Neonatal invasive candidiasis: A poorly understood entity

Orlando E. Flores-Maldonado, Miguel A. Becerril-García, Rogelio J. Treviño-Rangel,
Luis A. Rodríguez-Gómez and Gloria M. González*

Department of Microbiology, Autonomous University of Nuevo Leon, School of Medicine, Monterrey, Nuevo Leon, Mexico

Abstract

Candida spp. is the most common fungal pathogen that causes systemic infections in the pediatric population, mainly in premature newborns (Nbs). The first days of life of Nbs is crucial, as they move from a relatively sterile environment to a totally different one. In addition, the development and maturation of the neonatal organs and systems are in progress. These physiological and functional differences possibly make Nbs susceptible to mycosis. Compared to adults, neonatal invasive candidiasis (IC) is associated with a high incidence and elevated mortality rates. It is suggested that the *Candida*-Nb interaction is unique and different from that of adults and should be considered to further understand the immunopathological mechanisms during the early period of life, as well as risk factors and local epidemiology. The aim of this review is to provide information related to IC during the neonatal period, as well as differences that exist in comparison with adults.

Key words: *Candida* spp. Invasive candidiasis. Premature newborns. Immune response.

Introduction

Neonatal invasive candidiasis (IC) occurs within the first four weeks of life¹. During the neonatal period, important physiological and anatomical changes occur in the development of the newborn (Nb). Furthermore, premature neonates are kept to the hospital environment or the neonatal intensive care unit (NICU) to be subjected to medical procedures to avoid or treat complications^{2,3}. These factors make neonates an ideal target to be infected by pathogenic microorganisms (bacteria, fungi, viruses, and parasites). Among fungi, *Candida* is the most common pathogen causing fungal infections in Nbs⁴. The genus *Candida* includes a varied number of species. However, 95% of all mycosis is caused by *Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis*, and *Candida krusei*.

Candida spp. is an opportunistic pathogen. It is naturally part of the skin and mucosa microbiota since it can grow, proliferate, and coexist without causing damage to the host in these sites. The transition from a commensal microorganism to an opportunistic pathogen is associated with alterations in the host environment (e.g., hematological or solid malignancies, neutropenia, organ transplantation, surgical procedures, or immunosuppressive therapy), as well as virulence factors of the fungus that allows it to invade and cause candidiasis⁵. This fungal infection presents a wide range of clinical manifestations such as (a) cutaneous candidiasis that especially affects skin and nails, (b) mucocutaneous candidiasis that causes lesions in the gastrointestinal, genital, and oropharyngeal mucosa, and (c) IC, which refers to the presence of *Candida* spp. in the bloodstream (candidemia) and can affect or spread to several host organs (disseminated candidiasis).

Correspondence:

*Gloria M. González

E-mail: gmglez@yahoo.com.mx

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Candida spp. has been positioned as the third most common pathogen causing bloodstream infections in pediatric patients (9.3% of isolates), exceeded by *Staphylococcus* spp. (55% of isolates) and *Enterococcus* spp. (9.4% of isolates)⁶; while in adults, *Candida* spp. is the sixth most common pathogen associated with systemic infections⁷. Despite the drastic increase in the incidence of non-*albicans* species, *C. albicans* is currently the main etiological agent of IC in pediatric and adult patients. Furthermore, the species distribution is specific to the age of the patient. *C. parapsilosis* is ranked as the second most common pathogen causing IC in Nb and infants, while *C. glabrata* is the second most common pathogen in adult patients, particularly elderly adults⁸. The species distribution during systemic infection is an important factor to consider since some species are associated with a better clinical prognosis compared to others. For example, *C. parapsilosis* is associated with a lower mortality rate compared to *C. albicans*⁹.

Nowadays, there is limited information related to host-*Candida* interaction during the neonatal period. Experimental models of neonatal candidiasis have been established to obtain a deeper understanding of this complex relationship and the differences that may exist with respect to models performed in adults. This review aims to provide information related to IC during the neonatal period and the differences concerning mycosis in adults including epidemiology, risk factors, and immunopathogenesis and finally make a compilation of experimental models of neonatal candidiasis.

Epidemiology

The annual incidence of IC varies between 72 and 290 cases per million⁴. It is estimated that this fungemia affects approximately 250,000 people worldwide every year and is the cause of around 50,000 deaths. Several surveillance programs have allowed a comparison of IC rates between regions and countries. European surveys (Denmark, France, Italy, and Spain) have reported incidences of 0.17-0.96/1000 admissions, similar to that observed in the United States (0.27-0.91/1000 admissions)^{4,10,11}, while Latin America reports higher incidence rates (1.66-2.49/1000 admissions). In Mexico, rates of 8.6/100,000 inhabitants have been reported¹². The incidence of IC increased by 52% between 2000 and 2005; however, in the United States, trends have decreased in neonates from 31.5 cases/100,000 births in 2009 to 10.7-11.8 cases between 2012 and 2015, possibly due to advances in health care^{4,11-13}.

