELBW) neonates, the reported incidence rises up to 20% [1,2,9].

Invasive candidiasis in preterm neonates is associated with significant morbidity and mortality. A mortality rate of up to 50% has been reported among ELBW neonates [10,11]. A composite outcome of death or neurodevelopmental impairment was observed in 73% of a cohort of ELBW neonates with IC [12]. Moreover, a recent study demonstrated that 44% of neonates with IC exhibited adverse neurodevelopmental outcomes, a rate that was significantly higher than that observed in survivors of non-fungal infections [11].

1.2. Microbiology and Pathogenesis

Candida spp. represent a common constituent of the human mycobiome with the potential to manifest pathogenic behavior. The potential for *Candida* species to cause invasive infections has been

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associated with specific virulence factors, which may vary depending on the causative strain, the site of infection, and the host immune response. These factors include adherence and invasion of the host cells, formation of biofilms in tissues and indwelling devices, the transition from yeast to hyphae, and the production of tissue-damaging enzymes [13,14].

In neonatal invasive infections, *Candida albicans* is the most commonly identified strain, followed by *Candida parapsilosis* and, less frequently, *Candida glabrata, Candida tropicalis, Candida krusei,* and the recently emerging Candida auris. [8,11,15–17]. However, this varies according to geographical location. A higher proportion of non-albicans species is observed in low- and middle-income countries. It is worth noting that the susceptibility of different strains of *Candida* to antifungal drugs varies, and therefore, it is crucial to identify the causative strain [4–6,18–20].

Neonates in the NICU, especially preterm and low birth weight, represent a population with a high rate of *Candida* colonization. *Candida* spp. can be transmitted either vertically, during vaginal delivery from a colonized mother, or horizontally from the NICU environment [21–24]. It has been reported that up to 60% of very low birth weight (VLBW) neonates are colonized during the first month of their NICU stay, and about 20% of them will develop IC [21]. Colonization with *Candida* species represents the initial step in the pathogenesis of systemic infections. Although colonization does not invariably lead to invasive disease, colonization sites may act as a reservoir for *Candida* translocation and dissemination in the presence of predisposing conditions [23].

1.3. Risk Factors

The risk of IC is inversely correlated with gestational age and birth weight [9]. This is attributed to the immaturity of the immune system and the natural protection barriers of preterm neonates, as well as the need for prolonged NICU stay. Invasive procedures, including central venous catheters (CVC) and endotracheal tubes, disrupt epithelial barriers, thereby permitting the invasion of pathogens and subsequent dissemination [25–27].

The use of broad-spectrum antibiotics, especially third-generation cephalosporins, and carbapenems, which are known to suppress the normal flora of the gastrointestinal tract, is a well-recognized risk factor for IC [28,29]. Corticosteroids, due to their immunosuppressive effects, and H2-antagonists, due to the alkalization of gastric pH, which modifies normal bacterial flora, have been proposed to promote microbial dysbiosis [30–32]. Furthermore, the delay in the establishment of full enteral feeding and parenteral nutrition administration, particularly lipid emulsion, is a well-documented predisposing factor for Candida colonization and replication [33–35].

Gastrointestinal pathologies, such as prior abdominal surgeries and necrotizing enterocolitis (NEC), are known to predispose to IC due to the disruption of the epithelial intestinal barrier, which permits the translocation of Candida across the gastrointestinal tract and into the circulation [8,36,37].

It has been reported that colonization of more sites and increased colonization density represent risk factors for yeast translocation and dissemination, potentially leading to invasive disease [38].

Changes in NICU practice, including the avoidance of exposure to modifiable risk factors, such as reducing broad-spectrum antibiotic administration, accelerating enteral feeding advancement, and early removal of CVCs, along with the administration of antifungal prophylaxis in high-risk populations, have been demonstrated to be an effective strategy for reducing the incidence of IC.

1.4. Clinical Presentation

The clinical presentation of neonates with IC is often indistinguishable from that of a bacterial late-onset infection, as the symptoms are typically non-specific. Sepsis-like symptoms and signs, including apnea, respiratory distress, lethargy, temperature instability, feeding intolerance, and cardiovascular instability, may be presented [1,37,39].

Candidemia has the potential to disseminate in different organ systems hematogenous or by the formation of septic emboli, which can result in deep-tissue infections and the development of fungal masses [1,40]. Dissemination in the central nervous system (CNS) is a relatively common sequela, manifesting as meningitis or encephalitis or less commonly as ventriculitis or brain abscesses [40,41]. The spectrum of renal involvement extends from cystitis to parenchymal infiltration, calyceal

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mycetoma, and the formation of fungal masses, which can result in obstructive uropathy [42–44]. Endocarditis is a rare but serious complication, often associated with long-lasting candidemia and the presence of a central venous catheter [45]. Less common complications of IC include eye involvement (chorioretinitis or endophthalmitis), osteoarticular infections (arthritis or osteomyelitis), liver and spleen abscesses, and embolic skin abscesses [46]. A potential involvement of Candida infections in the pathogenesis of spontaneous intestinal perforation (SIP) has been proposed [47].

