Impact of Late-Onset Sepsis on Neurodevelopmental Outcome in Premature Infants: Strategies to Prevent It

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1 Introduction

The increased survival of Extremely Low Birth Weight (ELBW) infants has heightened awareness of the importance of assessing and improving long-term outcomes associated with prematurity. It is estimated that almost 15% of the most immature infants develop cerebral palsy (CP) and approximately half of them develop cognitive and behavioral deficits (Vohr et al., 2000).

Infections are frequent complications among ELBW preterm infants. They are associated with significant morbidity rates, including neurological outcome besides to short-term sequelae and increased risk of death (Stoll & Hansen, 2003). Few studies only reported data on neurodevelopmental follow-up, confirming that preterm infants with a history of sepsis show worse neurodevelopmental outcome compared to control group in early infancy, involving mental and psychomotor retardation, vision and hearing impairment and cerebral palsy (Stoll et al., 2004). Furthermore, differences in the outcome according to sepsis pathogens were reported and infants with Gram-positive sepsis had the poorest neurodevelopmental outcomes (Stoll et al., 2002). Sepsis may lead to poor neurodevelopmental outcomes by several mechanisms as: cytokine effects on cerebral hemodynamics with white matter injury, cerebral ischemia reperfusion injury after arterial hypotension, biological immaturity of preterm infants and vulnerability to brain injury during sepsis (Schlapbach et al., 2011). Prompt diagnosis and effective treatment do not protect septic neonates from the risk of late neurodevelopmental impairment. Thus prevention of bacterial and fungal infections is crucial in these settings of unique patients and it is related to the promotion of breastfeeding and hygiene measures, to the adoption of a cautious central venous catheter policy, to the enhancement of the enteric microbiota composition with the supplementation of probiotics and to the medical stewardship concerning H2 blockers. Additional measures may include the use of lactoferrin, fluconazole, nystatin and specific measures to prevent ventilator associated pneumonia (Manzoni et al., 2013).

The oral administration of probiotics reduces the incidence of necrotizing enterocolitis (NEC) in preterm infants, by inhibition or reduction of inflammatory signaling in the intestinal epithelia. But the limited number of clinical trials results in lack of definition of optimal strains, timing, dosage and duration of probiotics administered to VLBW (very low birth weight) preterm infants (Lin et al., 2013). Few studies report on the role of probiotics, used for the prevention of NEC, in neurodevelopmental outcome of preterm infants with sepsis. Sari (Sari et al., 2012) concludes that, although oral probiotics given to preterm infants reduce the incidence of NEC, they do not affect growth, neurodevelopmental and sensory outcomes between 2 and 3 years corrected age. One study (Romeo et al., 2011) reported a different neurodevelopmental outcome in a population of preterm infants between those treated with probiotics and those having no supplementation. The same affirmation is showed in Hunter’s study (Hunter et al., 2012). However these studies showed differences in term of based population, timing, dosage and duration of probiotics treatment and a clear comparison is difficult to make.

In the present chapter we wish to determine correlation between neonatal infections and adverse neurodevelopmental and growth results in early childhood, to suggest that a close neurological follow-up, especially during the first 12 months of life, is necessary for infants with a diagnosis of sepsis. We would also specifically explore the role of probiotics in the prevention of late-onset sepsis, of gastrointestinal colonization by Candida spp. and in the protection against fungal infections and their ability in reducing the risk of adverse neurological and growth sequelae in early infancy (12 months).
2 Intestinal Microbiota in Newborn

The normal human microflora is a complex ecosystem that somehow for establishing colonization depends on enteric nutrients, but also on the mode of delivery and on a possible administration of antibiotics. At birth, the digestive tract is sterile. Diet and environmental conditions can influence this ecosystem (Hammerman et al., 2004).

At birth intestinal colonization is derived from organisms in the birth canal and maternal faecal microflora. The microbial imprinting depends on the mode and location of delivery. Literature data shows that infants born in a hospital environment, by caesarean section, have a high component of anaerobic microbial flora (Clostridia) and high post of Gram-negative enterobacteria. Those born prematurely by vaginal delivery and breast-fed have a rather rich in Lactobacilli and Bifidobacteria microflora (Grönlund et al, 1999; Hall et al, 1990). Diet can influence the microbiota, while the breast-feeding promotes an intestine microbiota in which Bifidobacteria predominates, indeed in formula bottle-fed baby coliform, enterococci and bacteroides predominate. Escherichia coli and Streptococcus are among the first bacteria to colonize the digestive tract. Subsequently, strict anaerobes (Bacteroides, Bifidobacteri, Clostridium) are established during the first week of life, when the diet plays a fundamental role (Mackie et al, 1999). The pattern of bacterial colonization in the premature neonatal gut is different from the one of the healthy, full term infant gut. Aberrant preterm infants admitted to NICU, born by caesarean section, are more often separated from their mother and kept in an aseptic intensive care setting and treated with broad-spectrum antibiotics. This is the reason why they show a highly modified bacterial flora, consisting of less than 20 species of bacteria, with a predominance of Staphylococcus (Aureus and Coagulase negative) among aerobic microorganisms, and Enterobacteriaceae (Klebsiella), among enterococci and anaerobic Clostridia (Dai et al, 1999; Gothefors, 1989). It is believed that microbial diversity is an important factor in determining the stability of the ecosystem and that it is the fecal loss of diversity that predisposes the preterm gastrointestinal colonization of antibiotic-resistant bacteria and fungi with a consequent potential risk of infection and contribute to the development of necrotizing enterocolitis (Fanaro et al, 2003).