In general, the highest incidence is observed at the extremes of age. In fact, neonates are the most vulnerable age group to this mycosis¹³⁻¹⁵, they represent approximately between 30% and 40% of the IC (Table 1). Although some risk factors of neonatal and adult patients are shared¹⁶, there are unique factors that predispose Nb to develop IC such as follows.

Preterm Nbs and low birth weight

The incidence of IC varies inversely with gestational age and birth weight (Table 2). In fact, premature Nb with extremely low birth weight (<1000 g) has 10 fold-more the risk of developing IC in comparison with term infants and adults¹⁷, possibly to the immaturity and inconclusive development of tissues (e.g., skin and mucosa), together with admission to the NICU^{18,19}.

Invasive procedures

Invasive procedures such as the use of catheters, mechanical ventilation, and/or parental nutrition are often used during the neonatal period to prevent or treat health complications in Nb²⁰. However, the use of these medical devices is a risk factor for the development of microbial infections since it implies favoring the entry of pathogens into the host (Table 2).

Candida spp. colonization and the use of antibiotics

The previous colonization of the skin and mucosa by *Candida* spp. is an important factor in developing IC in hospitalized patients. Nb colonized with *Candida* present a higher risk of developing a systemic infection compared to adults (Table 2). The use of antibiotics as a routine therapy during a stay in the NICU reduces the bacterial density of microbiota, favoring colonization, or increasing the density of *Candida* spp. in the body sites²¹. The use of broad-spectrum antibiotics, such as third-generation cephalosporins, is associated with a 2-fold increase in the development of IC in Nb, and the use of cephalosporins during the first 7 days of hospitalization is considered an important risk factor for the disease's development²².

Neonatal immune system

The immune system of the Nb has been considered an important risk factor for the development of IC because neonates exhibit certain differences in their immune

Table 1. Incidence, mortality rates, and attributable costs due to invasive candidiasis by age group around the world

	Patients < 1 year old	Patients > 15 years old	References
Global incidence (Number of cases/1000 hospital admissions)	0.43-160	0.3-50	4,10,11
Mortality rate	35-55%	10-45%	4,6
Length of hospital stay (Average days per patient)	64.5 days	18.6 days	6,11
Hospital costs (Average per patient)	276,500 Dollars	70,000 Dollars	4

Table 2. Comparison of the incidence of invasive candidiasis by groups of risk factors

	Percentage of cases	References
Age of gestation Newborns < 32 weeks Newborns 32-37 weeks Newborns > 37 weeks	60 20-25 15-20	15,17,32
Birth weight Extremely low weight (<1000 g) Low weight (1000-1500 g)	50-75 13-20	17,32
Use of medical devices Neonate Adult	55-70 40-45	11,18
Colonization by <i>Candida</i> Neonate Adults	20-30 5-15	16,21

response compared to adults³. The neonatal immune response is characterized by the preference to induce a humoral (Th2) and/or regulatory response (Treg)²³, which makes Nb an ideal target to be infected by microorganisms that require a cell-mediated immune response for their elimination, such as *C. albicans*.

Pathogenesis of the neonatal IC

During the first day of life, *Candida* spp. colonizes the skin and gastrointestinal mucosa of the host. Infants born by vaginal delivery are more likely to be colonized than infants born by cesarean section²⁴. Colonization by *Candida* is a crucial step for the establishment of a systemic infection; it has been shown that 25% of premature babies colonized by *Candida* develop IC, while only 5-10% of colonized adults develop fungemia (Table 2). This high susceptibility in premature Nb is probably due to immaturity and/or the different functions

of the barriers of the Nb immune system (Fig. 1) compared to adults, where the fungal infection in these sites occurs only under certain conditions^{25,26}.

Tissue invasion by *Candida* begins with the penetration of the fungus and damage to the host epithelium, which is facilitated by the production of virulence factors as proteases, phospholipases, and lipases capable of degrading proteins and the lipids of epithelial cells, altering cellular integrity. Certain species of *Candida* have the ability to produce hyphae, facilitating tissue invasion through or between epithelial cells²⁷. Once it invades the skin/mucosa, the epithelial cells activate the immune response by recognizing components of *C. albicans* cell wall through pattern recognition receptors (PRRs). As with the toll-like receptors (TLRs) expressed in epithelial cells, the ligand-receptor interaction induces an intracellular signaling that ends in the production of pro-inflammatory cytokines and chemokines by the epithelial cells for the recruitment of immune cells as neutrophils and macrophages to the infection site²⁸. However, preterm infants show marked differences in the functionality of PRRs such as downregulated intracellular signaling due to low phosphorylation of intracellular molecules, resulting in altered cytokine production compared to adults (Fig. 1). In addition, the neutrophils and monocytes of the neonates present decreased chemotaxis capacity²⁹. It is possible that these functional differences in Nb promote the establishment of candidiasis and its subsequent dissemination into the bloodstream.