In consideration of the potential involvement of different organ systems, neonates diagnosed with IC should undergo a comprehensive evaluation to accurately determine the extensity of the disease. According to current Infectious Diseases of Society of America (IDSA) guidelines, a lumbar puncture and cerebrospinal fluid culture (CSF) and dilated eye examination should be performed in all neonates with positive blood or urine cultures for *Candida* spp. Moreover, imaging of the genitourinary tract, liver, and spleen is recommended in cases of persistent candidemia, as evidenced by persistent candida positivity [48].

This narrative review aims to summarise the existing and emerging knowledge regarding the diagnosis and treatment of invasive Candida infections in the neonatal intensive care unit (NICU). The PubMed and Google Scholar databases were searched for relevant studies up to August 2024 using the following terms: neonatal invasive candidiasis, preterm neonate, candidiasis diagnosis, candidiasis treatment, antifungal agents, amphotericin, fluconazole, and echinocandins. Ultimately, 176 articles were found, and 94 were included, particularly randomized control trials, systematic reviews, narrative reviews, and observational studies. Additionally, the reference lists of the retrieved articles were examined to identify any relevant studies that might have been missed in the initial search.

2. Diagnosis

An early and accurate diagnosis of systemic candidiasis, followed by the prompt initiation of antifungal treatment, is critical for survival and the elimination of long-term sequelae. However, the diagnosis is challenging due to the non-specific clinical presentation and, therefore, relies on diagnostic testing. While blood culture is considered the gold standard for IC diagnosis, it has significant limitations, and alternative laboratory methods have been investigated to facilitate a timely and precise diagnosis (Table 1).

Table 1. Advantages and disadvantages of laboratory techniques and biomarkers for the diagnosis of neonatal candidiasis.

	Advantages	Disadvantages
Blood culture [49–51]	Antifungal susceptibility testing Sensitivity threshold up to <1cfu/ml, depending on the blood volume	Sensitivity ~50% Difficult to obtain optimal blood volumes in neonates Slow turnaround time (1-3 days)
Mannan/anti-mannan antibody [52–54]	Early positivity High sensitivity and positivity (94.4%, 94.2% respectively) High NPV	Low sensitivity for *C.parapsilosis*, *C.krusei* infections Fast elimination and repeat testing may needed
1,3-β-D glucan [55–58]	Minimal amount of blood required (<100µl) High sensitivity (>80%) High NPV	The optimal positivity threshold in neonates is not yet determined Frequent false positive results

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	Useful in treatment monitoring	
T2MR assay [59–61]	High sensitivity and specificity Sensitivity threshold 1-3cfu/ml, depending on species Rapid turnaround time Useful in treatment monitoring	Detection of five Candida species High blood volume required
PCR techniques [49,50,62,63]	High sensitivity and specificity High NPV Minimal blood volume required	Limited data on neonates Technique optimization needed
NGS [64,65]	Detection of multiple microorganisms simultaneously	Inability to differentiate between colonization and infection Slow turnaround time High cost

NPV: negative predictive value; T2MR: T2 Magnetic Resonance; RCR: polymerase chain reaction; NGS: next generation sequencing.

2.1. Blood Culture

Blood culture is considered the gold standard for IC diagnosis in all age groups. However, considerable constraints exist in terms of the time required for diagnosis and diagnostic accuracy [63,66].

The sensitivity threshold for blood cultures is ≤1 colony-forming unit per milliliter (cfu/mL), with the detectability of Candida species contingent upon the volume of blood sampled [49–51]. Lancaster et al. employed in vitro techniques to investigate the minimum blood volume necessary for the isolation of Candida spp. from blood cultures exhibiting low and ultra-low concentrations. *Candida albicans* and *Candida parapsilosis* were recovered from blood specimens of 0.5 ml volume at a load of 1-10 cfu/ml. However, ultra-low concentrations (i.e., <1 cfu/ml) required a 3 ml blood volume for isolation [67]. In neonates, the detection of Candida is challenging due to the difficulty in obtaining adequate blood volumes [50]. According to the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) recommendations, three blood culture specimens should be obtained in a single session with a total volume of 2-4ml for neonates weighing less than 2kg [68]. The IDSA and the American Society for Microbiology recommend a single culture of 2ml for neonates < 1kg and two cultures of 2ml each for neonates weighing 1-2/kg [69]. Obtaining the recommended blood volume for culture in neonates is often unfeasible due to either hemodynamic instability or the difficulty of obtaining the sample. Harewood et al. reported that more than one-third of neonatal blood cultures contained negligible amounts of blood [70,71].