2.1 Necrotizing Enterocolitis (NEC)

NEC is a serious anoxic and ischemic disease affecting almost the ileo-colic area of premature newborns. It is characterized by bacteria proliferation and production of gas inside gastric walls (cystic pneumatosis) associated with edema and inflammation. Its incidence rate is 1-3 cases per 1000 newborns, with a mortality rate ranging between 10-50 %. The prematurity is the most important risk factor, as well as the low birth weight (<1500 g). This risk increases after the colonization or the infection of pathogens such as Clostridium, Escherichia, Klebsiella, Salmonella, Shigella, Campylobacter, Pseudomonas, Streptococcus, Enterococcus, Staphylococcus Aureus and coagulase negative Staphylococcus. Other factors that can increase its incidence are the intestinal immaturity, the decrease of the intestinal motility, the increase of permeability to macromolecules and an excessive volume of milk. Certainly breast feeding represents a protective factor, as it is shown by the decreased incidence of NEC in breast-fed infants. Moreover literature data supporting the benefits of probiotics are increasing in the last decades. The role of intestinal micro-organisms has been largely described, even if it is still not clear. Advances in molecular biology and intestinal microbiology allow a better characterization of the intestinal microbiota in children affected by NEC. Nowadays, literature data describes different methods of characterization of the microbial genotype and of identification of its genes, expression of the specific proteins and production of metabolites
(Deshpande et al. 2007). The application of these techniques on bioptic samples of infected and non-infected subjects could improve the comprehension of the persistence of NEC in premature newborns. Deshpande in 2010 published a meta analysis of 11 trials (N= 2176) which confirms the benefit of probiotic supplements in reducing NEC, death and disease in preterm newborns. Furthermore in this study the dramatic effect sizes, the tight confidence intervals, extremely low P values and overall evidence suggest that additional placebo-controlled trials are unnecessary if a suitable probiotic product is available (Desphande et al., 2010).

There is a correlation between NEC and neurodevelopmental impairment in newborns. A recent study of Shah (Shah et al., 2012) proves that the mental developmental index (MDI) and the psychomotor developmental index (PDI) values of the Bayley’s scale were significantly greater among infants without NEC than those with NEC. Even after adjustment for use of antenatal glucocorticoids, birth weight, gender, race, surfactant therapy, intraventricular hemorrhage (IVH), bronchopulmonary dysplasia (BPD), sepsis and postnatal steroid treatment, MDI and PDI values remained significantly different between no NEC and NEC groups. NEC provokes neurodevelopmental impairment by several mechanisms. Some of them are mediated by inflammatory cytokines and bacterial products, others by vasodilatation and arterial hypotension that may cause cerebral ischemia and reperfusion injury in an immature brain (Figure 1 g-h). As literature actually says we can conclude that the diagnosis of stage II or greater NEC is associated with an increased risk of adverse long-term neurodevelopmental outcome in preterm ELBW infants.

3 Neonatal Sepsis

Sepsis is the response of the organism at the invasion of tissues, fluids or body cavity by pathogens or potentially pathogens microorganisms. Clinical signs associated with sepsis are temperature instability, irritability, apathy, feeding difficulties, prolonged capillary refill, apnea, tachycardia and tachypnea; laboratory signs of sepsis are elevated C-reactive protein, left shift or leucopenia. Proven sepsis is defined as a positive result on one or more bacterial or fungal cultures in an infant with clinical and/or laboratoristic signs of infection. Suspected sepsis is defined by the presence of clinical and/or laboratoristic signs of infection without any positive bacterial or fungal culture. A sepsis is defined early-onset (EOS) if it occurs at \( \leq 72 \) hours of life; while it is late-onset (LOS) when it occurs at \( >72 \) hours.

3.1 Bacterial Infections

Bacterial infections continue to cause a high burden on neonatal morbidity and mortality (Stoll et al., 2003). Approximately 1.5 % of VLBW infants suffer from early-onset sepsis and about 36 % from late-onset sepsis, with mortality rates ranging from 10 % to 30 %. Mortality is higher in African-American preterm infants (24.4 %) (Shane & Stoll 2013).

