

Necrotizing enterocolitis and systemic candida infection in newborn with birth weight under 750 g.

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Abstract

Background: Recent literature shows the association between necrotizing enterocolitis (NEC), extremely low birth weight and fungal infection. Numerous studies reports very severe prognosis (100% mortality). Because of this we decided to analyze all babies with NEC. Our aim was to show the correlation between *Candida* species and gastrointestinal perforation in premature infants and to value the outcome.

Methods: We analyzed prospectively 41 infants admitted to the NICU and treated for invasive NEC from July 2006 to December 2017. Gender, gestational age, birth weight, perinatal asphyxia, umbilical catheter, intubation at birth, anti-fungal treatment, patent Botallo arterial duct, blood tests, urine, stool and blood cultures, bronchial washing, type of surgery and histological examination were evaluated in all patients.

Results: Of 41 patients 12 (29%) had a birth weight between 450 and 750 g and gestational age between 23 and 25 weeks. Of these 7 (58%) had systemic candidiasis. All these patients were intubated and submitted to gentamicin-ampicillin sublactam-fluconazole prophylaxis. In all 7 patients bowel resection and ileostomy were performed. All infants (100%) had blood culture positive for *Candida parapsilosis*; 2 patients (28%) presented co-infection with *Candida albicans* and 1 (14%) with *Candida tropicalis*. Spore of *Candida parapsilosis* were found in bowel lumen. 3 patients (43%) died for fungal sepsis and multi-organ failure.

Conclusion: Fungal sepsis is a source of significant morbidity and mortality in the newborn. Systemic candidiasis, especially by *Candida parapsilosis*, in ELBW with invasive NEC is a prognostic negative factor. So when early detected and appropriately treated the prognosis can be improved.

Keywords: Extremely very low birth-weight, *Candida parapsilosis*, Fungal sepsis, Ileostomy.

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Introduction

Necrotizing enterocolitis (NEC) is the most common gastrointestinal emergency in the NICU. The reported incidence of NEC varies between 1 and 15% of admissions to the neonatal intensive care unit (NICU). NEC is associated with high mortality and can led to multiple complications especially in the preterm under 1500 g [1,2]. This condition becomes more dangerous for neonates with a birth weight under 750 g, because of a premature intestine with decreased motility, absorption, mucosal integrity and incomplete immunological defenses [2,3]. The etiology of NEC remains largely unknown. Its pathogenesis is complex and involves impairment of multiple mechanisms. In 1975 Santulli was the first to describe, as factors responsible for

NEC, interaction among the intestinal bacteria, the injury to the intestinal mucosa and feeding. However the recent International Literature describes isolated cases of preterm patients characterized by NEC, with an extremely very low birth-weight and no bacterial but fungal cause.

Candida albicans is the most common fungal organism isolated in patient with NEC. Today also *Candida parapsilosis* is an emerging human pathogen and it is one of the main causes of invasive candidal disease. The population at greatest risk for infection with *C. parapsilosis* is the very and extremely low birth weight neonates (ELBW) [3-6]. The presence of intraluminal fungi with associated vascular occlusion may cause bowel ischemia, necrosis and perforation [3,6].

Therefore, based on our data, we think that there is a strong correlation between Candidemia, extremely very low birth-weight and a more aggressive and serious form of necrotizing enterocolitis.

Patients and Methods

From July 2006 to December 2017, 41 infants were admitted to the NICU of Siena and they were treated with surgical and/or medical approach for invasive NEC. We performed a prospective study, and we analyzed: gender, gestational weeks, birth weight, perinatal asphyxia, umbilical catheter, intubation at the birth, anti-fungal treatment, patent ductus arteriosus, blood tests (Thrombocytopenia and C-reactive protein) operative treatment and macroscopic findings of bowel at the surgery, urine, stool and blood cultures bronchial washing and histological features, with research of microbiological patterns. All patients were classified according to Bell's classification (Figure 1).

In particular we evaluated weight less 750 g, degree of inflammation, presence and degree of necrosis, presence of exudates and especially we research fungal microorganisms in perforated bowel and in the biological fluids.

Results

41 patients were submitted to surgical and/or medical treatment for invasive NEC. According to the data analyzed 12 patients (29%) had a birth weight between 450 and 750 g and gestational age between 23 and 25 weeks. According to Bell's staging criteria all 12 patients (100%) were at stage 3. 7 of these 12 patients (58 %) showed systemic candidiasis. 4 patients were female and 3 male. APGAR's scores were 3 at 1 min and all our 7 patients were intubated at the birth and treated with high frequency ventilation.