Even with a sufficient volume of blood cultures, the overall sensitivity of blood cultures in diagnosing systemic candidiasis is estimated to be below 50% [9,63,72]. A further limitation of blood cultures in the diagnosis of IC is the slow turnaround time, which typically ranges from 1 to 3 days [49,63]. A previous retrospective study demonstrated that in neonates diagnosed with IC, the median time to positive blood culture was 36 hours if not on antifungal drugs and 42 hours when antifungal therapy was initiated [73]. A delay in the initiation of therapy, pending culture results, has been associated with increased mortality [74]. Nevertheless, the *Candida* strain and the system employed influence the sensitivity rate and turnaround time [68,75]. The use of fungal selective media has been associated with higher sensitivity rates in a shorter time frame [76,77].

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2.2. Mannan/Anti-Mannan Antibody

Mannan is a high-molecular-weight polysaccharide that constitutes a component of the upper layer of the *Candida* cell wall [66,78]. The detection of mannan antigen and anti-mannan antibody has been proposed as a diagnostic tool for IC; however, there is a paucity of data regarding the neonatal population [52]. The most widely used testing assay is the combined mannan/anti-mannan antibody assay, PLATELIA™ *Candida* Ag *Plus* system (Bio-Rad Laboratories, Marnes-la-Coquette, France) [66,79]. Olivieri et al. studied the efficacy of PLATELIA™ in the diagnosis of IC in a neonatal cohort and reported a sensitivity of 94.4% and specificity of 94.2%. It is noteworthy that the test result was positive at a median of 8.5 days prior to the detection of positive cultures, which indicates the potential usefulness of this biomarker in the prompt diagnosis of IC in high-risk neonates [53]. In a prospective study, Montagna et al. reported the presence of positive mannan antigen in five out of seven neonates with IC. It is notable that in both neonates with IC and a negative mannan antigen result, *C. parapsilosis* was isolated [54]. The low sensitivity of mannan antigen in patients with *C. parapsilosis* and *C. krusei* has been observed in several studies and is likely attributable to variations in mannose epitopes [53,66]. A recent prospective case-control study examined the mannan antigen in *Candida* colonized and non-colonized neonates and observed that the test results were not influenced by the presence of *Candida* colonization [52]. However, due to the accelerated elimination of the antigen from the circulation, repeated testing is necessary [54,76].

2.3. 1,3-β-. D-Glycan

The 1,3-β-D-glycan (BDG) is a component of the inner cell wall of *Candida* species. Elevated levels of BDG have been observed in the serum of patients with systemic candidiasis, and thus, BDG has been proposed as a potential biomarker for early IC diagnosis [56].

The *Fungitell Assay* (Associates of Cape Cod, Inc., Massachusetts, USA) is the most widely used test for quantifying BDG [63,66]. Several studies have been conducted on neonatal populations, with the objective of investigating the utility of BDG as a biomarker for IC and the optimal cut-off levels for positivity. This method's significant advantages include prompt results and the minimal quantity of blood required for the assay (<100µl) [55]. As specified by the manufacturer of the *Fungitell assay*, a positive result is indicated by a cut-off level of 80 pg/ml [58]. Nevertheless, a number of studies have argued that this threshold may not be appropriate for use in neonates and have proposed a higher threshold for IC diagnosis [58,62,80,81]. In the CANDINEO study, utilizing the aforementioned threshold, the positive predictive value was estimated to be 14%, while the negative predictive value was 97.1% in a cohort of VLBW neonates [62]. Cliquennois et al., in a prospective cross-sectional study, reported a sensitivity and specificity of 85.7% and 51.9%, respectively, of BDG in the diagnosis of IC with a cut-off of 80pg/ml and proposed that the optimal threshold could be 174pg/ml [58]. According to the results of a recent review and meta-analysis, the sensitivity and specificity of the *Fungitell assay* in the neonatal population at a threshold of 80 pg/ml were estimated at 89% and 60%, respectively, and at a cut-off of 120 pg/ml were 81% and 80%, respectively. The authors concluded that BDG could be useful in excluding IC and potentially as an adjunctive method in the diagnosis of neonatal IC; however, they acknowledged the paucity of data in the neonatal population [55].

A further aspect of BDG as a biomarker of systematic candidiasis is monitoring the response to antifungal treatment. A limited number of studies in the neonatal population have performed serial measurements of BDG levels to assess the response to therapy and have observed an initial increase and then a progressive decline of serum BDG levels [56,80,82].