Group B streptococci cause 38 % of early-onset sepsis and they are followed by Escherichia coli (24%). In VLBW infants Gram-negative pathogens are isolated most frequently than in full-term newborns and they are correlated with an increased risk of death (Hornik et al., 2012).

Gram-positive organisms cause 70 % of late-onset infections. Of these, coagulase-negative Staphylococcus is the most common pathogen (48 % of all infections) and it is followed by Staphylococcus aureus (10 %). Enterococcus spp. causes the 3 % of late onset sepsis, group B Streptococcus the 2 % and Gram-negative organisms the 18 %. The most frequent are Escherichia Coli (5 %), Klebsiella (4 %),
Pseudomonas (3 %), Enterobacter (2.5 %) and Serratia (2 %). Sometimes more than one organism can be found (Stoll et al, 2002).

Neonatal risk factors of early onset sepsis are prematurity, low birth weight, male gender and the presence of other neonatal disease such as respiratory distress, asphyxia, congenital immunodeficiency disorders or congenital metabolic disorders. Maternal risk factors of early onset sepsis are rupture of membrane greater than 18 hours, maternal fever during labor or intra-amniotic infections, maternal drug addiction, group B streptococcal (GBS) vaginal colonization (Shane & Stoll 2013). The universal screen for GBS colonization during pregnancy and the recommendation for intra-partum antibiotic prophylaxis (IAP) in colonized women reduced significantly the rates of early-onset GBS sepsis (Boyer & Gotoff, 1986).

Late-onset sepsis complicate the extreme prematurity, they usually have slower onset and course, lower mortality but they more often involve the nervous central system (NCS). The risk of late-onset infections increases with decreasing birth weight and gestational age, with prolonged hospitalization and with the protracted use of central venous catheters (Stoll et al 2002).

Initial clinical manifestations of sepsis are nonspecific and they can be associated with complications such as respiratory distress, disseminated intravascular coagulation (DIC), necrotizing enterocolitis (NEC), persistent pulmonary hypertension of the newborn (PPHN) or septic shock. Group B streptococcal is often responsible of fulminant sepsis, Gram-negative organisms instead are often associated with an early endotoxin-associated septic shock while coagulase-negative Staphylococcus is the most common pathogen associated with a more insidious course of the sepsis. Symptomatic newborns, with or without risk factors, without any non-infectious cause that can explain the set of clinical signs, should undergo a careful physical examination, blood tests and cultures and should initiate empirical antibiotic therapy (Stolfi & Pedicino, 2009). The studying of the epidemiology of pathogens responsible of sepsis can be useful to identify infants with possible infection and to select appropriate empiric antimicrobial therapy while waiting a targeted therapy (Shane & Stoll, 2013).

### 3.2 Fungal Infections

Fungal infections (primarily Candida spp.) have become increasingly important as causal agents of infection in preterm infants. They currently are the third most common cause of late-onset sepsis in VLBW preterm infants at the NICU, with an estimated incidence of 1.6 – 9 % in VLBW infants, but up to 15% in ELBW, and with a crude mortality rate around 30 – 75 %. Candida Albicans has been and still is the species most frequently isolated, although Candida parapsilosis and other species of Candida are more often reported as a cause of invasive candidiasis. Clinical manifestations of systemic candidiasis may not be specific, especially in an infant already in critical condition, moreover blood cultures were positive just in 50% of the patients with invasive fungal disease (IFI) (Silva et al., 2009).

Among the known risk factors, colonization by Candida spp. is the most important predictor of IFI. This association between colonization and infection has been proven for all sites that can be monitored with serial cultures. In particular, previous colonization can often be found before any IFI, if properly investigated. It is estimated that about 60 % of VLBW infants can be colonized during the first month of life in the NICU, and many of these can be infected after being colonized. In this regard, in 1986 Baley calculated that out of 100 VLBW infants in NICU, 33 developed fungal colonization, and 7 of them proceeded to IFI (Baley et al., 1986).

Of all the sites of colonization, the gastrointestinal tract (investigated with cultures from throat swabs, gastric aspirates, fecal or rectal swabs) seems to be the one with the highest predictive value for
subsequent dissemination in case of colonization. The fungal colonization at different levels of the intestinal tube is a well-known risk factor for subsequent dissemination and systemic fungal disease in preterm infants. The positivity of the rectal swab was taken by some authors as an expression of fungal colonization in toto (Pappu-Katikaneni et al. 1990). Moreover, the preterm baby in NICU is highly at risk for intestinal microecology disorders with proliferation of pathogenic microflora (including fungi). This is due to the fact that the infant undergoes to long-term treatments with broad-spectrum antibiotics, and often has difficulties in establishing and maintaining the oral feeding. In these special patients, the enteric tube is considered the most important reservoir and site of colonization for all types of pathogens and it is also considered as the site from which most frequently a low systemic fungal dissemination can start.