All patients were submitted to gentamicin-ampicillin-sublactam-fluconazole prophylaxis through venous catheter to avoid bacterial and fungal infection. They were also treated with ibuprofen for patent ductus arteriosus and 3 of them were submitted to surgery for persistence of ductus.

All patients presented with abdominal distension, blue discoloration of the abdominal skin, bile-stained gastric aspirate and metabolic acidosis. Abdominal x-ray showed intestinalis pneumatosis and free intraperitoneal air. Laboratory tests showed thrombocytopenia and instable

values in all cases. C-reactive protein showed variable value and it was never normal. Also it not related to the sepsis and the prognosis.

Because of severe condition of NEC it was necessary to treat the patients with surgical approach. Surgical findings included hemoperitoneum, intestinal necrosis, discoloration of the serosal surface and multiple bowel perforations in all the 7 patients (100%). Bowel resection and ileostomy were performed in all 7 patients. All patients (100%) had blood cultures, stool cultures and bronchial washing positive for *Candida parapsilosis*; 2 patients (28%) presented coinfection with *Candida albicans* and 1 (14%) with *Candida tropicalis* (Table 1). 4 babies were submitted to two surgery and 3 to three surgery for reappearance of intestinal Candidiasis although they were treated by fluconazole therapy. Histological examination of resected bowel showed intestinal necrosis at the site of perforation and chronic phlogosis of bowel wall with extensive surface exudates. Besides fungal invasion and spore of *Candida parapsilosis* there were in bowel lumen and in submucosa (Figure 2). 3 patients (43%) died for fungal sepsis and multiorgan failure: 1 patient with *C. parapsilosis* blood infection, 1 *C. parapsilosis* and *C. albicans* and 1 *C. parapsilosis* and *C. tropicalis* (Table 2). The autopsy of patient died for *C. parapsilosis* and *C. albicans* sepsis and surgically treated 3 times, showed multiple liver abscesses. Finally urine cultures and research of fungal pathology of babies' mothers were negative in all patients.

Discussion

NEC is a multifactorial process of the gastrointestinal tract of premature neonates that may result in inflammation of the bowel wall and in mucosal and/or transmural necrosis. Although the etiology of this disorder is unknown, International Literature shows clearly that NEC occurs primarily in infants born before 36 weeks gestational age. Incidence of NEC is about 1 in 1000 live births, but it occurs in up to 7% of very low birth weight infants. Indeed the immaturity of gut in premature represents an important contribution to pathogenesis of NEC for the absence of coordinated peristalsis that exposes the intestinal epithelium to a prolonged contact with potentially noxious substances. Immature mucous coats and improperly formed tight junction leads also to altered permeability and regeneration of the mucosa after an insult. Furthermore the immature immune system of the preterm neonate adds to an insult, like bacteria but also fungi, resulting in high risk of invasion and dissemination of the gut. In fact in the last years virulent properties of the fungus, especially

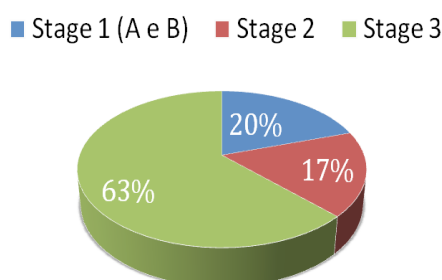


Figure 1. Classification according Bell's staging criteria

Table 1. Classification according blood culture

Blood culture	Percentage
<i>C. parapsilosis</i>	7 (100%)
<i>C. parapsilosis</i> + <i>C. albicans</i>	2 (28.5%)
<i>C. parapsilosis</i> + <i>C. tropicalis</i>	1 (14.2%)

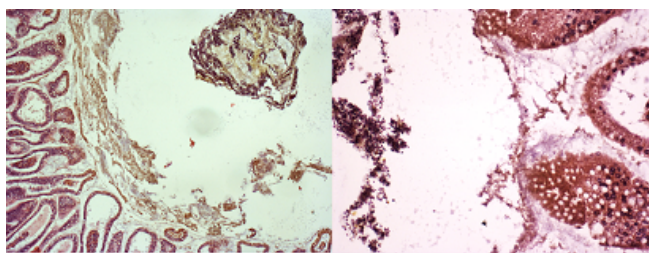


Figure 2. (A, B) Hematoxylin-and-eosin stained slide showed spores of *Candida parapsilosis* in the bowel lumen and wall