One notable limitation of the BDG as a biomarker for IC is the high proportion of false-positive results. A number of potential contributors have been identified, including glycan-containing gauzes, hemodialysis membranes, and the administration of specific beta-lactam antibiotics, blood products, intravenous immunoglobulin, albumin, and postnatal corticosteroids. Moreover, it has been proposed that gram-positive and gram-negative sepsis and *Candida* colonization may be associated with elevated BDG levels [55,57,62,63,66,80].

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2.4. T2 Magnetic Resonance (T2MR) Assay

T2 magnetic resonance (T2MR, T2 Biosystems, Lexington, MA, USA) technology is an innovative molecular technique that utilizes magnetic resonance combined with nanotechnology to identify pathogens. The T2Candida system, an FDA-approved assay, can detect five *Candida* species (*Candida albicans, Candida tropicalis, Candida parapsilosis, Candida glabrata, and Candida krusei*) in whole blood specimens using T2MR technology [49,76]. The sensitivity threshold varies between *Candida* species and has been defined as 1cfu/ml for *C. tropicalis* and *C. krusei*, 2cfu/ml for *C. albicans* and *C. glabrata,* and 3cfu/ml for *C. parapsilosis* [59,61].

In a retrospective study in a pediatric cohort, T2Candida showed a 100% sensitivity and 94.1% specificity [60]. One of the most notable advantages of this assay is the rapid turnaround time and speciation, which facilitate the prompt and targeted administration of antifungal therapy [59]. In the aforementioned study, the mean time for *Candida* identification was 3.7 hours using the T2Candida assay, significantly shorter than the mean time of 125.5 hours for positive blood culture results [60]. Despite the paucity of data regarding the utility of T2Candida as a diagnostic tool for IC in neonatal and pediatric populations, the available data are consistent with those of larger studies involving adults. A recent meta-analysis of eight studies on adult populations revealed a pooled sensitivity of 91%, a specificity of 94%, and a time to positivity of 3-4 hours [83].

According to the manufacturer's specifications, the quantity of blood required for the assay is 3ml; however, in studies with pediatric populations, a reduced volume of blood has been employed, either by pipetting samples directly into the T2Candida cartridge or by diluting with General Purpose Buffer, without compromising the assay's sensitivity [60,84].

According to the current literature, previous antifungal treatment does not impact the T2Candida assay's results, in contrast to the effects observed in blood cultures. Therefore, T2Candida may represent a useful tool for monitoring the response to treatment in patients treated with antifungal agents [60,85].

2.5. Polymerase Chain Reaction (PCR) Assays

A variety of PCR techniques have been investigated to facilitate the early and accurate diagnosis of IC, either by targeting specific strains of fungi or by detecting in general fungal DNA ("panfungal" PCR) [79,86]. Despite the availability of several commercial PCR assays, they are not FDA-approved for *Candida* infections, and their role in the diagnostic pathway of IC remains undefined, especially in neonatal populations in which data are very limited [62,86]. The principal benefits of PCR-based methodologies are the rapid turnaround time, providing accurate strain identification in 2 to 4 hours, the increased sensitivity compared to blood cultures, the reduced blood volume necessary, the high negative predictive value (NPV) in low prevalence settings, and the ability to monitor patient's response to antifungals [49,50,62].

In the CANDINEO study, a multicenter study involving VLBW, the sensitivity and specificity of PCR in diagnosing IC were reported to be 87.5% and 81.6%, respectively. The reported NPV of the PCR assay was 98.8%, underscoring the potential clinical utility of these techniques in the cessation of unnecessary antifungal treatment. Moreover, in 17.4% of cases, PCR was positive despite the negative blood cultures [62]. Furthermore, the enhanced diagnostic yield of PCR was demonstrated in another study conducted on a pediatric population, in which PCR was positive in 24% of cases suspected of candidemia and blood cultures in 14.8% [87]. The limited data available regarding the PCR in neonates and children are consistent with the evidence from studies conducted in adults. In a meta-analysis, Avni et al. reported a PCR sensitivity and specificity of 93% and 95%, respectively, and positivity rates of PCR of 85% in patients with proven or probable systemic candidiasis, compared to 38% of positive blood culture results [88].

The novel Droplet Digital PCR (ddPCR) technology, which involves randomly encapsulating pathogen nucleic acid in microdroplets and a separate reaction in each one, offers several advantages, including the ability to detect pathogens rapidly, even in minute quantities, and to quantify the target genetic material with great precision in biological samples [89]. The utility of ddPCR has been

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investigated in a neonatal population, with reported sensitivity and specificity of 86% and 100%, respectively, and a detection limit of 3.2 copies/µl [90].