It has been shown that you can sometimes prevent the incidence of IFI by reduction of intestinal fungal colonization. Therefore research has focused its efforts towards the identification of the optimal prophylactic strategy especially in premature infants. However, at date, there is still no clear indication: prophylaxis with antifungal agents in fact still raises concerns about the tolerability and about the potential selection of resistant strains (Manzoni et al. 2013).

4 Pathogenesis

The body’s response to infections is the pathophysiological basis of sepsis (Hotchkiss & Karl, 2003). Severe sepsis is the syndrome due to the complication of the organ failure in sepsis (Skrupky et al. 2011). The theory of an uncontrolled immunitary response is not enough to explain the pathophysiology of sepsis. Many animals studies where guinea-pigs died from “cytokine storm” and where the administration of drugs that block these mediators improved survival, do not reflect the clinical pictures in humans (Flink & Heard, 1990; Deitch, 1998). This is due to a revaluation of the fundamental meaning of the pathophysiology of sepsis. Cytokines, such as TNFα and IL1, have damaging effects in sepsis but also beneficial ones (Hotchkiss & Karl, 2003): the use of TNF or IL1 antagonists in fact is often associated with a higher mortality except for specific subgroups of patients (Fisher et al, 1996). Other news about pathophysiology of sepsis gather from recent discovered about toll like receptors (TLRs). These receptors exist to serve as an early warning of infection and to help mount an expeditious response. It is possible that in selected individuals down modulation of this pathway may be advisable but it must be done in a graded fashion and perhaps only after the initial immune response has been activated (Skrupky et al. 2011).

Although there is still considerable debate, a growing consensus is that sepsis initiates both a pro- and anti-inflammatory response that begin rapidly after life threatening infection. Although both, pro- and anti-inflammatory processes, begin promptly after sepsis onset, in general there is predominance of an initial hyperinflammatory phase whose magnitude is determined by a number of factors including pathogen virulence, bacterial load, host genetic factors, and host comorbidities. Sepsis can be considered a race to the death between invading pathogens and the host immune response where pathogens seek an advantage by disabling selected aspects of host defenses including inducing apoptotic death of immune cells, decreasing monocyte major histocompatibility complex class-2 expression, increasing expression of negative co-stimulatory molecules, inducing anti-inflammatory cytokines production, and increasing suppressor cells (Skrupky et al. 2011). This could explain the results of the Hotchkiss’s autopsy study that shows a discordance between histologic findings and the degree of organ dysfunction in patients who died from sepsis (Hotchkiss et al 1999). Skrupky et al. in their work of 2011 affirm that much organ dysfunction in patients with sepsis can be explained by “cell hibernation” which results from defence mech-
anisms reducing cellular processes to basic roles. To date, although occasionally a patient with sepsis may die of refractory shock, more frequently the exact cause of death remains elusive even after the autopsy (Hotchkiss & Karl, 2003).

4.1 Sepsis May Lead to Poor Neurodevelopmental Outcome by Several Mechanisms

Preterm infants are at risk for infection throughout their hospitalization with various long-term complications, including adverse neurodevelopmental (ND) outcome (Adams-Chapman, 2012). A study on extremely premature infants born earlier than 28 weeks of gestational age, shows that proven sepsis independently increases the risk for poor neurodevelopmental outcomes at 2 years of age. The presence of sepsis, BPD, brain injury, and retinopathy of prematurity (ROP) is highly predictive of adverse outcomes. Sepsis is among the 4th main risk factor influencing long-term outcomes in this population, together with BPD, brain injury, and ROP. All of these have a greater impact on the outcome than the gestational age, the birth weight and the gender (Schlapbach et al., 2011). Mechanisms by which sepsis can lead to poor neurodevelopmental outcome are:

1. **Bacterial products and the cytokine storm.** Pathogens stimulate the dendritic cells which induce TCD4+ cells to produce pro-inflammatory cytokines, including TNFα and IFNγ, and to activate B lymphocytes to produce specific antibodies. Pathogens also activate the NFkB gene in macrophage with the production of TNFα, IL1 and IL10. In the course of the systemic inflammatory response syndrome, this cytokine storm can directly damage the highly vulnerable premature brain (Wu & Colford, 2000) and other organs, such as the lung and retina. Additional support for this hypothesis comes from magnetic resonance imaging studies demonstrating white-matter injury associated with bacterial infections and NEC in premature infants (Shah et al., 2008). In accordance, the recent study by Martin, reports a higher risk of neurodevelopmental dysfunction and microcephaly in NEC infants with late bacteremia (Martin et al., 2010). Of note, postnatal infections result in increased white-matter injury in premature infants (Chau et al., 2009).