Once *Candida* crosses the skin and mucosa, it reaches the host blood vessels and, with the help of its virulence attributes, spreads to different tissues. On the other hand, the development of IC in neonates and adults is facilitated by the accidental administration of *Candida* directly into the bloodstream by contaminated implantable medical devices³⁰. The principally affected organs in adult patients by the fungus are the heart,

Figure 1. Differences in the immune response during adulthood and the neonatal period. Immunological mechanisms involved in the recognition of *Candida* in adults (black) and newborns (red, the text in parentheses refers to the function of the immune system during the 1st day of life after the application of different bacterial stimuli).

(1) *Candida* colonizes the mucosa and the skin as yeast, under certain conditions invade in the form of hypha allowing it to penetrate the epithelial barriers (neonates present important differences in epithelial barriers compared to adults). (2) After the invasion, *Candida* is detected through pattern recognition receptors and induces the production of pro-inflammatory cytokines, which favors the recruitment of innate immune cells to the site of infection (decreased levels of cytokines and immune cells in neonates). (3) Invasion of *Candida* leads to the development of invasive candidiasis, the immune response during the invasive infection begins with the activation and opsonization of the complement (reduced activity in neonates). At the same time, the recruitment and activation of the innate cells initiate (different function in neonates). (4) The presentation of *Candida* antigens to T helper cells (reduced in neonates), results in differentiation in Th1/Th17 effective against the fungus and its subsequent elimination (polarization toward a Th2/Th2 response in neonates).

lungs, brain, spleen, liver, and kidney, among others. Meanwhile, in neonatal patients, the central nervous system is one of the main sites of dissemination, causing meningitis³¹. Neurological alterations such as cerebral palsy, alterations in psychomotor movement, and blindness are very common in pediatric patients who survive the IC³².

Immune response in the IC

After entry of the fungus into the bloodstream of the host, it activates the complement system, resulting in the

opsonization of the microorganism by deposition of C3b fragments and the release of C5a and C3a, which promote the recruitment of immune cells to the site of invasion³³. The anti-*Candida* activity of the complement system has been evidenced in mice deficient of C3 and C5 that were susceptible to IC³⁴. The arrival of neutrophils, macrophages/monocytes, and dendritic cells to the site of invasion is crucial for the control of fungemia, as well as the activation of the adaptive immune response and elimination of fungal structures. Monocytes in the bloodstream and macrophages in tissues recognize mannan and glucans from *Candida* cell wall through PRRs such

as TLR2, TLR4, Dectin-1, and Dectin-2, inducing the production of essential pro-inflammatory cytokines such as tumor necrosis factor- α , interleukin-1 beta (IL-1 β), and IL-6, which promote the recruitment and activation of neutrophils²⁸. Neutrophils are the main defense against *Candida* since they can inhibit the transition from yeast to hyphae. In addition, they can phagocytose and eliminate *Candida* intracellularly, mainly through oxidative mechanisms such as the production of reactive oxygen species. At the same time that neutrophils perform their functions, macrophages phagocytose and destroy the fungus, as well as modulate the adaptive immune response together with dendritic cells²⁸.

The adaptive immune response of T cells against *Candida* is diverse and depends on multiple factors such as the morphology of microorganisms that are recognized by the antigen-presenting cells, as well as the site of invasion. The Type 1 T helper (Th1) cells response is characterized by the production of interferon gamma (IFN γ), which is associated with the control and elimination of the fungus because this response potentiates the fungicidal activities of neutrophils and macrophages³⁵. Similarly, the response of Type 17 T helper (Th17) cells is crucial for the control of IC³⁶ through the production of cytokines such as IL-17 and IL-22, which induce the recruitment and activation of neutrophils. Therefore, the Th1 and/or Th17 cellular immune responses are associated with a protective response against IC. The Type 2 T helper (Th2) cells and/or immune regulatory response characterized by the production of IL-4 or IL-10, respectively, suppress the cellular immune response (Th1/Th17) and promote the proliferation of *Candida* and tissue invasion³⁷.

Regarding the neonatal immune system, most of the research focuses on evaluating the ontogeny and function of the immune system, as well as the immune response to other microbial infections, evidencing important differences compared to adults³⁸. These phenotypic and functional differences probably make Nb an ideal target for developing IC (Fig. 1). However, it is important to conduct studies focused on the immune response of Nb against *Candida* to describe the cellular and molecular mechanisms involved.