The cationic conjugated polymer-based fluorescence resonance energy transfer (CCP-FRET) technology has recently been developed as an innovative method for diagnosing IC. The two components of the CCP-FRET assay are a water-soluble conjugated polymer and a fluorescence dye-labeled pathogen-specific DNA. This technique is rapid, providing pathogen identification within three hours, with a detection limit as low as one-tenth that of real-time PCR. It has been demonstrated to have a sensitivity and specificity of up to 100% in clinical specimens. Moreover, the assay necessitates a minimal blood volume of 0.2ml, which is of particular significance in neonates. It is important to note that the selection of appropriate primers is essential for the efficacy of the assay. However, more research is required to optimize the technique [63,91].

2.6. Next Generation Sequencing (NGS)

Metagenomics (mGNS) refers to the application of NGS to detect the genomic material of multiple microorganisms simultaneously in various biological samples [86]. In a retrospective study of pediatric patients with hematological diseases and probable sepsis, the rate of positive results using mNGS was significantly higher than that of blood cultures (57.2% vs 12.5%) [92]. A recent meta-analysis of studies conducted in neonatal and pediatric populations concluded that mGNS could be a valuable tool for identifying pathogens in cases of sepsis, offering a particular advantage in cases where the causative pathogen is an unusual or difficult-to-isolate organism, such as fungi [64]. Despite the indisputable advantages of metagenomics as a diagnostic method for the identification of fungi, bacteria, viruses, and mixed infections from a single specimen, this method presents several noteworthy limitations. These include its inability to differentiate between colonization and infection, the lengthy turnaround time, and the high cost [65,66].

3. Treatment

The timely initiation of antifungal treatment in neonates with disseminated candidiasis has been demonstrated to have a critical impact on survival rate. The efficacy and safety of agents from four classes of antifungals have been evaluated in infants and neonates: polyenes, triazoles, echinocandins, and nucleoside analogues. The various classes of antifungal drugs act via disparate mechanisms, including the disruption of cell membrane biosynthesis, cell wall synthesis, and stability, and fungal DNA/RNA synthesis [93,94].

3.1. Antifungal Agents

3.1.1. Polyenes

Polyene macrolides are the oldest category of antifungal drugs. Amphotericin B deoxycholate (D-Amb), derived from *Streptomyces nodosus*, has been used since 1950 to treat fungal infections and still represents one of the first-choice agents for neonatal systemic candida infections [93,95]. Amphotericin B exerts its antifungal activity by binding to ergosterol, a component of the fungal cytoplasmic membrane. This leads to pore formation, increased permeability of the membrane to electrolytes, and, ultimately, cell death [96]. Nevertheless, D-Amp has the potential to bind to cholesterol within the membranes of mammalian cells, which is postulated to be the cause of the observed side effects, including nephrotoxicity [97].

In order to decrease the incidence of side effects, new formulations of the drug combined with lipids were developed: amphotericin B lipid-complex (ABLC), amphotericin B colloidal dispersion (ABCD), and liposomal amphotericin B (L-Amb) [95]. However, in most settings, D-Amp is preferred over lipid formulations in neonatal systemic candidiasis [48,98]. A multicenter observational study reported a significantly higher mortality rate in neonates with IC treated with L-Amb than with D-Amb. The authors hypothesize that this is probably attributable to the poorer penetration of L-Amb to the kidneys or inappropriate dosing in neonates [99]. In comparison to D-Amp, L-Amp demonstrates a restricted capacity to penetrate the urinary tract, which is frequently

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implicated in neonatal systemic candidiasis [48]. However, in a prospective historical control multicenter study in a VLBW population, the two formulations of amphotericin showed comparable efficacy [100].

D-Amb lacks enteral absorption and is administered intravenously [93,95]. It is characterized by a high affinity for plasma proteins, extensive distribution throughout tissues, and clearance via the urinary and biliary systems [93]. Based on the limited available data on the pharmacokinetics in the neonatal population, D-Amb is characterized by faster clearance, a larger volume of distribution, lower concentrations, and longer half-life compared to adults [98,101]. However, these pharmacokinetic parameters have been shown to have high inter-individual variability among neonates [102]. The current recommended dosing regimen for D-Amb is 1mg/kg once daily, although doses up to 1.5mg/kg/day have been proposed in resistant infections [48,98,103]. Moreover, the faster elimination observed in neonates is presumably the reason for the reduced nephrotoxicity compared to older children and adults [93]. Le et al. reported an incidence of nephrotoxicity of 16% in neonates treated with D-Amp. In the majority of cases, the nephrotoxic effects were transient [99]. In accordance with the observations of previous studies, Ambreen et al. noted that maintaining adequate hydration and sodium intake above 4 mEq/kg/day throughout the course of D-Amb therapy exerts a protective effect with regard to the development of nephrotoxicity in neonates [104]. In addition to nephrotoxicity, hypokalemia, infusion-related reactions, and hepatotoxicity are reported side effects of amphotericin, although these appear to affect neonates less frequently than older patients [105].