2. **Arterial hypotension during sepsis may cause cerebral ischemia-reperfusion injury.** Because of varying institutional practices in the definition and treatment of hypotension (Barrington, 2007). Arterial hypotension is mediated by pro-inflammatory cytokines and by the activation of neutrophils that increased vascular permeability and by the induction of nitric oxide synthase (iNOS) with the subsequent increase of the nitric oxide (NO).

Moreover sepsis may be an indicator of disease severity in extremely preterm infants. For example, prolonged mechanical ventilation is associated with a higher sepsis risk (Stoll et al., 2002). Susceptibility to sepsis may also reflect the biological immaturity. These developing brain of these infants may be more vulnerable to injury (Figure 1 g-h).
Figure 1: Role of probiotics in decreasing the incidence of necrotizing enterocolitis (NEC) (g.), sepsis (h.) and neurological impairment in preterm newborns. Probiotics (green sticks) can prevent NEC or sepsis by several mechanisms: a. Forming a physical barrier against the pathogens (brown sticks). b. Stimulating the Goblet Cells to produce a barrier of mucus (blue rounds). c. Reinforcing the apical tight-junctions of the enterocytes. d. Producing antimicrobial factors which kill pathogenic microorganisms. e. Stimulating the innate immune system by signaling dendritic cells. f. Preventing or triggering an innate immune response by initiating TNF production by epithelial cells and inhibiting (or activating) NFκB in macrophage and dampening (or priming) the host immune response. 1 g-h During NEC (g.) or sepsis (h.) neurological damage can be caused by the direct action of pathogens and their products; by the cytokine storm.

5 Neonatal Sepsis and Neurodevelopmental Outcome

Pre and postnatal infections in preterm infants represent a risk for inflammatory-mediated white matter injury (WMI) to the immature brain, with possible neurobehavioral impairments (Shah et al., 2012). These brain abnormalities are seen even when the infection is only clinical without positive cultures. But in these cases only serial quantitative brain imaging could detect the impact of postnatal infection at term-equivalent age, suggesting an impairment of the brain in developing (Chau et al., 2009).

Shah et al. demonstrated that sepsis/NEC in preterm infants is associated with a higher prevalence
and severity of WMI on MRI at term-equivalent age and a delayed cognitive and motor development at 2 years of age (Shah et al., 2008).

So far, the largest study to date to evaluate the impact of neonatal infection on adverse outcomes in early childhood was conducted on more than 6,000 preterm newborns with infection and followed at 18-22 months of corrected age, in which 41% of infected infants (any clinical sepsis, bloodstream infection, or meningitis) and 57% of infants with fungal sepsis had at least one adverse neurodevelopmental outcome. The prevalence of adverse neurodevelopmental outcome in infants with fungal sepsis (Stoll et al., 2004) were as follows:

- Mental developmental index of less than 70 - 34%
- Psychomotor developmental index of less than 70 - 24%
- Cerebral Palsy - 18%
- Visual impairment - 14%
- Hearing impairment - 5%

On the other hand, in a recent analysis, the Swiss Neonatal Network evaluated neurodevelopmental outcomes of preterm infants with a GA of 24-27 weeks at 2 years of age. 25% percent of these infants had culture-proven sepsis, which was independently associated with an increased risk of cerebral palsy. (Schlapbach et al., 2011)

Thus, rigorous neurodevelopmental follow-up of these high-risk newborns is needed, to early identify those infants at risk of ND outcome and possibly reduce the risk of neurological impairment (Romeo et al., 2013). The identification of differences and variations in development of preterm infants should be interpreted cautiously, as it could identify infants as having problems when their developmental course is simply different (Rosenbaum et al., 2006). Therefore finding appropriate instruments for the assessments of these infants is crucial not only for individual patient but also for families and society; furthermore the possibility for an early identification of neurodevelopmental delay implies an early intervention with beneficial effects on development.

Body’s response to infections is the pathophysiological basis of sepsis (Hotchkiss & Karl, 2003). Severe sepsis is the syndrome due to the complication of the organ failure in sepsis (Skrupky et al. 2011). The theory of an uncontrolled immunitary response is not enough to explain the pathophysiology of sepsis. Many animals’ studies where guinea-pigs died from “cytokine storm” and where the administration of drugs that block these mediators improved survival, do not reflect the clinical pictures in humans (Flink & Heard, 1990; Deitch, 1998). This is due to a revaluation of the fundamental meaning of the pathophysiology of sepsis. Cytokines, such as TNFα and IL1, have damaging effects in sepsis but also beneficial ones (Hotchkiss & Karl, 2003): the use of TNF or IL1 antagonists in fact is often associated with a higher mortality except for specific subgroups of patients (Fisher et al., 1996). Other news about pathophysiology of sepsis gather from recent discovered about toll like receptors (TLRs). These receptors exist to serve as an early warning of infection and to help mount an expeditious response. It is possible that in selected individuals down modulation of this pathway may be advisable but it must be done in a graded fashion and perhaps only after the initial immune response has been activated (Skrupky et al. 2011).