Table 2. Findings of patients with NEC

Patient	Birth Weight (g)	Infection by <i>C. parapsilosis</i>	Other Infections	Number of Surgery	Localization Perforation	Survival
1	680	Yes	No	3	Term ileum, colon	Yes
2	450	Yes	<i>C. albicans</i>	2	Term and Mid ileum	No
3	720	Yes	<i>C. albicans</i>	2	Term ileum	Yes
4	640	Yes	No	2	Term ileum	Yes
5	470	Yes	<i>C. tropicalis</i>	3	Term ileum	No
6	450	Yes	No	2	Term ileum	No
7	650	Yes	No	3	Term and mid ileum	Yes

of *Candida*, were described in Literature as responsible of intestinal damage and also of NEC [1,2,7]. The first neonatal *Candida* peritonitis with multiple perforations was described by Johnson in 1980. Today *Candida* incidence is increasing and in Literature there are some Authors that describe intestinal candidiasis like a new cause of NEC and spontaneous intestinal perforation (SIP). Normally, *Candida* species are commensal organisms colonizing skin, gastro-intestinal and female genito-urinary tract but they become opportunistic pathogen responsible of fungal sepsis and fatal complications in extremely low birth weight neonates (ELBW) [8,9]. According to Literature our 7 patients of 12 with ELBW (58%) have blood, stool culture and bronchial washing positive for *Candida*.

Candida ability to form filamentous (hyphae) explains its pathogenic mechanism. In fact it can adhere to the skin, mucosal and catheter surfaces as commensal organisms, with damage of the skin or the mucosal membranes. So the presence of hyphae of *Candida* in the bowel produces endothelial damage, blood stagnation, platelet aggregation and it causes a necrosis of gut with very particular features. The phlogosis of bowel wall, like confirmed from histological examination, is chronic with extensive surface exudates around the site of perforation. *Candida* is present in bowel lumen but also in the submucosa showing to be directly responsible of NEC. Also *Candida* may seeds tissues and forms abscesses which are difficult to eradicate (1). So according to Authors, all our patients (100%) showed these histological features. Over the past decade, today the main *Candida* species responsible of intestinal colonization is *Candida parapsilosis*. Our data confirmed this trend; indeed *Candida parapsilosis* was identified in all patients (100%). Furthermore, 2 patients

(28%) presented co-infection with *Candida albicans* and 1 (14%) with *Candida tropicalis*. Obviously, the co-presence of different species of *Candida* worse the prognosis of the patients like our data confirms. About mortality of NEC, it is major of 30% in the patients with ELBW but it became of 100% when there is also intestinal Candidiasis. In our retrospective analyzes we report mortality of about 50%. *Candida parapsilosis* is able to perform biofilms on devices used in NICU (endotracheal tube, umbilical artery catheterization, bladder catheterization) and may contaminate glucose-containing solutions such as parenteral nutrition mixtures. However, prolonged and multiple antibiotic and corticosteroid treatment, typical hyperglycemia of preterm newborns, pharmacologically decreased gastric acid and immature immune system, can increase the risk of Candidiasis with bowel damage [6-9]. *Candida* Species, often, have got a vertical transmission from mother to child; instead, *Candida parapsilosis* has got horizontal transmission. In fact it cannot be accounted in maternal isolated and the major vector is the hands of health workers. According to the Literature, none of the mothers of our patients showed fungal infection by vaginal buffers. For these reasons *Candida parapsilosis* often is responsible of nosocomial infection. To confirm this, a study performed about NICU of Pisa in 2002, showed that the same genetic type of *Candida parapsilosis* isolated in newborn there was on hands of two nurses of NICU.

Finally about the fluconazole prophylaxis the major of authors advice the routinely use for the patients with ELBW. In our experience 7 patients of 12, equal to 58%, developed a *Candida* sepsis despite fungal prophylaxis. Despite our data however, we think that this therapy must be always used for these patients [10-12].

Conclusion

Our results and review of Literature support the idea that *Candida*, particularly *Candida parapsilosis*, promotes inflammation and invasion of immature enterocytes and that fungal-enterocyte interaction should be considered in models of gastrointestinal inflammation and so of NEC.

Also, we think that there is a strong correlation between *Candida parapsilosis*, ELBW and more aggressive and serious NEC. For these reasons, a strong collaboration between neonatologist and pediatric surgeon is able to avoid Candidiasis intestinalis in NICU. So we confirm that the management of patients with EBLW should provide: feeding of prebiotics and probiotics (lactobacillus), fluconazole prophylaxis, acidification of feeds, and to decrease the use of devices and antibiotic therapy, if possible. However the proper education of health staff about hygiene during the management of these patients is certainly the main ally to decrease Candidiasis in NICU.

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