Amphotericin has been reported to penetrate CSF well in neonates. Although studies in adults report CSF levels as low as 2-4% of serum concentration, Bailey et al. detected CSF levels of amphotericin B in preterm neonates at 40-90% of serum values. In a rabbit model of *Candida* meningoencephalitis, D-Amb and L-Amb exhibited superior antifungal efficacy relative to alternative amphotericin formulations. However, higher concentrations of L-Amb were achieved in brain tissue compared to those of D-Amb [106]. The IDSA guidelines recommend D-Amb as the agent of choice for the treatment of *Candida* CNS infection in neonates, and the ESCMID guidelines recommend these formulations as equivalent (Table 2). In patients beyond the neonatal age, both societies recommend L-Amb for the treatment of *Candida* CNS infections [48,103].

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Table 2. Infectious Diseases of Society of America (IDSA) and European Society of Clinical Microbiology and Infectious Diseases (ESCMID) guidelines for the management of neonatal invasive candidiasis.

	IDSA (2016) [48]		**ESCMID (2012)** [103]
	Candida bloodstream infection	Candida CNS infection	
Antifungal agent Agents of choice	D-Amb 1mg/kg/day or fluconazole 12 mg/kg/day if not on fluconazole prophylaxis	D-Amb 1mg/kg/day	D-Amb 1mg/kg/day or L-Amb 2.5-7 mg/kg/day or fluconazole 12 mg/kg/day if not on fluconazole prophylaxis (loading dose 25 mg/kg/day can be considered)
Alternatives	L-Amb 3-5mg/kg/day as an alternative (caution if urinary tract involvement)	L-Amb 5mg/kg/day as an alternative	ABLC 2.5–5 mg/ kg/day as an alternative
	Echinocandins with caution, as salvage therapy or when D-Amb or fluconazole cannot be used due to toxicity or resistance.	Flucytosine, 25 mg/kg 4 times daily, may be added as salvage therapy in patients who have not had a clinical response to initial AmB therapy	Micafungin 4-10 mg/kg/day
		After response to initial treatment, step down to fluconazole 12 mg/kg daily is recommended for susceptible isolates	Capsofungin 25 mg/m^2/day (limited data available)
Implanted devices	CVC removal is strongly recommended	It is recommended CNS devices, including ventriculostomy drains and shunts, be removed if feasible.	Removal or replacement of intravenous catheters and/or other implanted prosthetic devices should be considered
Therapy duration	2 weeks after blood culture sterilization and resolution of signs	Continue therapy until all signs, symptoms, and CSF and radiological	2 weeks after blood culture sterilization provided that no unresolved deep infection remains

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	attributable to candidemia	abnormalities have resolved	

D-Amb: Amphotericin B deoxycholate; L-Amb: liposomal amphotericin B; ABLC: amphotericin B lipid-complex; CVC: central venous catheter; CNS: central nervous system; CSF: cerebrospinal fluid.

3.1.2. Triazoles

Triazoles represent a major class of antifungal drugs widely used in the neonatal population. The antifungal activity of triazoles is achieved through the disruption of ergosterol biosynthesis, a crucial component of the fungal cell membrane. This is achieved by the inhibition of 14-a-sterol demethylase, a cytochrome P-450 enzyme [94,95].

Among triazoles, fluconazole, a first-class triazole, is the most thoroughly studied and widely used agent for the prophylaxis and treatment of IC in neonates. Fluconazole has been demonstrated to be effective against the majority of *Candida* species; however, resistance has been documented in *Candida glabrata* and *Candida krusei* [93,94]. Fluconazole shows minimal affinity to proteins and distributes widely through tissues, demonstrating excellent penetration into the CNS and vitreous body [95]. A significant benefit is the high oral bioavailability (>90%) of the drug [107,108]. Given the greater volume of distribution and prolonged half-life in neonates compared to older patients, it has been suggested that administration of a loading dose of 25 mg/kg may facilitate rapid achievement of therapeutic concentrations [107]. This approach has been shown to be effective and well-tolerated in neonatal cohorts [109,110].

The most common adverse effects of fluconazole in neonates are gastrointestinal irritation and hepatotoxicity [107,111]. As fluconazole is renally eliminated, dose adjustment is necessary in patients with renal impairment [93,107]. The inhibition of cytochrome P450 enzymes by azoles may result in interactions with other pharmaceutical agents, potentially affecting the therapeutic efficacy [95].