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an initial hyperinflammatory phase whose magnitude is determined by a number of factors including pathogen virulence, bacterial load, host genetic factors, and host comorbidities. Sepsis can be considered a race to the death between invading pathogens and the host immune response where pathogens seek an advantage by disabling selected aspects of host defenses including inducing apoptotic death of immune cells, decreasing monocyte major histocompatibility complex class-2 expression, increasing expression of negative co-stimulatory molecules, inducing anti-inflammatory cytokines production, and increasing suppressor cells (Skrupky et al. 2011). This could explain the results of the Hotchkiss’s autopsy study that shows a discordance between histologic findings and the degree of organ dysfunction in patients who died from sepsis (Hotchkiss et al. 1999). Skrupky et al. in their work of 2011 affirm that much organ dysfunction in patients with sepsis can be explained by “cell hibernation” which results from defence mechanisms reducing cellular processes to basic roles. To date, although occasionally a patient with sepsis may die of refractory shock, more frequently the exact cause of death remains elusive even after the autopsy (Hotchkiss & Karl, 2003).

6 Prevention is Possible

Prompt diagnosis and effective treatment do not protect septic neonates from the risk of late neurodevelopmental impairment. Thus prevention of bacterial and fungal infection is crucial in these patients. Prevention strategies are based on the promotion of breast-feeding and of hygiene measures, the adoption of a cautious central venous catheter policy, the restriction of the use of H2 blockers or of proton pump inhibitors and the enhancement of the enteric microbiota composition with the supplementation of probiotics. Additional measures may include the use of lactoferrin, fluconazole, and nystatin and specific measures to prevent ventilator associated pneumonia (Manzoni et al. 2013).

6.1 Prophylaxis of Bacterial Mediated Sepsis

Preterm newborns at risk for sepsis, either because of clinical signs and symptoms or pregnancy-related risk factors, must be submitted to empirical antibiotic therapy. Before the 72nd hours of age the combination between ampicillin and aminoglycoside is recommended (Polin RA, 2012). Cephalosporins should be never used as empiric therapy except if gram-negative meningitis is suspected. They in fact can due the development of multi-resistant gram negative bacterial strains and of candidiasis (Manzoni et al. 2011). Among third-generation cephalosporins, ceftriaxone interferes with the bilirubin-albumin binding so the use of cefotaxime is preferred (Shane & Stoll 2013). Antimicrobial therapy must be reassessed as soon as culture results became known. Duration of therapy recommended is of 10 days in case of bacteriemia without a focus, while it is prolonged to 21 days in case of meningitis. In suspected sepsis with negative cultures, optimal therapeutic strategy is unknown. Some studies recommend to prolong antimicrobial therapy until clinical normalization (Stolfi et al. 2009) but several others describe the association between antimicrobial treatment for greater than 5 days in newborns with culture-negative clinical sepsis with increased risk of death or NEC (Shane & Stoll, 2013; Kuppala et al, 2011). If there is no clinical evidence of infection and if any organism is not isolated, the antibiotic therapy can be discontinued after 48-72 hours (Stolfi et al. 2009). Intra-partum antibiotic prophylaxis (IAP) with ampicillin in women, who are colonized by GBS, reduced significantly the rates of EO GBS sepsis but it does not prevent LOS (Boyer & Gotoff, 1986).
6.2 Prophylaxis of Fungal Mediated Sepsis

Because of the high mortality rate and the neurodevelopmental impairment associated with fungal sepsis in VLBW infants, prevention with nystatin, miconazole, and fluconazole has been studied in the highest-risk patients. Manzoni and colleagues in Italy published the results of their multicenter, randomized, placebo-controlled trial investigating two different fluconazole doses (3 mg/kg and 6 mg/kg) compared with a placebo group. In 322 infants who weighed less than 1500 g, investigators reported a significant difference in the incidence of fungal infections between the fluconazole prophylaxis groups compared to the placebo patients. There were no significant differences between the 3 mg/kg and the 6 mg/kg group (Manzoni et al., 2007).

Fluconazole is an excellent drug for prophylaxis because of its long half-life, high tissue concentration, low lipophilicity, and low protein binding. The one concern with fluconazole prophylaxis is the potential for the emergence of resistance over time, and this issue is under further study. Dosing with 3 mg/kg twice weekly is effective and limits exposure, cost, and potential adverse effects. When initiated around birth, prophylaxis should be administered for 6 weeks or less in patients with a birth weight of less than 1000 g or less than 6 weeks if intravenous access is no longer needed. For patients with a birth weight of more than 1000 g, prophylaxis must be continued until intravenous access is no longer needed and until adequate enteral feedings are achieved (Manzoni et al., 2007).