Prevention and treatment of IC in neonates

At present, there are several international guidelines for the clinical management of IC/candidemia; these guidelines propose that antifungal prophylaxis may be an appropriate strategy to prevent IC in the main risk

groups, especially for premature Nb with extremely low birth weight. Here, we highlight three prophylactic strategies^{13,14,39}.

Use of oral non-absorbable antifungals

Non-absorbable antifungals (nystatin and miconazole) are used to decrease the burden of *Candida* on the gut and, therefore, the probability of translocation into the bloodstream.

Administration of *Lactobacillus* and lactoferrin

The administration of *Lactobacillus casei* prevents the establishment of *Candida* spp. in the gut. Studies in which oral probiotics were administered from the 3rd day of life until either the end of the 6th week of life or until discharge from the NICU suggest that this approach prevents enteric colonization by *Candida* but has no impact on the overall incidence of IC. Lactoferrin is an alternative agent that may be effective through the abrogation of the invasive potential of *Candida* spp.

Fluconazole prophylaxis

Prophylactic administration of fluconazole (intravenous or oral) results in a reduction in *Candida* colonization and a decrease of approximately 40-50% of IC cases in the NICU. However, the prophylactic use of fluconazole increases the risk of the appearance of fluconazole-resistant strains and selection of non-*albicans* *Candida* species.

Other suggested prevention strategies are avoiding horizontal transmission in the NICU, treatment of maternal vaginal candidiasis before delivery, and rational use of broad-spectrum antibiotics (especially third-generation cephalosporins)³⁹.

International guidelines for the treatment of IC show discrepancies concerning antifungal therapy, but all agree that treatment depends on the patient's characteristics (Reviewed 14,39). These guidelines promote the use of amphotericin B formulations, echinocandins (micafungin and caspofungin), and fluconazole formulations for the treatment of neonatal IC.

Experimental models of neonatal IC

The development of experimental models that emulate human invasive infections has been a helpful tool for researchers, allowing them to explore the

mechanisms associated with pathogenesis, the immune response, therapeutic efficacy, and virulence factors of different strains.

Neonatal models have been developed where systemic infection can be established by the orogastric administration of *C. albicans* and other non-*albicans* species to mice (4-8 days old) and rats (4-10 days old), observing that gastrointestinal candidiasis persists for at least 30-40 days post-infection, which shows that neonatal animals are susceptible to gastrointestinal colonization without the need to use antibiotics or immunosuppressive agents⁴⁰⁻⁴². These neonatal models reveal that *Candida* is able to colonize the oral cavity, esophagus, stomach, and intestine of neonatal animals, causing superficial lesions characteristic of stomatitis (sores or ulcers). Furthermore, it has been detected from the first 12 h post-infection in several organs of neonates, highlighting the liver, spleen, and kidney as the principally affected tissues. On the other hand, the systemic infection has been established by intraperitoneal administration of *C. albicans* in rats (4-10 days old) and mice (2-4 days old). In both models, the animal survival, fungal burden, and histopathology of various tissues were evaluated^{43,44}. Animal mortality was dependent on the dose of yeast administered and occurred 72 h after infection. Spread to the spleen, liver, and kidney occurred in the first 24 h of infection, with the liver and spleen being the most affected tissues, with only some neonatal animals showing dissemination to the brain.

Different authors suggest that the standard model to emulate IC is to administer *Candida* directly into the bloodstream to mimic the candidiasis that occurs in pediatric patients with intravenous catheters or other medical devices, as well as reproduce the clinical course of the condition since medical devices are the main route for the development of neonatal IC. At present, two experimental models of intravenous infection have been developed in 2-4 days old rats and mice, inoculating *C. albicans* and *C. parapsilosis* through the external jugular vein^{40,45}. Both models showed that neonatal animals were highly susceptible to fungemia and died during the first 48 h of infection due to *Candida* dissemination to multiple tissues such as liver, brain, kidney, spleen, and lungs, with the neonatal liver being the most affected tissue. Thus, the use of these and additional experimental models may generate new knowledge related to invasive neonatal candidiasis and the development of timely diagnosis, as well as biomarkers for prognosis and new treatments against the disease.

Conclusion

We consider that the incidence of neonatal IC is due to multiple factors associated with Nb, which leads us to suggest that the neonate-*Candida* interaction is unique and may be different from its counterpart in adults. It is essential to understand the pathophysiology of this systemic infection during the neonatal period, as well as to know the roles of the various virulence factors of *Candida* spp. implicated in the pathogenesis of neonatal candidiasis. The use of experimental neonatal models is a potential tool to generate more information and to have a greater understanding of neonatal IC.

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Conflicts of interest

The authors declare no financial or commercial conflicts of interest. The authors alone are responsible for the content of this paper.

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Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

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