The latest IDSA and ESCMID guidelines recommend fluconazole 12 mg/kg/day as an alternative to amphotericin B as a first-line treatment for neonatal candidiasis in neonates not receiving fluconazole prophylaxis [48,103]. The literature on the comparative efficacy of fluconazole and amphotericin B in neonatal candidiasis is limited, resulting in a lack of consensus regarding the optimal first-line agent in NICUs [112–114]. However, fluconazole, in addition to its high oral bioavailability, has the advantage of being compatible with drugs commonly used in the NICU, as opposed to amphotericin, when administered intravenously [107].

Itraconazole, another first-generation triazole that demonstrates fungistatic and fungicidal activity, is generally well tolerated in pediatric patients and available in an oral formulation [111]. However, oral bioavailability is variable and dependent on gastric pH and food intake [66]. Mondal et al., in an RCT involving 43 pediatric patients with systemic candidiasis, reported comparable efficacy and safety of itraconazole and fluconazole [115]. Moreover, a systematic review of 32 studies concerning systematic fungal infections in infants revealed a similar conclusion regarding the use of itraconazole [116]. However, the use of itraconazole in neonates is limited due to its highly variable pharmacokinetics, the lack of sufficient data on neonates, and the availability of alternative agents that have been subjected to more extensive investigation [111].

Voriconazole, a second-generation triazole, is a synthetic derivative of fluconazole with a broader spectrum of activity among *Candida* species, including *Candida glabrata* and *Candida krusei* [94]. It has also been demonstrated to exhibit efficacy against *Candida auris* [117]. Voriconazole demonstrates about 90% oral bioavailability, is characterized by moderate protein bound, and distributes well into tissues, including the CNS [93,118]. Adverse effects of voriconazole include hepatotoxicity, photosensitivity, and visual disturbances, which are reported to be transient in adults. Due to the paucity of safety data in the neonatal population, voriconazole is not recommended and should be used only in refractory cases as second-line therapy. However, it is not approved for use in children younger than 2 years [93,94].

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3.1.3. Echinocandins

Echinocandins are more recently developed antifungal drugs. They exhibit their fungicidal action by inhibiting the 1,3-b-glycan synthase complex, which leads to disruption of cell wall stability and, ultimately, lysis. As the target enzyme is absent in mammalian cells, echinocandins are generally well tolerated [94,95]. The results of a meta-analysis indicate that the incidence of adverse effects necessitating treatment discontinuation was lower in pediatric patients receiving echinocandins than in those treated with amphotericin B [119]. Echinocandins show poor oral bioavailability and are administered parenterally [93]. These agents have been demonstrated to exhibit a broad spectrum of activity against *Candida* species that are resistant to other antifungal agents and have also been shown to be effective in the treatment of *Candida* biofilms [120]. Echinocandins are characterized by high-protein binding, liver metabolism, and a wide distribution to tissues, with the exception of the CNS and kidneys. In neonates, these sites are often affected in disseminated candidiasis, and high doses of echinocandins may be required to achieve optimal efficacy [66,94]. Micafungin is the only echinocandin approved for infant use [95].

A limited number of studies have been conducted to examine the efficacy, optimal dosing, and safety of micafungin in the neonatal population. Two RCTs involving neonates have documented that micafungin exhibits comparable efficacy to D-Amb and L-Amb in the treatment of systemic *Canida* infections [121,122]. The half-life has been demonstrated to be shorter, and the elimination process faster as gestational age and birth weight decrease [123]. Compared to older children and adults, neonates exhibit accelerated clearance, necessitating higher doses to achieve the target serum concentration [66,94,112]. A dose of 4 mg/kg has been demonstrated to achieve serum concentrations comparable to those achieved by a dose of 100 mg/kg in older children and adults [124]. Pharmacokinetic studies have demonstrated that micafungin exhibits a dose-dependent penetration in the CNS and that higher doses, up to 10mg/kg, are required in neonates and young infants to achieve CNS *Candida* eradication [124,125]. Although the urine excretion of active micafungin has been reported to be 0.7%, it has been postulated that the high plasma concentrations that are achieved may yield sufficient elevated levels in the urine to eradicate *Candida* from the urinary tract [126,127]. It is noteworthy that micafungin has been demonstrated to exert significant inhibitory activity against the adhesion and biofilm formation of various Candida species [128].

Micafungin is generally well tolerated, with a minimal propensity for drug-to-drug interactions [125]. The most common adverse effects are gastrointestinal disturbances, hepatotoxicity, and hypokalemia [129]. The European Medicines Agency (EMA) has issued a “black box” warning due to the reported increased incidence of hepatocellular tumors in experimental animals after prolonged administration [103]. Nevertheless, a systematic review of 9 studies reported a 73% efficacy of micafungin in infants with systemic candidiasis and an acceptable safety profile for both term and preterm neonates [130]. The current guidelines recommend micafungin 4-10 mg/kg/day for the treatment of neonatal IC [103]. However, due to the limited data regarding its safety, micafungin is proposed as a salvage therapy when first-line drugs are contraindicated or when resistance is present [48].