6.3 Probiotics: a Viable Weapon

Neonatal infections are frequent complications of ELBW infants receiving intensive care. The most valid indication of the probiotic remains the decrease of intestinal infections. In fact, the literature shows that the probiotic can reduce the severity and number of episodes of diarrhea.

6.3.1 Mechanism of Action

Probiotics can prevent sepsis by several mechanisms:

- forming a physical barrier against the pathogens (Figure 1 a).
- stimulating the Goblet Cells to produce mucus that forms a mechanical barrier (Figure 1 b).
- reinforcing the apical tight-junctions of the enterocytes and thus preventing the penetration of the pathogens (Figure 1 c).
- producing antimicrobial factors which kill pathogenic microorganisms (Figure 1 d).
- stimulating the innate immune system by signaling dendritic cells which then lead to the induction of TREG cells and to the production of anti-inflammatory cytokines including IL-10 and TGF-β
- preventing or triggering an innate immune response by initiating TNF production by epithelial cells and inhibiting (or activating) NFκB in macrophage dampening (or priming) the host immune response by influencing the production of IL-8 and subsequent recruitment of neutrophils to sites of intestinal injury (Figure 1 f).
6.3.2 Probiotics and Necrotizing Enterocolitis

Weizman made a double-blind placebo-controlled study using a formula supplemented with *Lactobacillus* (L.) *reuteri* or *B. bifidum* for 12 weeks. In the group of infants in therapy with probiotics less gastrointestinal infectious episodes have been demonstrated, fewer episodes of fever compared to placebo, with consequent reduction of antibiotic therapy. The improvement of perinatal care has led to increased survival of high-risk infants (ELBW, respiratory distress, surgery). For this reason one of the neonatal research priorities is the prevention and the treatment of sepsis in NEC and bronchopulmonary dysplasia (Weizman & Alsheikh, 2006). In view of the role of mediators of inflammation in BPD and in sepsis is therefore important to modulate the immune response in these young patients. Some studies have shown that probiotics can alter the intestinal microflora and reduce the growth of pathogenic microorganisms in the intestines of preterm infants, decreasing the incidence of necrotizing enterocolitis and sepsis (Figure 1). Deshpande in 2010 found a significant difference in developing definite NEC between neonates in the control group (no probiotics) compared with neonates in the probiotics group. Moreover he affirmed that the numbers needed to treat (NNT) with probiotics to prevent 1 case of NEC was 25 and confirmed the benefits of probiotic supplements in reducing death and disease in preterm neonates (Deshpande et al, 2010).

6.3.3 Probiotics and Fungal Infections

A study performed in rats with immune deficiency has been shown that the administration of *Lactobacillus rhamnosus* GG (LGG) reduced the risk of colonization and sepsis by *Candida*. One of our retrospective study, performed in 2002 at the University of Catania, showed that supplementation from birth for at least 4-6 weeks of a symbiotic (*Lactogermine plus* 3.5 x10^9 ucf/day) decreased the incidence and the intensity of gastrointestinal colonization of *Candida*, and subsequently its related infections in a group of preterm infants. Another randomized study on 80 preterm infants has confirmed that the administration of LGG (at a dose of 6 billion ufc/day) from the first day of life for a period of 6 weeks reduced the fungal enteric colonization with no side effects (Romeo et al, 2011). Newborns submitted to greater surgical interventions (esophageal atresia, diaphragmatic hernia, intestinal malformations) have an increased risk of bacterial and/or fungal infections due to the use of drains, central venous catheter, tatal parenteral nutrition (TPN), persistent nose-gastric probe that can be the cause of serious sepsis or pneumonias. In a recent study we presented at ESPHGAN, we demonstrated that surgical infants admitted to our NICU and supplemented with probiotics have a reduced risk of bacterial and *Candida* infections and an improved in clinical outcome (Betta et al, 2007). Furthermore in our study newborns treated with probiotics healed in 19±9.3 days of anti-mycotic treatment while newborns who didn’t received probiotics healed in 40.7±16.2 days (p<0.05). In our experience the administration of probiotics has allowed a more rapid eradication of the infection with a lower number of total days of anti-mycotic therapy (Romeo et al.2006) (Figure 2). In another recently published study on preterm infants, the use of probiotics appeared to be effective in the prevention of both bacterial and fungal infections, in the attenuation of gastrointestinal symptoms and in a more rapid weaning from TPN with a reduction in the central venous catheter time and the number of days in hospital. These results were evident both in a group of preterm baby and in a group of surgical newborn treated with a supplementation of probiotics. A new prospector, multicenter, double-blind, randomized placebo-controlled trial in 11 tertiary care NICUs evaluated the capacity of bovine lactoferrin (BLF) supplementation in VLBW newborns with birth weights of <1000 grams. Infants treated with BLF or with BLF and *Lactobacillus rhamnosus* GG (LGG) compared with placebo had
lower incidence of the first LOS episode (P< 0.001 for BLF plus LGG vs control) (Manzoni et al. 2009). The secondary analysis of data from this study show that in newborns treated with BLF or with BLF and LGG compared with placebo, invasive fungal infections (IFI) (P< 0.002 for BLF plus LGG vs control) and the progression rate colonization-infection (P< 0.02 for BLF plus LGG vs control) was significant lower (Manzoni et al. 2012). However in Desphande meta-analysis no significant difference in the risk for sepsis between the probiotics and control group neonates was found (Deshpande et al., 2010). Only one of the 11 trial included in his study reported significantly lower risk for sepsis in the probiotic group (Stratiki et al., 2007).

6.3.4 Probiotics and Neurodevelopmental Outcome

Although oral administration of probiotics has proven to be effective in reducing the incidence of sepsis and NEC in preterm infants, the role on neurodevelopmental outcome in this population of infants is controversial and studied only by a few authors.

Both the studies of Sari (Sari et al., 2012) and of Chou (Chou et al., 2010) reported that oral probiotic administered to VLBW infants reduce the incidence and severity of NEC but did not affect growth, neuromotor, neurosensory, and cognitive outcomes at 18 to 22 months corrected age using the Bayley Scale of Infant Development Second Edition (BSID-II). On the other hand, in a recent work (Romeo et al. 2011) reported a study on 249 preterm infants with sepsis with supplementation of two types of probiotics (Lactobacillus (L.) reuteri, and L. rhamnosus), they performed a structured neurological assessment.
using the Hammersmith Infant Neurologic Examination (HINE) at 12 months. A statistically significant higher incidence of suboptimal scores in the control group was found rather than in both the probiotic ones, concluding that probiotic supplementation may improve the neurological status of these preterm infants. However these studies present significant differences on based population, timing, dosage, and duration of probiotics treatment and to make a clear comparison is quite difficult. In the study of Romeo et al the supplementation of probiotics was given within 72 hours after hospitalization, whereas in the others studies probiotics were given later, on the 7th day after birth when most complications like IVH and periventricular leukomalacia (PVL), and even the inflammatory processes, had already occurred. Furthermore, the based population was clearly different with higher incidence of high risk infants in the studies of Chou (Chou et al., 2010) and Sari (Sari et al., 2012). Romeo in his work shows the potential beneficial effects of L. reuteri supplementation on clinical and physiological variables related to gut function, consistently higher than L. rhamnosus and control groups. L. reuteri improves feeding tolerance, bowel habits and gastric motility by increasing the gastric emptying rate and reducing the fasting antral area, and then reducing episodes of regurgitation. The first and foremost factor is the bacterial tolerance to both acid and alkaline environments. L. reuteri is reported to be highly resistant to the acid pH and to the antibiotic treatment (Romeo et al. 2011). L. reuteri has also the ability to successfully inhibit the growth of pathogens by a combination of different mechanisms including excretion of lactic and acetic acids or other short chain fatty acids, hydrogen peroxide, antimicrobial substances and bacteriocins. Furthermore L. reuteri, when comes in contact with other bacteria found in the human gut, converts glycerol into a potent, broad-spectrum antimicrobial that was termed ‘reuterin’. It is a low molecular weight, neutral, water-soluble compound (3-hydroxy propionaldehyde) capable of inhibiting growth of species representing several bacterial genera as well as yeasts, fungi, protozoa and viruses. Reuterin action is local and it is not systemically absorbed. (Connolly E, 2004). Finally in the study of Romeo, the follow-up was based on a structured neurological assessment at 12 months corrected age, possibly underlining the role of probiotics at early ages. Longitudinal follow-up is therefore needed to explore the effect of probiotics from the first months to pre-school age using structured psychomotor scales, as BSID-II (Romeo et al., 2011).

7 Conclusions

Sepsis is still a serious problem for the neonatal intensive care units. Today improvements in neonatal care allow survival of lower birth weight and lower gestational age infants who have a major duration of hospitalization and more need of parenteral nutrition and of others invasive maneuvers. This is related with a major risk of late onset-sepsis. Despite their usefulness in preventing bacterial sepsis is still under discussion, probiotics in addition to other strategies of prevention, are useful to prevent fungal infection. They in this way decrease morbidity and mortality in ELBW and especially reduce long-lasting neurological sequelae. Certainly more randomized and controlled studies about longitudinal follow-up from the first months to pre-school age of newborns who were admitted at the NICU, are therefore needed to explore long-lasting effects of probiotics.

Abbreviations

BLF—bovine lactoferrin
References


Betta P.; Sciacca P.; Trovato L. et al. (2007) Probiotics in the prevention of Bacterial and Candida infections in newborns submitted to greater surgical interventions and admitted in NICU. Retrospective Group Controlled Study. ESPGHAN, Barcelona, May 9-12, 2007