Caspofungin, another echinocandin, is not FDA-approved for infants younger than three months. Data on the use of caspofungin in neonates are limited, but the available literature suggests that the drug is both safe and effective [119,131–133]. Pharmacokinetic studies have reported reduced clearance of caspofungin with decreasing age, and a dose of 25mg/m^2/day has been proposed for infants [93,132]. Due to the lack of data, the ESCMID guidelines give a weak recommendation for the use of caspofungin in the treatment of neonatal IC [103].

Anidulafungin, a semi-synthetic lipopeptide, has the unique property that it is not metabolized but undergoes a process of slow degradation and biotransformation [134,135]. Therefore, dose adjustment is not required in cases of impaired hepatic or renal function [134]. Anidulafungin is not currently licensed for neonatal use. The high doses that need to be administered to attain therapeutic CNS levels are associated with polysorbate 80 (PS80) accumulation [136]. However, in a recent prospective multicenter study, no PS80 accumulation was detected in pediatric patients aged > 1 month who received anidulafungin [137].

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3.1.4. Nucleoside Analogues

Flucytosine, a synthetic fluorinated analogue of cytosine, exerts its antifungal activity by disrupting RNA and inhibiting DNA synthesis in the fungal cell [134,138]. It is characterized by low protein binding, high hydrophilicity, and a wide distribution, including the CNS, the vitreous body, and urine [93,138]. Flucytosine is primarily excreted by the kidneys, and the elimination rate is proportional to the renal function. Thus, dose adjustments are necessary in cases of renal impairment, and caution is needed when administering the drug to premature neonates due to their immature renal function [64,138]. Another concern with the use of flucytosine is dose-related toxicity, including hepatotoxicity, bone marrow suppression, and gastrointestinal disorders [138].

Flucytosine monotherapy is not recommended due to the rapid development of resistance [64]. Due to its excellent CNS penetration, flucytosine has been proposed as an adjunctive therapy in combination with amphotericin B in cases of candida meningitis [93,94]. However, data on the effectiveness of this approach are conflicting [12,139]. Current IDSA guidelines recommend the addition of flucytosine as salvage therapy in neonates with CNS candidiasis unresponsive to amphotericin monotherapy [48].

3.2. Central Venous Catheters (CVC)

CVCs are a common practice in the care of preterm and low birth weight neonates during their stay in the NICU, primarily for the administration of parenteral nutrition and intravenous drugs. It is well documented that systemic *Candida* infections are often associated with the formation of biofilms on implanted medical devices [13,140,141]. Biofilms are attachment complexes composed of microbial cells integrated within an extracellular polymeric matrix composed of water, polysaccharides, proteins, lipids, and extracellular DNA [142,143]. The eradication of *Candida* biofilms represents a significant challenge, given that these structures provide protection for the fungus from antifungal agents and the host immune response. Consequently, biofilms act as reservoirs for the systemic dissemination and end-organ dissemination of pathogens, thus prolonging the infection [140,141].

According to the current guidelines, prompt removal of CVC in cases of neonatal systemic candidiasis is strongly recommended [48]. Benjamin et al. observed that prompt removal of CVC was associated with a shorter duration of candidemia and improved survival and neurodevelopmental outcomes in ELBW neonates [12]. In a recent study, Chen et al. identified delayed CVC removal as an independent risk factor associated with mortality in neonates with IC [144].

However, CVC removal is not always feasible, and the decision to proceed with removal should be made considering the necessity of maintaining central venous access in critically ill neonates [144,145]. In cases where maintaining central venous access is essential, lock therapy, i.e., instilling high concentrations of antifungals into the catheter lumen, has been proposed, but data on neonates are limited, and efficacy and safety have not been established [95]. The efficacy of various antifungal agents and combinations has been studied for lock therapy, including caspofungin, micafungin, anidulafungin, and L-AmB [146]. Ethanol-based solutions have also been shown to be highly effective and are a reasonable alternative [147].

4. Conclusions

The diagnosis and management of invasive Candida infections remain a significant challenge in the NICU. Since blood culture, the long-standing gold standard for IC diagnosis, has severe limitations, biomarkers and innovative molecular diagnostic methods have been investigated, but the implementation of these techniques in routine clinical practice remains a future prospect. Accurate and early diagnosis is the key to effective and timely treatment, which improves outcomes, particularly for preterm neonates, who are at particular risk of mortality and long-term sequelae. A limited number of antifungal drugs have been approved for use in neonates, and future studies evaluating drugs currently used in adults and recently developed drugs will provide more opportunities for effective treatment. The diagnosis and management of invasive Candida infections

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